

HEPATOCARCINOGENIC PROPERTIES OF HEPATITIS B VIRUS

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ABSTRACT

Approximately two billion people are infected with hepatitis B (HBV) worldwide. Infection due to the virus can be influenced by host immune status, age, and vigorosity of viral replication. Majority of patients recover from acute infection while a larger percentage of new infections, contracted mostly in utero, advance to a chronic form of the disease and therefore stand the chance of developing complications. The mechanisms through which chronic HBV infection causes hepatocellular carcinoma (HCC) are however unclear, though evidence exist to show that the virus itself is hepatocarcinogenic in action. With an estimated 85% worldwide cases of HCC attributed to HBV, the virus is said to be one of the leading causes of cancer-related deaths. Although several discoveries have been made in the physiology and pathology of cancer in relation to disease progression, mortality rate of HCC is still high due to late diagnosis. This occurrence is however low in developed countries than developing countries where the endemicity of HBV strongly predisposes to the development of chronic liver disease and subsequent progression to HCC. It is indeed necessary to encourage HBV vaccination as well as treatment of chronic hepatitis B, to facilitate the application of many of the advances that have been made in the understanding, prevention, and treatment of hepatocellular carcinoma.

Key words: tumor, cirrhosis, HBV protein, HBV DNA, hepatocyte, chronic hepatitis B

INTRODUCTION

Liver infection due to Hepatitis B virus (HBV) is a major threat to public health worldwide. It is estimated that there are more than 350 million HBV carriers worldwide, with about one million deaths recorded annually from liver diseases due to the virus. Implementation of effective vaccination programs in many countries has, however, significantly reduced the incidence of acute hepatitis B the virus still remains an important cause of worldwide morbidity and mortality (1).

HBV causes both acute and chronic infection in humans with varying clinical manifestations (2). During the acute phase, manifestations range from subclinical or anicteric hepatitis to icteric hepatitis and, in some cases, fulminant hepatitis, while in the chronic phase, manifestations range from an asymptomatic carrier state to chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Extrahepatic manifestations, including serum sickness, polyarteritis nodosa, and essential mixed cryoglobulinemia, membranous glomerulonephritis, and aplastic anemia can also occur with both acute and chronic infections (3; 4).

Hepatocellular carcinoma (HCC), characterized by rapid tumor growth and a high rate of vascular invasion, is the fifth most common malignancies worldwide (5) with majority of cases largely due to chronic infection with HBV (6; 7). In addition chronic hepatitis infection could cause cirrhosis with global incidence of 57% estimated to be due to infection with HBV (8). Cirrhosis could be present in 80–90% of patients with hepatitis B-related HCC, but there is also evidence that HBV infection could directly damage DNA in liver cells, potentially leading to cancer when cirrhosis is not present (9).

Due to differences in the rate of prevalence of viral hepatitis, there is high incidence of HCC in low and middle income countries which collectively account for about 85% of cases, and low in high income countries where 40% of cases are recorded (10; 11).

There are two major HBV-specific mechanisms that contribute to the development of HCC. The first is the integration of the viral genome into the host chromosome with the resultant loss of tumor suppressor gene functions, and/or activation of tumor-promoting genes, while the second is associated with the expression of trans-activating factors obtained from HBV genome, which have the capacity to affect intracellular signal transduction pathways and change host gene expression (12).

Transmission of HBV is through infected blood or other body fluids, from mother-to-child, household contacts; sexual intercourse; needle-sharing; and occupational/health-care-related issues. The highest concentrations of infectious HBV are found in blood and serum. However, other body fluids, such as semen and saliva could also be infectious. Individuals with chronic HBV infection are the major sources of infection, although any person testing positive for HBsAg could also be infectious to both household and sexual contacts (13).

HBV is a partially double stranded circular DNA virus in the family *Hepadnaviridae*. The DNA, which is organized in a compact manner, is 3.2 kb, with four partially-overlapping open reading frames (ORF) including surface (S), core (C), polymerase (P) and X genes. These viral genes can be translated to different viral proteins from four major transcripts, such as surface antigen (HBsAg), e antigen (HBeAg) and core antigen (HBcAg). The host immune system produces corresponding antibodies against these viral proteins, including anti-HBs, anti-HBe and anti-HBc. These antigens and antibodies lay the basis for serological diagnosis of HBV infection (2). HBV as well as other members of the *Hepadnaviridae* family including the duck hepatitis virus, woodchuck hepatitis virus and ground squirrel hepatitis virus are species specific, infecting only humans and higher primates (14).

