

Hepatocarsinogenesis in Viral Hepatitis B Infection: The Role of HBx and p53

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ABSTRACT

Infection of Hepatitis B Virus (HBV) is a risk factor of chronic active hepatitis (CAH), hepatic cirrhosis and hepatocellular carcinoma (HCC). Infection of HBV may develop to HCC without antecedent hepatic cirrhosis. Pathogenesis of HBV causing malignant changes has not been fully understood. HBx, a protein of HBV, is an activator of transcription process involved in hepatocarcinogenesis. Most of human cancer associated with mutation of p53, a Tumor Suppressor Genes, a protein serves as cellular protection for growth and cell division, which is one of predisposition factor of hepatocarcinoma. Some studies indicate the correlation between mutation / inactivation of p53 and HBV protein x (HBx) in hepatocarcinogenesis. In that process, HBx will suppress p53 function, which will lead to ineffective liver cell division and resulting in HCC.

Key words: Hepatitis B virus (HBV), hepatocellular carcinoma (HCC), tumor suppressor genes, HBxAg, p53.

INTRODUCTION

More than 2 billion people have been infected by Hepatitis B Virus (HBV) and more than 350 million of them have developed asymptomatic carrier and are at risk to have chronic active hepatitis (CAH), cirrhosis, and hepatocellular carcinoma. The prevalence of HBV infection in the world has been decreased. It is associated with better vaccination and hygiene and incessant anti-AIDS campaign which emphasize the danger of free and unfaithful sex, concomitant usage of syringe needle. WHO estimates that 80% patients will have carrier state in 2001. However, patients with active infection should receive treatment.^{1,2,3,4}

There is a correlation between increased world's prevalence of hepatoma and the prevalence of HBV and HCV. The increased prevalence of hepatoma is obvious in the United States in the last two decades, which is also obvious in France and England. Hepatoma is placed on 5th rank of malignancy in the world and there is an estimation of 437,000 new cases in 1990. The prevalence is estimated approximately 7.4% of all male malignancy and 3.2% in the female. East Asia has the highest prevalence including Japan, China and sub Sahara, Africa. In America, the highest incidence is found in immigrants come from high risk countries.^{4,5}

The HBsAg prevalence in Indonesia is very various because Indonesia has a very vast archipelago area, a lot of tribes and various behaviors and cultures. The current study found the mean prevalence value of HBsAg infection in Indonesia is about 9.38%; the highest is found in Kupang (25.61%) and the lowest in Banjarmasin (2.50%).⁶

The risk factor of HCC has been identified based on epidemiologic study i.e. chronic HBV and HCV infection and prolonged exposure to aflatoxin. In HCC, aflatoxin is assumed as etiologic factor of gene P53 mutation on codon 249 (arginine into serine). Chronic hepatitis B and liver cirrhosis have been known as risk factor of hepatocellular carcinoma (HCC) development. In endemic area, the incidence rate of liver tumor in 100 people per year is 0.1 of asymptomatic carrier HBsAg patient, 1.0 of untreated chronic hepatitis B patient and has no cirrhosis before, as well as 3.0-8.0 of untreated Asian patient with compensated HBV-cirrhosis. Other literatures show that actually there is only 61.3% among more than 1073 HCC patients who also have cirrhosis, while among African HBV-HCC patients, 30% of them has no liver cirrhosis, as well as in England there is 30% HCC patient without preceding liver cirrhosis. These data indicate that tumor may develop without any preceding liver cirrhosis.^{4,5,7}

Pathogenesis of HBV causing changes regarding malignancy has not been fully understood. HBx, a HBV

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protein, is an activator of transcription process in hepatocarcinogenesis. Most of human malignancy demonstrates mutation of p53; a protein serves as cellular protection for growth and cell division, which is one of predisposition factor of hepatocarcinoma.^{8,9,10}

Current studies indicate a strong correlation between mutation/inactivation of p53 and HBV protein x (HBx) in hepatocarcinogenesis. In that process, there is a binding of both proteins, which will suppress p53 function and will lead to ineffective cell division.^{11,12}

HEPATITIS B VIRUS

Genomic Structure of HBV and Its Function

Hepatitis B Virus is also known as Hepadnavirus and it is also included in a group of virus replicating through RNA. Apart from human HBV, there are also woodchucks Hepatitis Virus (WHV), ground-squirrel Hepatitis Virus (GSHV), and Duck Hepatitis B Virus (DHBV), etc.^{3,5,13}

