

Insights of HBV Pathogenesis and Emerging Clinical Therapies for HBV Induced HCC Patients for a Better Prognostic Outcome

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ABSTRACT

Chronic hepatitis B (CHB) virus infection is a global epidemic and a major cause of hepatocellular carcinoma (HCC). Approximately, 400 million people with 1.5 million Americans are chronically infected with this virus and around 1 million deaths from HBV annually have been estimated. High serum viral load has been detected between 75-80% of HCC patients. Epidemiological studies revealed that there are geographical variations in the incidence rate of hepatitis B virus genotype and subgenotypes. Furthermore, HBV induced HCC has a direct correlation with the recurrent genomic integration of this virus and cross-talk of its oncogenic protein HBV x (HBx) with different cellular signaling pathways. With the help of an extensive pathological study, whole genome sequencing and molecular insights of HBV induced HCC patients, suitable medications providing high-genetic barrier to resistances are made available. These medications can prevent HCC by inhibiting viral replication, modulating the immune response or both. The evolution of drugs with superior efficacy and less toxicity are providing important tools for clinicians to reduce HBV related HCC. This review sheds new perspective on molecular insight of HBV life cycle and clinical updates to treat sustained virus infection and associated cancers.

Key words: HBV, Epidemiology, HCC, peg-IFN- α , Tenofovir, Entecavir, Clevudine, Clinical trials

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INTRODUCTION

Hepatitis B is a systemic infection with major pathology in the liver. Caused by HBV, a DNA virus with human as the only reservoir, it is a serious worldwide public health problem and major cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma. Of the estimated 2 billion people who have serological evidence of past or current HBV infection, more than 400 million people are chronic carriers of whom there is ~1 million deaths annually from HBV related diseases.¹⁻⁴

HBV is usually transmitted by parenteral route and contact with infected body fluids such as blood, semen and saliva.⁵ In comparison to low endemic areas where transmission is in adulthood which is self-limited in most, in highly endemic countries it is transmitted early in life by vertical or horizontal mode increasing the chronicity rate.⁶ Chronic hepatitis B (CHB) is the major etiological factor of HCC

globally with more than one half of HCC patients being chronic carriers.⁷ The prevalence of CHB is highly variable, ranging from 0.01% in United States to 20-30% in some Pacific Island Nations.⁶ From virological point of view, CHB is defined as the active viral replication with sustained hepatitis B surface antigen for more than 6 months with or without HBe antigen (HBeAg).⁸

The availability of an efficacious and safe vaccine since 1982 with implementation of effective vaccination programmes in various countries, ensued in a significant decrease in the incidence of acute as well as chronic HBV infection. But because of the high morbidity and mortality rate among chronic carriers worldwide, hepatitis B still remains a disease of considerable importance.

Knowledge of the epidemiology of HBV will be an initial direction towards prevention and treatment of this global infection. It is known that prevalence of chronic HBV

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infections varies according to geographical locations. Related to this, it has been categorized as high, intermediate and low endemicity.⁵ This geographical variability showed a positive correlation with the risk of HBV induced HCC in patients.^{5,9} In the pathogenesis of HCC, virological factors contribute an important role. The complex life cycle of HBV initiates with viral entry into the cell by binding to Na⁺ taurocholate cotransporting polypeptide (NTCP) on the surface and being endocytosed.¹⁰ Thereafter the virus modulate hepatocytes by genomic integration and its stages of replication. Appropriate understanding of the HBV life cycle can help in the development of novel antiviral drugs and design effective therapeutic measures. This review highlights important aspects of HBV induced HCC such as epidemiology, life cycle and current clinical therapies.