Preventive measures adopted to reduce cases of HCC are targeted against new cases of HBV, protection of susceptible individuals, and early detection of tumor. Wide spread vaccination against HBV has subsequently lead to reduction in cases of chronic hepatitis B and HCC in children and adolescents. In addition, randomized controlled trials and meta-analysis has shown that successful administration of relevant antiviral drugs targeted against chronic hepatitis B could reduce the risk of HCC and cirrhotic complications (11).

As a global public health problem this study was undertaken to examine the various properties that aid hepatitis B virus (HBV) in its ability to cause hepatocellular carcinoma (HCC).

BRIEF HISTORICAL BACKGROUND

An aetiological association between cirrhosis and HCC was suspected as early as the beginning of the 20th Century (15). By the 1950s it was realized that it was posthepatic cirrhosis that was most closely linked to tumour formation (16; 17). Following the introduction of serological tests for HBV in the late 1960s it soon became evident that chronic HBV infection was directly responsible for the induction of HCC. Refinements of molecular techniques for identifying viral DNA, availability of HCC cell lines harbouring integrated HBV DNA, and a variety of animal models, either infected with viruses that belong to the same family as HBV or transgenically propagated with specific HBV genes, has made it possible to investigate in depth the role of chronic HBV in the pathogenesis of HCC (18).

MECHANISM OF HCC INDUCTION BY HBV

Multifactorial mechanisms are responsible for the development of chronic HBV-induced HCC (Fig 1). This includes factors contributed by the virus, the host, as well as viral DNA integration-induced genetic alterations, expression of oncogenic viral proteins and hepatitis that result from chronic activation of the immune system (19).