Hepatitis B Virus is the etiology polymorphism in human liver disease, from asymptomatic to acute fulminant hepatitis, chronic hepatitis and cirrhosis. Moreover, to date, the correlation between chronic hepatitis and hepatoma has been clearly explained. Hepatitis B Virus may be detected in liver cells in the form of free DNA molecule or in the integrated form. The plasma of infected patient contains virus particles with different size and form. A 22nm particle contains viral envelopes, which is called as Hepatitis B Surface Antigen (HBsAg). While a 42 nm particle (Dane Particle) consists of: 3 different surfaces, known as major protein, middle protein and large protein. Inside the viral envelope, there is a 27 nm core particle formed by core protein sub-units and known as HBcAg consisting viral polymerase virus and DNA molecule, where proteins adhere by covalent binding.^{3,4,13,14,15}

HBV genome is a circular double stranded molecule consists of L (-) strand with the length of 3200 nucleotides and S (+) strand with varied length between 50-100% L strands. L(-) strand consists of 4 genes or 4 Open Reading Frames (ORFs), which is encoded by S,C,P, and X. regions.^{1,4,14,15}

The S regions encode polypeptides which form HBV envelope and divided into S, Pre S1, and pre S2. The C regions encode protein of Hepatitis Core Antigen (HBcAg). HBcAg is an important target of recognition process in immune system and HBV elimination from the hepatocytes. Moreover, every mutation occurs in the core may cause infected cell avoiding the cellular immune response. The P regions encode 65 to 75 KD polypeptides responsible for DNA polymerase activity

in virus particle. This peptide also has important role in reverse transcriptase activity in the synthesis of L (-) strand. The X regions encode 145-156 amino acids, which are known as HBxAg. HBxAg has an important role in the development of hepatocellular carcinoma because of a high HBxAg concentration is found in transgenic rat correlates to liver tumor and there is a complex structure of HBxAg and tumor suppressor genes p53.^{12,13,14,15}

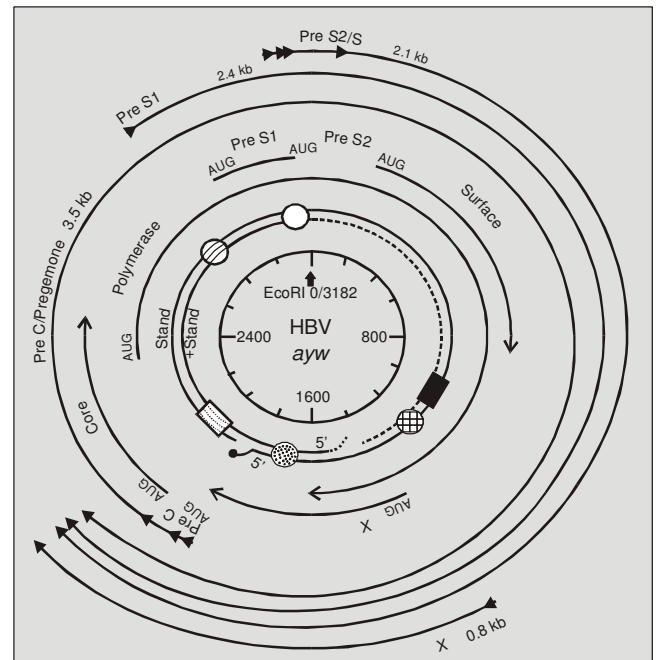


Figure 1. The Structure of Hepatitis B Virus (J Gastroenterol. Hepatol 2000; 15: 357-6)

The S Region (HBsAg/Envelope Structure)

The envelope of Hepatitis B Virus consists of 3 protein i.e. major protein, middle protein and large protein. HBsAg has several determinant group of an a code, along with sub-type specific determinant antigens d, y, w, and r. Three major sub-types, namely, adw, adr, and ayw is the most common but they are not well distributed around the world, ayr is rarely found.

Small (Major) HBs = (SHBs). Small/major protein consists of 226 amino acids and is encoded as S gene. This gene contains a lot of hydrophobic amino acids, which consists of 3 hydrophobic regions in amino acid sequence of 1-23, 80-98, and 169-226, which respectively separated by 2 hydrophilic regions. Protein between 122 and 155 residues in the second hydrophilic region is an exposed region and contains HBsAg group and sub-type determinant. A specific determinant of a code is placed between 124 and 147 residues, and it has a double loop structure. Cysteine 124-137 and 139-147 creates both loops. Cysteine is bound and creates

disulfide bridges. It has been proven to have an antigenic characteristic. Sub-type d or y determinant is placed on 122 position (lysine on d, or arginine on y), while w or r on position 160 (lysine on w, or arginine on r).