Epidemiology of HBV induced HCC

The prevalence of HBV varies markedly in different parts of the world. The main clinical marker of HBV which indicates chronic or acute infection, prevalence as well as endemicity is HBsAg. A huge load of HBsAg infection was recorded in all sub-Saharan African regions, East Asia and to a lesser extent, in Oceania and Andean Latin America.¹¹ In other regions such as Tropical Latin America, Central Latin America, North America and Western Europe throughout all age groups the prevalence was below 2%.¹¹ The development of HCC has been strongly associated with chronic HBV infection since 1970's.¹² Similar studies showed that in high incidence areas such as east Asia and sub-Saharan Africa, more than 80% patients who developed HCC patients were seropositive for HBsAg.¹²

Molecular insight of HBV pathogenesis to induce HCC

The HBV is classified as the type species of the *Orthohepadnavirus*, though the *Hepadnaviridae* family of viruses has not been assigned to a viral order.¹³ The virion or the virus particle of HBV consists of an outer lipid envelope and an icosahedral nucleocapsid core composed of protein; the nucleocapsid encloses the viral DNA and a DNA polymerase that has reverse transcriptase activity similar to retroviruses.¹⁴ Also this virus has filamentous and spherical bodies lacking a core, which are composed of the surface antigen HBsAg. The genome of the HBV has several distinctive features which contain: (a) a partially double stranded DNA, (b) circular DNA conformation, (c) dependence on a reverse transcription step in the viral cycle of replication, and (d) persistence of the viral genome in infected cells as either integrated forms, or as episomal form.¹⁵

Recent studies help us to provide a comprehensive idea about the mechanism of integration of HBV DNA into host genome, and further possible rearrangements for its

oncogenicity.¹⁶ HBV replicates productively in the liver (unintegrated in host cell DNA) and the infectious virions circulate in the plasma along with an excess of HBsAg (Figure 1). The HBV genome has recombinant proficient areas based on the distribution of DNA junctions.¹⁷ All the integrated viral genomes are defective at least around the cohesive end region, particularly within the X gene. Experimental evidence suggests that capsids with mature genome interacts with nucleoporin 153 in nuclear basket to get inside the nucleus.¹⁸ The partial DNA strand of the genome is synthesized and a complete double-stranded DNA covalently closed circular molecule (cccDNA) is formed in the nucleus.¹⁹ During the initiation of infection of the liver by HBV, both linear DNA and rcDNA are converted into cccDNA.²⁰ The cccDNA is transcribed into mRNA by host cell RNA polymerase II. The mRNA is translated into structural proteins (envelope and core proteins) and non-structural proteins (enzymes) in the cytoplasm. Within the core, the negative sense DNA strand is synthesized by reverse transcriptase. The plus-strand is not synthesized prior to the virion release, as a repair damage mechanism to produce a fully dsDNA during initiation of a subsequent round of infection.

Apart from HBV infection, this virus induced hepatocarcinogenesis is the most important aspect which has a direct correlation with its life cycle and its 17 KDa molecular weight HBV x protein (HBx) (Figure. 1). The genomic integration of HBV virus causes multiple effects such as chromosomal instability, copy number variations, aberrant expression of nearby genes and interruption of normal function. Previous studies reported that HBV preferentially incorporated into *TERT*, *CCNE1*, *FAR2*, *ITRP1*, *IRAK2*, *MAPK1*, *MLL2* and *MLL4* to cause aberrant expression for liver cancer.²¹⁻²⁶ With the help of next generation sequencing (NGS), more comprehensive pictures of HBV integration into human chromosomes are revealed. Whole genome wide studies of HBV induced HCC patient samples revealed that the virus integration is occurring in both tumor and normal cells in hepatic tissue.^{27,28} HBV integration was predominantly detected at chromosome 10 in the tumors whereas random distribution was observed in normal tissues.²¹ Notably, HBV integrations are predominantly occurred at enhancer, transcription factor binding sites and exon regions to induce genomic and transcriptomic aberrations in the tumors.^{16,27,29} Also, recurrent integration around 31-40% has been detected in tumor areas in certain genes that are predominantly associated with cell communication, differentiation, transcriptional regulation and negative regulation of cell death.^{16,27} Furthermore, non allelic recombination between two copies of integrated viral sequences causes genomic instability and loss of pro-apoptotic associated genes.²⁹ Contrary to this, viral integrations were detected in intronic