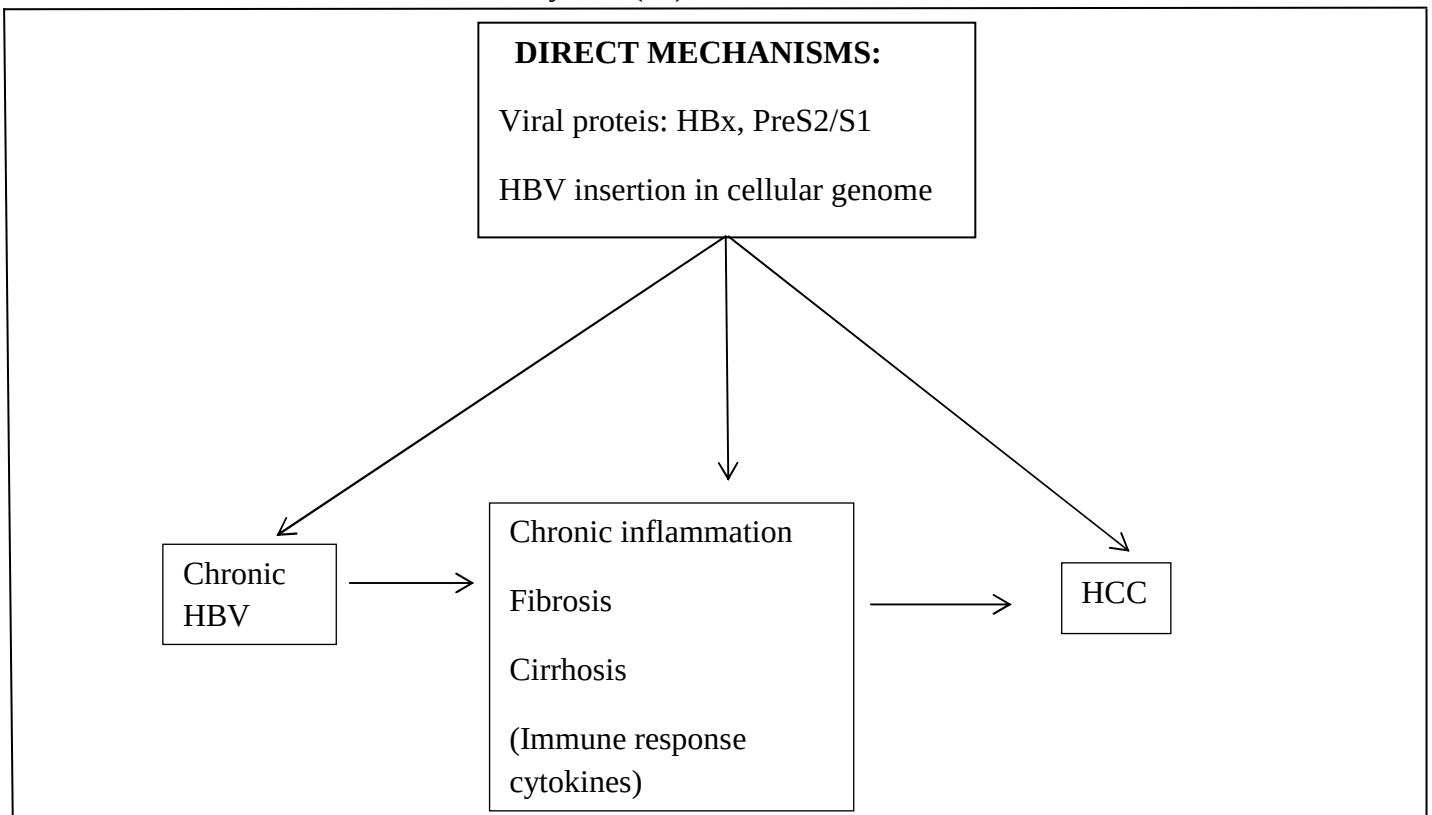


Fig 1. Schematic representation of the mechanisms involved in HBV-related chronic liver lesions and HCC. The figure underlines the dual effects of HBV. Expression of the viral proteins stimulates the host immune response and triggers liver inflammation; some viral proteins as well as insertion of the viral DNA into liver cell genome also directly interfere with cell proliferation and viability and induce genetic alterations.

The course of chronic hepatitis B leading to HBV-related hepatocarcinogenesis is indeed dynamic due to periodic down-regulations of viral titers and associated immune-mediated liver injuries known as flares which result in repeating cycles of death and proliferation of hepatocytes (20). Liver injuries due to chronic hepatitis B are immuno-mediated caused by HBV-specific T cells. Some studies have also implicated non-specific chemokine-mediated infiltration of neutrophils, natural killer cells and activation of resting lymphocytes, all inflammatory cells known for their release of cytokines and chemokines that may enhance the development of cancer. It is indeed the high rate of hepatocyte proliferation that is directly responsible for hepatocellular carcinoma development in the cirrhotic liver (21).

Studies have shown that chronic inflammation results in increased production of reactive oxygen species (ROS) which can damage DNA, leading to gene mutations (22) as well as activation of several signal transduction pathways that regulate proliferation, differentiation and apoptosis, including the mitogen-activated protein kinase/activated protein-1 and nuclear factor kappa-light-chain-enhancer of activated B cells (MAP-kinase/AP-1 and NfκB) pathways (22). Inflammation-induced oxidative stress and influx of Kupffer cells can promote the activation of stellate cells which are the main producers of extracellular matrix in the liver (23), leading to cirrhosis, characterized by the co-existence of regenerative nodules, irreversible fibrosis and severe liver injury (20).

Cirrhosis is indeed an important predisposing condition for HCC of all etiological backgrounds, approximately 3–5% annually. In HBV endemic areas, infected patients with cirrhosis have a greater risk of developing HCC than those with chronic hepatitis B only, or asymptomatic carriers (24). Chronic occult HBV could also become indirectly oncogenic through the sustainance of a mild necroinflammation that contribute to cirrhosis and HCC development (25).