Middle HBs (MHBs). Middle Protein consists of 281 amino acids and is encoded as pre-S2 gene and S gene with pre S2 region containing 55 hydrophilic amino acids and epitope domain in the surface of envelope. This epitope has a characteristic of disulphide bond-independent without any cysteine. This region is more immunogenic than the major protein region.

Pre-S2 is very sensitive against protease and it may be selectively removed from HBs particle without damaging the S gene region. The pre-S2 region also consists of receptor for Polymerase Human Serum Albumin (pHSA). Hepatocytes also have a similar receptor, therefore it is postulated that pHSA receptor is the attachment/insertion site of HBV into hepatocytes.

Middle HBs is believed to be more immunogenic compared to SHBs in B cell and PreS2 level containing HBs particle has been exploited as prophylactic vaccine.

Large Protein (LHBs). Large HBs consists of preS1 gene, pre-S2 gene, and S gene. Pre-S1 region is located on surface and it is the most variable region in HBV. Large HBs has an important immunogenic part either for B cell or T cell, which both of cells have important role in healing process from viral infection or in protecting body against infection. The main immunogenic epitope on pre-S1 region is encoded by amino acids in sequence 27-35, 72-78, and 95-107. Large HBs is also very immunogenic in human T cell i.e. on 21-48 and 81-108 amino acids.^{13,14,15}

The C Region (Core/HBcAg)

The C region encodes core protein, known as Hepatitis Core Antigen (HBcAg). HBcAg is HBcAg is an important target of recognition process in immune system and HBV elimination from the hepatocytes. Moreover, every mutation occurring in the core may cause infected cell avoiding the cellular immune response

The HBc protein has two domains, of which the domain of 1-144 amino acids has a role in the formation of 32 nm nucleocapsid while domain loaded by arginine of 150 amino acids has a role in binding amino acid, viral encapsidation and DNA replication.^{13,14,15}

The P Region (Precore)

The P region encodes 65 to 75 KD polypeptides responsible for DNA polymerase activity in virus particle. This peptide also has important role in reverse transcriptase activity in synthesis of L (-) strand.^{13,14,15}

The X Region (HBx)

The X region encodes polypeptides of 154 amino acids with 17kDa molecular weight. HBx is an important virus component in infection process, and also has a role as a potent HBV cofactor in the development of hepatoma. The data supported by study findings showed that HBx attached and caused inactive transcription factor and tumor suppressor p53.

Antigen x of Hepatitis B Virus (HBxAg) induces viral gene expression and replication, which are important for survival and chronic state of this carrier. Integrated virus DNA, namely HBxAg in chronic infection, causes increased expression of this antigen x. HBxAg over-expression may cause alteration of signal transduction pathways, which is very important to regulate cell growth for hepatocytes regeneration. HBx was found to be attached and it may inactivate inhibitor molecules of these cell regulation such as tumor suppressor p53. This data improve our estimation that HBx has an important role in the development of hepatoma. HBxAg might also induce gene expression of addition growth regulating molecule such as insulin-like growth factor II and insulin-like growth factor I receptor. It is found that HBx may disturb DNA repairing process and this will affect the regulation process of normal growth molecule in the proteasome and it may contribute in hepatocarcinogenesis process.^{13,14,15}

TUMOR SUPPRESSOR GENES

Mutation causing malignancy is a result of an altered protein, which is a product of gene controlling cell beginning in cell division cycle. The genes involved are genes working on opposite regulatory control, which is called proto-oncogenes if it is normal and oncogenes if it undergoes mutation, or genes that negatively regulate cell cycle known as **tumor suppressor genes**. Because there is a multiple security system in cell, it needs more than one mutation before leading a malignancy. The encoded protein originated from tumor virus may inactivate the tumor suppressor gene products. Protein p53 is one part of the tumor suppressor genes.^{16,17}

Gene encoding tumor suppressor protein p53 is the most common mutated genes of human malignancy. Mutation of p53 is found in half of all human malignancy cases and mutation p53 is found in approximately 75% human hepatocarcinoma cases. Gene p53 is a single-copy gene and it is located in short division of chromosome 17. Gene p53 encodes certain protein phosphor produced in a very low concentration in the normal nucleus cell.^{8,16,17}