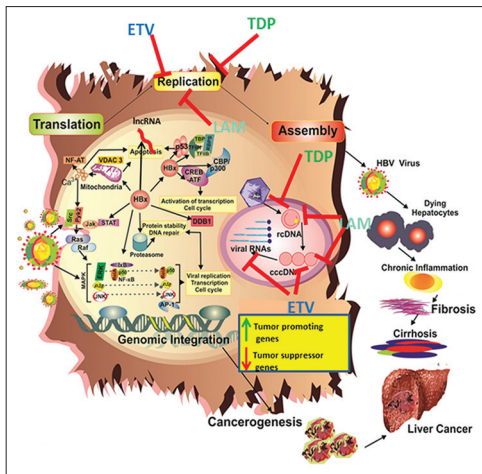


Figure 1: Molecular insight of HBV life cycle to induce HCC and novel antiviral therapies to alleviate liver cancer. Hepatotropic HBV infects hepatocytes to initiate its pathogenic life cycle inside the host. During the initiation of infection of the liver by HBV, both linear DNA and rcDNA are converted into cccDNA. The cccDNA is transcribed into mRNA by host cell RNA polymerase II. The mRNA is translated into structural proteins (envelope and core proteins) and non-structural proteins (enzymes). Also, HBV virus undergoes genomic integration to regulate tumor promoting and suppressor genes to induce HCC. Sustained viral infection promotes chronic inflammation in hepatic microenvironment to drive sequentially fibrosis to cirrhosis to liver cancer. Therapeutically, third-generation nucleotides analogues (tenofovir and entecavir) maintained superior viral suppression in long-term therapies post tumor resection to first-generation drug (lamivudine). These drugs are inhibiting viral replication by chain terminator to inhibit DNA synthesis or interfere with priming or acts as a competitive inhibitor of dGTP. cccDNA, Covalently closed circular DNA; rcDNA, Relaxed circular DNA; HBx, Hepatitis B virus X protein; LAM, Lamivudine; ETV, Entecavir; TDP, Tenofovir disoproxil phosphate; IncRNA, Long non coding RNA.

regions in normal tissues that might explain a favorable clonal expansion of most integrated positive hepatocytes in tumor areas.²⁷

Besides genomic integration, the multifunctional regulatory protein HBx plays central role for the pathogenesis of HBV-induced hepatocarcinogenesis. During HBV integration into host genome, approximately 40% of breakpoints on the HBV genome were detected around 1800- base pair region where HBx and core genes are located.²⁷ Thus chimeric transcripts of HBx with human gene can be detected that might promote tumorigenic potential and corrupt tumor suppressors. One such chimeric transcript HBx-LINE1 was identified in 23.3% of HBV associated HCC.^{30,31} Functional characterization of HBx-LINE1 revealed that it might have a role in metastasis by promoting epithelial-mesenchymal transition. Besides chimeric fusions the classical oncoprotein HBx can modulate cell proliferation, apoptosis, migration, cell cycle progression and epigenetic regulations due to its prolonged expression^{32,33} (Figure 1). The HBx protein is a dual-specificity activator of transcription, stimulating signal transduction pathways in the cytoplasm and transcription

factors in the nucleus.³⁴ Extensive studies on the functional roles of HBx showed its transactivation property to upregulate viral and cellular genes including enhancers, class II and III promoters and proto-oncogenes through a wide variety of cis-elements.³⁴ Cytoplasmic protein interaction with HBx has been shown to induce numerous signal transduction pathways such as NF- κ B,^{35,36} NFAT,³⁷ RAS/RAF/MAPK,³⁸ JAK-STAT,^{39,40} Wnt/ β -catenin⁴¹ and anti-apoptotic activity by inhibiting p53 dependent activities⁴² and modulating the serine proteases hepsin activities⁴³ and activation of surviving⁴⁴ (Figure. 1). Inside the nucleus, HBx interferes with epigenetic changes by directly regulating DNA methyl transferase-3A and histone deacetylase-1,^{45,46} DNA repair mechanism⁴⁷ and cell cycle related kinases⁴⁸ to promote hepatocarcinogenesis (Figure 1). Furthermore, recent characterization of HBx interaction with miR-148,⁴⁹ long noncoding RNA-Drch⁵⁰ and ERK/CREB pathway to upregulate FOXM1 expression⁵¹ provides a better insight about the functional role of HBx in invasiveness and metastasis of HCC. Overall, the recent findings on the functional role of HBx protein and HBV induced genomic changes greatly improved our therapeutic strategy to alleviate HBV driven liver cancer.