The predisposing factors to cirrhosis development and mortality include HBeAg-negative rather than HBeAg-positivity, old age, persistent viral replication, and genotype C rather than genotype B as observed in Asian patients (26). Factors observed to contribute to cirrhosis progression include alcohol ingestion and HCV co-infection. Once cirrhosis is established, liver decompensation follows after a brief period. This is observed in a EUROHEP cohort study where observations made for a period of 5 years shows that hepatic decompensation was 16%

with incidence per 100 person years shown to be 3.3 while the mean interval between the time of diagnosis of cirrhosis and the onset of first episode of decompensation was 31 months (27). After decompensation, rate of survival may drop to 55% from 70% at the first 1 year and about 14% from 28% after 5 years (24).

HCC of all etiological background develop after a long term activation of the immune system as a result of hepatitis (28). This is characterized by persistent cycles of gradual liver cell destruction and regeneration, leading to fibrosis, cirrhosis, and HCC (29). In one study to establish this finding, transgenic mice which was observed to produce hepatotoxic quantities of L-HBs (28) was also shown to display hepatocellular injury, regenerative hyperplasia, chronic inflammation, Kupffer cell hyperplasia, oxygen radical production, glutathione depletion, oxidative DNA damage, transcriptional deregulation and aneuploidy that subsequently progressed to HCC (30). Similarly transgenic mice that were shown to express nontoxic concentrations of HBV envelope proteins in the hepatocyte without liver disease also developed HCC after a long period of chronic hepatitis attributed to HBV-specific cytotoxic T lymphocytes (31).

HCC still occurs in these conditions despite the lack of cofactors such as viral integration and X gene expression. Immunological, virological and histological features of this chronic immune-mediated HCC model is nearly similar with the features of human chronic hepatitis, thus suggesting that an ineffective immune response could adequately influence the oncogenicity of chronic HBV infection in man. The significance of the pathogenesis of immune-mediated hepatocellular injury in the hepatocarcinogenesis of HBV is further supported by the fact that HCC is preceded by cirrhosis in several human liver diseases due to hepatitis B, chronic hepatitis C (32), alcoholism (33), hemochromatosis (34), glycogen storage disease (35), α -1-antitrypsin deficiency (36), and primary biliary cirrhosis (37).

However irrespective of etiology or pathogenesis of HBV-induced HCC, chronic liver cell injury is therefore a premalignant predisposing condition that initiates a series of events that lead to increased rates of cellular DNA synthesis, production of endogenous mutagens, and compromised cellular detoxification and repair functions. Sustainance of these processes for a sufficiently long period of time would cause multiple genetic and chromosomal changes necessary for the initiation of HCC (31).

The cycle of hepatocarcinogenesis in about 85–90% of HBV-related HCCs begins with HBV genome insertions into the DNA of host hepatocytes (38). However the extent by which this process contributes to HCC is still unclear (12) although studies have shown that integration events precede the development of tumor in individuals with both chronic and acute HBV infection (19).

The insertion of HBV genome are facilitated by cellular conditions such as disruption of the stability of host DNA, increase of cellular DNA replication, exposure to oxidative stress or DNA-damaging agents, deficiency of DNA repair machinery, co-infection with other viruses and chronic liver injuries that increase the total number of hepatocyte (38). With the exception of the woodchuck hepatitis B virus (WHV), which prefers to insert its DNA into c-Myc or N-Myc genes located in its DNA, no other hepatitis viruses including HBV have been observed to show any specific preference for insertion of its gene into the human genome (38). The insertion can, however, cause general genomic instability in host cells and as a consequence lead to chromosome deletions, duplications, amplifications, translocations and transpositions of viral and surrounding host genes between chromosomes (19). In addition, regulatory proteins such as HBx and PreS2 activators can function as an enhancer of tumor initiation, leading to positive selection of cells producing a functional regulatory protein (39).

Liver biopsies of HCC patients shows that cancer-causing cellular genes are altered by HBV DNA insertion, especially those regulating cellular immortalization or hTERT, proliferation or MAPK1, cycline A, and viability or TNF receptor-associated protein 1, suggesting that hepatocytes that harbor these insertions are preferred during hepatocarcinogenesis (38). One of the consequences of rearranging viral and host DNA sequences during integration is the production of aberrant protein products whose functions may predispose hepatocytes to oncogenicity. This condition, observed as mutation at the C-terminal of the X protein caused by deletion of inserted viral DNA, has been reported to enhance oncogene-induced transformation, whereas the wild-type full-length protein suppresses such transformation (40). HBx mutations is however common among occult HBV infections (41). Though the insertion of HBV DNA into the host DNA might randomly contribute to the formation of an unstable environment, and sometimes causes genomic mutations that facilitate hepatocyte transformation even in the case of

occult HBV infections, HBV integration is still partially implicated in the development of most HBV-induced HCCs (12).