Cell division is divided into four time phases, namely, G1, S, G2, and M. Phase S is a synthesis, when DNA duplication occurs and phase M is when cell mitosis occurs. Phase G1 is a phase between M and S, while G2 is a phase between S and M. Before a cell goes through the phase S, the cell DNA should be normal and has no damage either by carcinogenic component, ultra violet or gamma radiation. Duplication of damaged DNA will increase the mutation frequency and mostly will be destroyed. Destruction of the damaged DNA is completed by p53. As a response of DNA damage, the p53 concentration in cell will be increased. Protein p53 is a protein functioning as a transcription factor and it will recognize adjacent DNA sequence in various different genes. By attaching those DNA sequence, p53 will regulate genes through activating the product of messenger RNA. One of the genes working on this regulation system is coded by a protein, known as p21. Protein p21 will attach on cdk2 and cdk 4 and it will inhibit cyclin-dependent phosphorylation, therefore the cell is unable to leave the G1 phase and it is also unable to go through the S phase. The p21 expression provides opportunity for the cell to repair the DNA damage and it reduces mutation possibility in DNA replication. If the damaged has been repaired, the p53 level will decrease, p21 is not produced anymore, and cyclin-cdk complexes may phosphorylate a signal to go through the S phase. When there is DNA damage, p53 will have a role to inhibit the progressiveness of cell division at the end of G phase, and it will also cause apoptosis that will bring cell death of the damaged cell. Inhibition of cell division may occur because of trans-activational properties in p53, which will activate several genes in cell cycle. Protein p53 is assumed to have a direct role in recognizing the damaged DNA and in its repairing process. In the tumor cell, the process mentioned above does not occur because there is disturbance of p53 function, therefore a terrible DNA repair occurs and the cell is genetically unstable. ^{8,10,11,12,16,17,18}

PATHOGENESIS OF HEPATOCELLULAR CARCINOMA

Research showed evidence that several factors indicating HBV as the main that etiologic factor to develop chronic liver disease and hepatocellular carcinoma (HCC). In the chronic liver disease, HCC occurs only after HBV marker is found in the blood plasma. In carrier state, HCC often occurs after CAH episode and cirrhosis has been developed. Chronic infection in WHV may develop into CAH and HCC up to 100% after being infected for 2-3 years. This observation assumes that infection of hepadna chronic

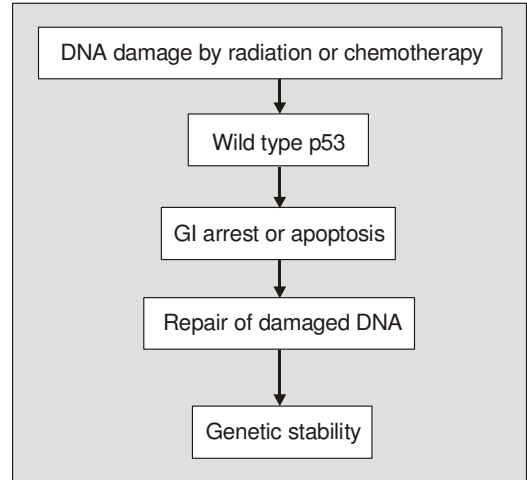


Figure 2. Normal Cell (Levine JA. Tumor suppressor genes. Science Am. January 1995; 28-37)

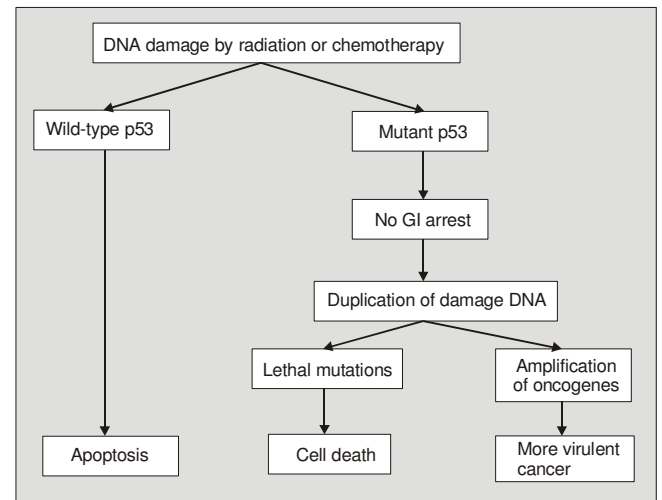


Figure 3. Cancer Cell (Levine JA. Tumor suppressor genes. Science Am. January 1995; 28-37)

virus is extremely correlated to genetic instability. The present evidences indicate a strong correlation between infection hepadna chronic virus and the HCC development.^{6,7,12}