Therapeutic approaches towards HBV induced HCC

The objective of HBV treatment is to prevent cirrhosis, liver degeneration and hepatocellular carcinoma. The paradigm of anti-viral therapy is continuously improving with an emphasis on long-term viral suppression, targeted, potent, minimal toxicity, suitable biomarker and less chance to emerge drug resistant mutant HBV strain.⁵²⁻⁵⁷ Vaccination for HBV is the most common mode of prevention. However, it has certain limitations as several patient populations are noted to have suboptimal seroprotective rates after HBV vaccination.⁵⁸

Presently, surgical resection along with concomitant antiviral therapy is considered suitable therapeutic strategy to reduce viral load and alleviate liver inflammation for extending the overall survival of HBV induced HCC patients. In case of antiviral treatment, there are several approved therapeutic medications like cytokine based therapy (interferon- α (IFN- α) and pegylated IFN- α) and nucleotide/nucleoside analogues (lamivudine, adefovirdipivoxil, entecavir, telbivudine, tenofovir and clevudine). The selection of specific treatment is based on patient characteristics and stage of the disease. Clinical studies showed that IFN- α based treatment causes higher rate of HBsA gloss among patients having HBV genotype A due to its immunomodulation. Also, lack of drug resistant viral strain and sustained seroconversion facilitate the overall survival period of HCC patients in this therapy.^{59, 60} However, IFN- α has certain side effects- short plasma half-life that was improved by

using pegylated-IFN- α . Moreover, doses of 5-10 MU might cause influenza like symptom and organ toxicities-central nervous system disturbances, thyroid dysfunction and blood cytopenias.⁶¹ Modified version peg-IFN- α showed improved therapeutic efficacy and more suitable dose regimen.⁶² Based on large scale randomized studies, peg-IFN- α therapy exhibited considerable loss of HBV DNA (~25%), significant amount of HBeAg seroconversion (~20-32%), optimal viral suppression maintained (a mean of ~3 yrs) and a higher clearance rate of HBsAg (~6%).⁶³⁻⁶⁶ Moreover, peg-IFN- α therapy for 52 weeks showed significant sustained virological responses in patients (25%) having advanced fibrosis compared to without fibrosis (12%) and cirrhotic patients (30%) compared to without cirrhosis (14%).⁶⁷ Despite lot of advantages of peg-IFN- α therapy, this is not widely approved for HBV treatment due to its limited specificity towards genotype A and B of HBV and substantial side effects. Thus, IFN- λ could be clinically beneficial for non-responding patients towards peg-IFN- α therapy after its successful inhibition of HBV replication in preclinical model.^{68,69} These immunomodulatory based cytokine therapies showed an important step towards therapy of HBV induced HCC patients. However, persistence HBV infection causes downregulation of RIG-1 mediated innate immunity and upregulation of immunosuppressive cytokines- transforming growth factor- β and interleukin-10.⁷⁰

From clinical data obtained from these chronic HBV patients, it was concluded that HBV specific T cells are either deleted or exhausted and there is substantial increase of immunosuppressive regulatory T cells.^{71,72} These could be a potential explanation of the limited success of IFN based therapy or vaccination approach for chronic HBV patients. To reactivate adoptive immune system, T-cell receptor gene transfer technique and HBV antigen-pulsed monocyte-derived dendritic cells could be used as an emerging therapy for lysing HBV infected hepatocytes that express viral antigens.^{52,73,74}

Immune activation based therapies are important aspect to alleviate HBV related HCC by increasing sustained virological response, baseline ALT value and histological improvements. However HBV reactivation is frequently occurred in patients with baseline HBV replication status. Antiviral therapies are considered to reduce viral load and ameliorate hepatic inflammation to improve postoperative survival benefit. The first approved drug lamivudine showed seroconversion in both HBeAg positive and negative patients around 44-47% approximately and 56% respectively after 2-5 years of lamivudine therapy.⁷⁵⁻⁷⁷ The results of this study clearly suggested that lamivudine treatment improved the overall survival of patients but not recurrence free survival after radical hepatectomy

in these patients. Based on Sung et al meta-analysis of 5 studies (n=2289) suggested that lamivudine therapy greatly reduced the risk of HCC in 78% of treated patients and this drug acts more effectively on patients without cirrhosis than with cirrhosis.⁷⁸ Though, this drug showed amelioration of fibrosis and decrease the incidence of liver cancer, long-term therapy causes drug resistant HBV strain. Also, HBV reactivation was also detected in >50% patients during post-therapeutic period.^{79,80}