In addition HBV insertions in HBV-related HCCs can also occur in genes that target telomerase and mixed lineage leukemia encoding genes, suggesting potentially common pathways in HBV-related carcinogenesis (42). HBV DNA integration has also been observed in patients with chronic hepatitis suggesting that integration is an important step in the initiation of hepatocarcinogenesis (43). However, HBV integration does not occur in resting hepatocytes further suggesting that integration of HBV genome is a predisposing condition to hepatocarcinogenesis, preceded by procarcinogenic events that lead to excessive hepatocyte generation (29).

Recently, it was reported that HBV is easily integrated into liver tumor cells than in normal liver tissue cells. Similarly it has been observed that multiple integrations may be responsible for rapid and aggressive disease progression and reduced chances of survival. In one study Sung *et al.*, (43) compared parallel sequencing of 81 HBV-positive and 7 HBV-negative HCC tumors with adjacent normal tissues, and revealed that the incidences of HBV integrations were high with 76 out of 88 patients observed to have HBV integration. They observed that HBV will integrate into the following genes: CCNE1, SENP5, and ROCK1. This causes an increase in the expression levels of these genes, and thereby enhances tumor growth. This observation is beside that reported previously about integration into TERT and MLL4 genes. The report concluded that HBV integration induced chromosome instability, resulting in the scrambling of the genome.

In addition to the integration of viral genome into host DNA, another direct role played by HBV in hepatocarcinogenesis is associated with the long term persistence of its surface (HBsAg) and X (HBx) proteins. This observation is supported by the fact that in a large number of HCC patients, HBV DNA sequences encoding these proteins are found to be integrated into the host genome (19). The oncogenic potential of HBsAg is due to mutation of the large surface protein which increases hepatocyte proliferation by enhancing the expression of cyclin A. Similarly, mutation at the M surface protein can specifically activate c-Raf-1/Erk2 signaling and increase the excessive growth of hepatocytes (44). Furthermore HBV surface proteins accumulate in the endoplasmic reticulum (ER) and induce ER stress thereby contributing to the the development of HCC. ER stress is caused by the imbalance in the number of unfolded or misfolded proteins that

enter into it as well as the capacity of its machineries to arrange the folding of the proteins. Such imbalance activates intracellular signaling pathways that are sometimes referred to as unfolded protein response (UPR) (45) that can lead to various biological outcomes depending on the intensity and duration of ER stress. When the ER stress is mild or temporary, the ER burden is lowered by UPR by weakening the process of protein synthesis, increasing protein degradation, and increasing the expression of genes that refold misfolded proteins (46; 45). In the case of severe or prolonged ER stress, UPR could cause cell death through a mechanism that is not yet fully understood (45).

A very significant side effect of UPR as it concerns carcinogenesis is the induction of oxidative stress since it has been observed to increase the production of intracellular ROS by enhancing the oxidative folding machinery (47). ROS is responsible for oxidizing thiol groups to form disulfide bonds which are important sources of intracellular ROS (48). UPR-mediated enhancement of ROS has also been reported to contribute to ER stress induced cell death (47) which can lead to various oncogenic consequences, including DNA damage, activation of mitogenic response and deregulation of signaling pathways that control proliferation, cell survival and cell death (21).

The ER is an essential organelle for viral replication and maturation during the infectious cycle. Studies have shown that infection by many types of viruses, including HBV and HCV, can induce ER stress due to the large amount of unfolded or misfolded viral proteins synthesized in cells infected by such viruses (46). Chronic HBV infection in human liver tissues leads to cytoplasmic accumulation of HBsAg which, for a long time, is known to cause “ground-glass” hepatocytes (49) which often demonstrate ER proliferation and distortion (50) and are responsible for mutations in the pre-S region of the HBV genome which may lead to excessive production of large surface protein that suppresses the secretion of all forms of surface proteins from the ER (40). ER stress is also caused by accumulation of viral proteins in the organelle which lead to oxidative stress and DNA damage, and consequently predisposes the hepatocytes to oncogenic transformation (51).