There are a lot of evidences indicating that tumor suppressor protein such as p53, a negatively regulate cell growth protein, prevents uncontrolled cell proliferation, which is a characteristic of tumor cell. The main role of p53 maintaining genome integrity at cellular lever emerges because from observation, there is increased p53 expression in cell that undergoes radiation (DNA damage occurs) and p53 prevents cell division until the DNA repairing is completed. When the function of p53 is inhibited, the cell with damaged DNA carries on replication and usually it will die or change. The important role of p53 is highlighted by a mutation which inactivate this protein has been found in various kinds of tumors. Moreover, p53 inactivation is the most common

condition in carcinogenesis. The p53 integrity is very important in HBV and HCC development. Integration of HBV DNA in human genome is common and the integration is correlated to chromosome instability. Therefore, it is assumed that chronic HBV infection is extremely correlated to prolongation period of DNA damage, which requires the p53 function for DNA repairing so that mitosis may occur. The p53 integrity is likely an important factor reducing DNA damage caused by HBV integration and HCC development.

It is assumed that HBxAg is a protein kinase with autophosphorylation activity and the phosphorylated HBxAg in human hepatoma cell indicates that phosphorylation has a contribution in carcinogenesis. Although the target of HBxAg phosphorylation has not yet been known, but phosphorylation of tumor suppressor gene product and protein cell cycle have been recognized to cause activity changes in controlling cell growth. There are evidences indicating that HBxAg has ATPase and deoxy-ATPase activity *in vitro*.^{12,19}

Correlation between HBxAg and p53 was studied in human with HCC carrier state by immunoprecipitation method for peptides of both proteins by using anti-HBx or anti-p53 and it has been confirmed by a similar study method *in vitro*. The study indicates that HBxAg and p53 may work in synergy to stimulate HBV replication, and this depends on p53 binding site in HBV DNA. Evidence indicates HBxAg inhibiting p53 attachment in responsive element *in vitro* seems as a contradiction with previous statement, but the disparity depends on HBxAg: p53 ratio. A low HBxAg: p53 ratio will cause HBxAg to attach to its responsive element and subsequently it will stimulate transcription.

High ratio causes HBxAg to eradicate p53 from the responsive element. Low HBxAg: p53 ratio dominates early infection; a small amount of free HBxAg in the cell will stimulate HBV gene expression and replication. High HBxAg: p53 ratio is dominant at the end of infection when HBxAg is produced in large amount from integrated and accumulated template in the infected cell.

Further studies indicate that HBxAg does not cause p53 degradation which is similar to HPV 16 E6. Therefore, it is assumed that inactivation of p53 function causes p53 adjustment from its complex formation or HBxAg causes p53 substrate substitution. P53 substrate may be in the form of deregulating expression correlated to several tumors, complex promoter growth inhibitor in DNA damage, TATA-binding protein, HSP 70 and correlated promoter, as well as other important genes in regulating cell growth and differentiation. Furthermore, the HBxAg-p53 complex may alter genetic stability and cell cycle

control, which is the basic carcinoma development and growth.^{11,12,18,19,20}

HBxAg-p53 complex has been found in HCC developed from transgenic x-rats. HBxAg-p53 coloration from the same cell of adenomas and HCC nodule indicates a strong correlation. In the experimental rats, it is found that HBxAg inactivates p53 in cytoplasm. In human HCC, increased p53 level is seen in the nucleus of infected cell and in several cases, HBxAg is also found in similar cells. The sequence of exon indicates that p53 coloration in most cases is a wild type of p53. These data are appropriate to our conclusion that HBxAg stabilizes p53 either the wild type p53 or mutant p53.

There is a correlation between p53 inactivation and disturbance of DNA repairing, which will cause genomic instability. Normal p53 attachment on ERCC3 (a transcription factor which has a role in DNA repairing transcription) appears to be disturbed by HBxAg. It is possible that p53 suppresses the transcription process and inhibits the activity of DNA helicase and ERCC3 has an intrinsic helicase activity. As a result, p53 inactivation by HBxAg will cause irregular transcription and it is not well-regulated. Replication and transcription of damaged DNA will support the mutation which has contribution in carcinoma development. At molecular level, p53 inactivation by HBxAg through complex formation and ERCC3 disturbance may have a contribution for chromosome changes, and it is reported that it correlates to the HCC.^{11,12,18,19,20}

CONCLUSION

Hepatoma development in HBV infection may occur without any preceding liver cirrhosis. In HBV infection, hepatoma may occur because of mutation on tumor suppressor genes either through radiation, or through chemotherapy of prolonged aflatoxin diet.

p53 is a protein which has a function in protecting, maintaining and repairing cells. HBx will cause inactivation of p53 function, therefore if the HBxAg-p53 complex is formed, p53 will lose its function, and it is the beginning of cancer development.

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