Due to various deleterious side effects of lamivudine, alternative antiviral drugs were explored with lower risk of drug resistance, more potent and affordable for long-term use. Clinical trial evaluations of several nucleoside and nucleotide analogs identified adefovirdipivoxil, entecavir, telbivudine, tenofovir and clevudine to consider superior antiviral agents to lamivudine. Based on the clinical experiences with these drugs, tenofovir and entecavir are considered the most potent first-line monotherapies for treating CHB and its associated HCC patients. Mechanistically, both of these drugs, tenofovir and entecavir, undergo phosphorylation multiple times by human cellular kinases to transform into their active metabolite with a half-life of 95 and 15 hrs respectively.⁸¹ Both drugs showed virological response rate around 95% with minimal drug-resistant, well-tolerated and superior safety profile upon long-term therapy.

The long-term therapy with tenofovir disoproxil fumarate (TDF) is showing promising results for reducing the risk of HCC in CHB infected patients. In a large-scale study with 641 patients treated with TDF for 6 years showed that the incidence of HCC is reduced compared to predicted HCC risk.⁸² Also, there was a progressive divergence occurred between the predicted and observed number of HCC incidences after 3.3 years of follow-up with a standardized incidence ratio of 0.55 (95% CI) at the latest follow-up (median 5.52 years).

In another two prospective studies of Tenofovir where patients were showing phenotypic and genotypic resistances towards adenovir or lamivudine or both retain significant activity against HBV.^{83,84} Patients in one of these studies had resistance to multiple nucleotides- lamivudine and adefovirdipivoxil (~40%), lamivudine, adefovirdipivoxil and entecavir (~60%).⁸³ Tenofovir containing treatment regimen for 12 and 24 months in these patients showed complete virologic response (serum HBV DNA level \leq 60 IU/mL) of 86.2% and 96.6% respectively. In other clinical trial, 63% patients had adefovirdipivoxil and 37% had adefovirdipivoxil and lamivudine resistance with a median duration of drug exposure between 2-3.5 years.⁸⁴ Tenofovir containing mono or combination therapies for 96 weeks in these 60 enrolled patients achieved an undetectable viral load <15 IU/mL.

in 64.4% of cases. Given these clinical results, it can be concluded that tenofovir can be administered either as monotherapy or rescue therapy where first line of antiviral treatment did not show any improvement.

The effect of third-generation drug entecavir showed lot of promising results on HBV related cirrhotic patients with a risk of liver cancer. One retrospective-prospective cohort clinical study was conducted with 1446 entecavir-treated patients with a follow-up for 36±13 months.⁸⁵ Overall, there was no significant difference in hepatic events between entecavir-treated patients compared to control cohorts. Nevertheless, entecavir-treatment significantly reduced the risk of HCC in 78% of the 482 cirrhotic patients compared to untreated patients. Compared to tenofovir, entecavir was unable to achieve viral suppression in patients who previously received antiviral drug therapies- lamivudine, adefovir, telbivudine or cytokine based therapy peg-IFN- α .

Both tenofovir and entecavir are able to achieve long-term response against virus with low rates of viral breakthrough and well-tolerated among patients except tenofovir treatment showed nephrotoxicity.^{86,87} However, superior therapeutic efficacy between tenofovir and entecavir is not widely studied in clinical trials. One retrospective case-control study was conducted to compare between 62 patients treated with tenofovir and 199 patients treated with entecavir.⁸⁸ Though there is no significant difference in rates of viral suppression, tenofovir treated patients achieved viral suppression faster (16% at 6 months, 50% at 12 months, and 71% at 18 months) than those taken entecavir (11% at 6 months, 13% at 12 months, and 39% at 18 months). This study is definitely recommending