Mice also demonstrate ground-glass alteration in their hepatocytes similar to human chronic HBV infection, due to the ER accumulation of the large surface protein resulting in a series of oncogenic events such as liver dysplasia. In addition, increased levels of oxidative DNA damage (53), severe prolonged liver damage, regenerative hyperplasia, nodule formation leading to HCC,

was also observed in mice to be directly related to the amount of HBsAg accumulated in its hepatocytes (52). These observations, in combination with data obtained using human HCC cells, strongly support the claim that HBV surface proteins harbor oncogenic potentials that are mainly mediated by ER stress consequently leading to elevation of intracellular ROS levels (50).

Another oncogenic property of HBV is seen in the possession of HBx protein which has been shown to interfere with numerous cellular activities especially proliferation, apoptosis, senescence and DNA repair (54). HBx is a small protein of 154 amino acids encoded by the X ORF which is highly conserved among Hepadnaviruses infecting only humans and is absolutely necessary for infectivity in vivo in woodchuck viruses as well as enhancing HBV DNA replication in tissue culture. Tumorigenic properties of HBx in vivo have been reported, with HCC developing in some, but not all of transgenic mice carrying the X gene especially in the presence of c-Myc expression or upon exposure to low levels of carcinogens. They could also moderately activate in trans, a variety of cellular gene expression, suggesting that the viral protein may assist hepatocarcinogenesis in an environment considered conducive for cancer development (54). Furthermore, HBx interacts with DNA repair proteins or UVDDDB1, inhibit apoptosis induced by p53, TNF, Fas, or TGF-beta, promote cell death by inducing mitochondrial aggregation and cytochrome C release, or by sensitizing cells to TNF-mediated killing (Fig 2).