for tenofovir as a suitable medication for HBV induced HCC however more worldwide studies especially patients with genotypic variations are required for definitive recommendation. Apart from these two drugs with extensive clinical trials, a new antiviral pyrimidine nucleoside analogue clevudine has been approved in South Korea.⁸⁹ Initial multicenter clinical trials demonstrated that daily dose of 30 mg for 24 weeks maintained virologic response in 59% of HBeAg positive and 92% in HBeAg negative CHB patients without any viral breakthrough.^{53,90} In another retrospective study, clevudine therapy showed comparable ALT normalization of 83.9% at 48 weeks and 91.5% at 96 weeks to entecavir treated patients 80.9% and 91.2% respectively.⁹¹ Also, serum HBV DNA level was lowered in 72.6% and 83.1% in clevudine treated group compared to entecavir treatment group 74.4% and 83.8% respectively during the same time points. The initial outcomes of clevudine therapy are as good as entecavir upto 96 weeks study. More long-term and rescue therapies are required to understand its optimal clinical efficacy. In this review we have also provided updated results of completed clinical trials in Table 1 which might be a better opportunity for HBV infected HCC patients in near future.

CONCLUSION

Over the last decade several seminal studies have provided numerous breakthroughs in molecular pathogenesis of HBV related liver cancer and its effective management strategies. Extensive genomic sequencing of HBV induced HCC patients have suggested the complexity of HBV infection and its targeted multiple signaling pathways that

Table 1: An overview of recently completed clinical trials for HBV and HCC drugs

Drug name	Type of CLD	Phase of trial	Outcome of trial	References
HBsAg-HBIG immunogenic complex therapeutic vaccine	HBV	III	HBeAg seroconversion is decreased. Overdosing caused immune fatigue	[83]
Besifovir	HBV	IIb	Similar effect as entecavir	[84]
HEPLISAV	HBV	III	Complete seroprotection after three doses	Dynavax Technologies
NASVAC	HBV	III	Complete seroconversion as early as in 30 days and seroprotection with highest α -HBs titer at day 90	[85], NCT01374308
Molixan	HBV	II/III	Immunomodulating and anti-inflammatory. More than 700 Russian patients were treated successfully	[86]
X-linked inhibitor of apoptosis (XIAP) antisense AEG3515 and sorafenib	HCC	II	More promising than sorafenib alone	[87]
Tivantinib	HCC	II	Potential alternative option for advanced HCC patients	[88]
Sorafenib and Gemcitabine	HCC	II	Not better than sorafenib alone	[89]
Sorafenib and Tegafur/Uracyl	HCC	II	More efficacious than sorafenib alone	[90]
MGN-3 with interventional therapy	HCC	II	Useful for the treatment of HCC	[91]
Pegylated Arginine deiminase	HCC	II	Promising and well tolerated	[92]
TRC105 anti-angiogenic monoclonal Antibody	HCC	II	Well tolerated in patients. Suitable as a combination therapy	[93]
5-fluorouracil/leucovorin (FOLFOX4)	HCC	III	Increased response rate and median overall survival	[94]

drive tumor progression. Also, the protein HBx regulates in cis- and trans- tumor promoting and suppressor proteins which are often found to play critical roles in early tumorigenesis and development. Parallel to molecular insights, long-term therapy with immunomodulators or nucleotide analogues or in combinations showed significant viral suppression in numerous clinical trials. The treatment by high genetic barrier nucleotide analogues is highly beneficial to HBV patients with high baseline risk of cirrhosis. In addition these next generation nucleotide analogues promote virological remission in patients, thereby reducing the risk of HCC. Along with therapeutic advancements there is also a serious requirement of global multicenter clinical trials among different ethnic groups and compilation of the results to predict drug efficacy.

HIGHLIGHTS

- HBV mediated liver infection is affecting approximately billion of people across the world based on hepatitis foundation.
- Approximately 75-80% of HCC cases are associated with chronic infection of HBV.
- Whole genomic sequencing analysis revealed lot of new insights about the HBV induced HCC
- This review provides the latest promising results of clinical trials and long term therapies with tenofovir, entecavir and clevudine for HBV induced HCC patients.

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