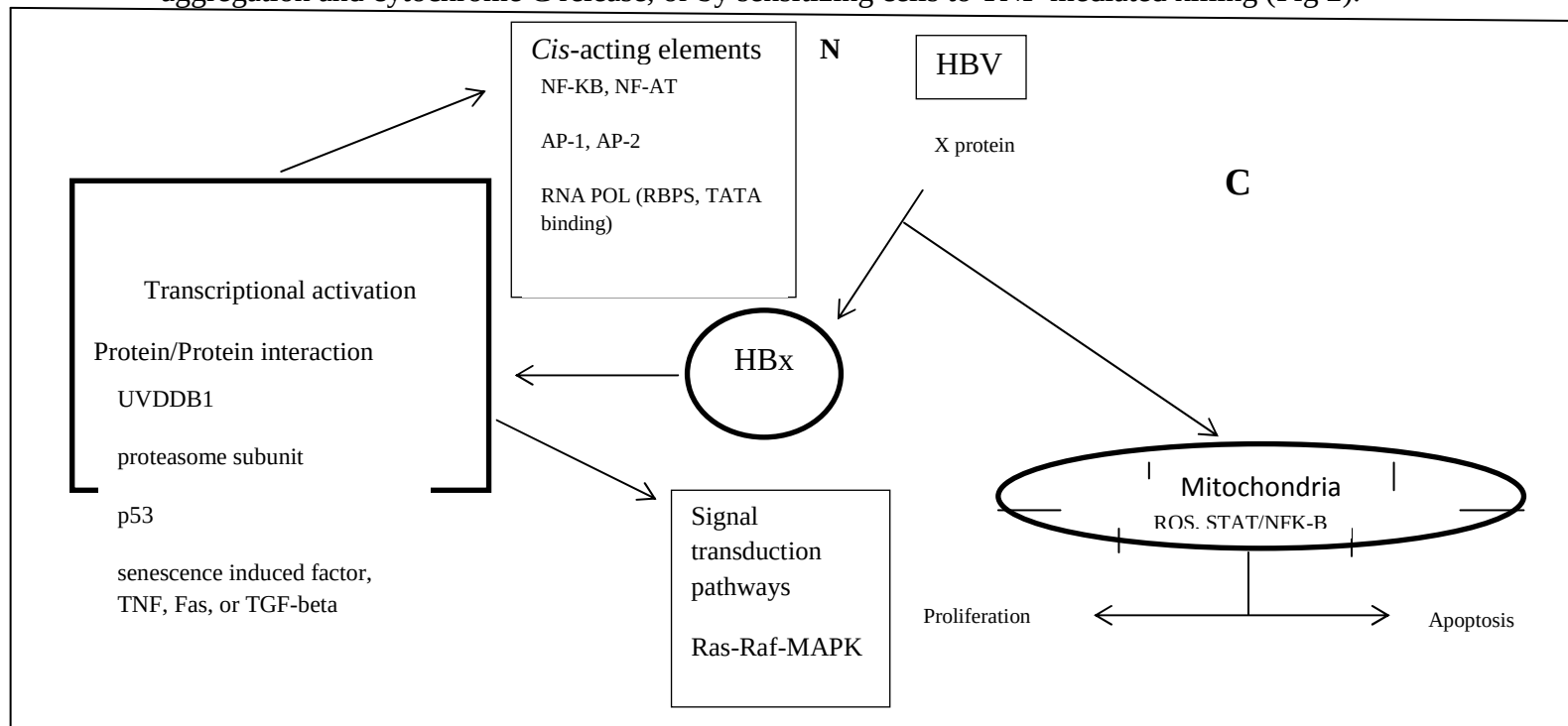


Fig 2. Schematic representation of the different targets of HBx. The figure shows the cytoplasmic, mitochondrial and nuclear transduction cascades activated by HBx.

The activities of HBx is also important in the epigenetic control of viral and host gene expression, increase in cell proliferation by causing transition from G₀ to G₁, and stimulating cell cycle progression in differentiated hepatocytes as well as causing arrest of S phase in dedifferentiated cells (55). The G₀ phase or resting phase is a period in the cell cycle in which cells exist in an inactive undividing state, while the G₁ phase, or Gap 1 phase, is the first of four phases of the cell cycle that takes place in eukaryotic cell division, while the S-phase or synthesis phase is the period in the cell cycle in which DNA is replicated and it occurs between the G phase and G₂ (56) (Fig 3).

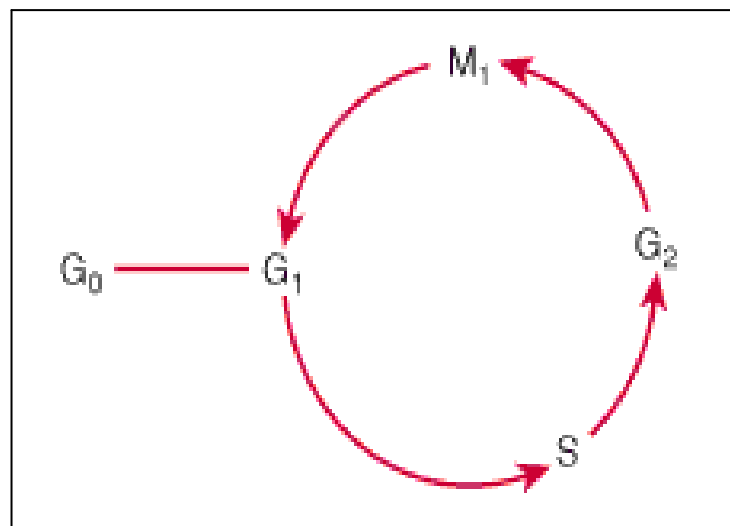


Fig 3. HBV DNA cycle in host cell

G₀ = resting phase (nonproliferation of cells); G₁ = variable pre-DNA synthetic phase; S = DNA synthesis; G₂ = post-DNA synthesis

Studies have shown that coinfection of HBV and HCV increases the risk of developing HCC than with HCV or HBV alone (57; 58), with the chances of coming down with full blown cancer increasing with each passing year (58). In a study to investigate HCC risk in subjects coinfecting with HBV and HCV (59), the OR for developing HCC in HBsAg positive and anti-HCV/HCV RNA negative subjects was 20.4, while it is 23.6 in HBsAg negative, anti-HCV/HCV RNA positive subjects, and 135 in subjects positive for both markers. These observations suggest a combined effect of HBV and HCV coinfection in increased chances of developing HCC. It is possible that the two viruses act through a common or different pathways to achieve their common carcinogenic objective (60).

Similarly, HDV coinfection with HBV increases the risk of liver damage as revealed by Kao *et al.*, (61) who showed that HBsAg positive patients with HDV superinfection develop cirrhosis and HCC at an earlier stage (mean age 48 years) compared to HBsAg carriers without HDV infection (mean age 62 years).

RISK FACTORS IN HBV-INDUCED HCC

Chronic hepatitis B is the major etiology of HCC in developing countries (62), accounting for majority of all global cases (10). This observation was confirmed when, in a population-based cohort study involving 22,708 Taiwanese men who were followed up for over eight years, the incidence of HCC was 98.4 times higher in HBV carriers than in non-carriers (63). In a follow up study to establish the involvement of chronic HBV in HCC development, also in Taiwan, it was observed that in inactive HBV carriers with normal liver tests, slow HBV replication and undetectable viremia, there is also an increased risk of HCC compared to healthy controls (64).

Similarly in a 5-year retrospective study in Ado-Ekiti, Nigeria, it was discovered that in addition to positive HBsAg and HCV-Ab positive, other risk factors for HCC in the country included heavy alcohol ingestion, multiple scarification marks, multiple sexual partners, history of blood transfusion, and a possible aflatoxin exposure through consumption of foods stored under humid conditions (65) (Table 1).

Table 1: Independent risk factors for the development of HCC in HBV infection

Types	Risk factors
Host	Male Older age (>45 years old) First degree relatives with HCC
Clinical	HBeAg positive Detectable HBV DNA Cirrhosis Persistent HBV infection (HBsAg positive)
Viral and environmental	Coinfection with HCV or HDV Alcohol intake Aflatoxin in diet

Ethnicity is another risk factor that predisposes to HCC in chronic HBV. For instance HBV carriers in western countries develop HCC at old age after a period of progressive liver cirrhosis, whereas in Asians and Africans, which may show fewer signs of cirrhotic liver disease compared to western HBV carriers, the cancer develops in adolescence and middle age. The differences observed in these different ethnic groups may be as a result of genetic variation, the age of acquisition of infection, and mode of transmission of the virus. While vertical transmission is the major route of HBV acquisition in Asia, horizontal transmission in early life is the predominant route of transmission in Africa, and in western countries HBV is mostly transmitted in adolescence and adulthood through high-risk behaviors, such as intravenous drug use, sexual intercourse, blood transfusion, hemodialysis and organ transplantation (66).

Similarly the class II cytokine receptor gene, IFNAR2 and IL10RB, located on chromosome 21 that encodes the type I interferon receptor and the receptor for Il-10-related cytokines respectively, including the interferon λ family (67) has been strongly implicated in hepatitis B persistence. Chromosomes that protect this locus are more frequently found in Europeans than in

West Africans suggesting further why HCC is more prevalent in this sub region in the African continent. In addition variation in the HBV genotype may also partly explain the differences in the risk and presentation of HCC between different populations. For instance, HBV genotype C is most commonly found in Asians and is associated with the persistent presence in serum of HBeAg, a decreased rate of spontaneous HBeAg clearance, increased rates of reversion to HBeAg seropositivity after HBeAg clearance, and a raised risk of HCC, irrespective of HBV DNA viral load levels (68).

Finally infection with specific HBV genotypes or HBV strains that carry common mutations, including the basal core promoter mutation and pre-s deletion mutation may also increase the risk of HCC, as do a high HBV load, old age, male sex, persistent HBV replication and chronicity of infection (67; 68; 69) (Table 1).

CONCLUSION

HBV is the major etiology of HCC worldwide especially in developing countries such as Eastern Asia and sub-Saharan Africa. Although the pathway towards HBV-related HCC is not well understood, studies have shown that HBV is directly involved in the induction of HCC. Prevention of HBV through adequate vaccination programs in adults and infants will indeed reduce the incidence of HCC in future. Though the impact of antiviral therapy in controlling HBV is unclear it is believed that sustained and prolonged suppression of HBV replication with nucleoside or nucleotide analogues may reduce the risk of HCC in patients with chronic hepatitis B.

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