Hepatocellular carcinoma and the underlying mechanisms

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Abstract

The incidence of hepatocellular carcinoma is increasing worldwide as well as the associated risk factors, some of which include exposure to aflatoxin B1, Hepatitis B (HBV) virus and hepatitis C (HCV) virus. Mutation of tumour suppressor gene p53 at codon 249^{ser} at exon 7 has been found to contribute significantly to replication of damaged DNA and subsequent tumour progression. The x gene of HBV (HBx) is the most common open reading frame integrated into the host genome in hepatocellular carcinoma and the integrated HBx is frequently mutated in hepatocellular carcinoma. Mutant HBx proteins still retain their ability to bind to p53 thereby attenuating DNA repair and p53-mediated apoptosis.

Keywords: hepatocellular carcinoma, aflatoxin B1, HBV, HCV, p53

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. It is the fourth leading cause of cancer-related death in the world.[1] The major risk factors include chronic infections with the hepatitis B (HBV) or C (HCV) virus and exposure to dietary AFB1 or alcohol consumption. A link based on circumstantial evidence has been divulged between high exposure to AFBI and mutation at the 3rd nucleotide base of codon 249, which is located on the 7th exon of p53 gene of cells of primary liver cancer from patients in tropical countries of the world and activation of the WNT signal transduction pathway.[2-6] AFB1 frequently induces G: C to T: A transversions at the third base in codon 249. Interestingly, mutant DNA in plasma is a biomarker of both AFBI exposure and potential risk factor for HCC with subsequent p53 mutation.[7] The tumour suppressor gene *p53* is the most commonly mutated gene in human cancers. [8]

Chronic infections with HBV and HCV viruses and oxyradical disorders including hemochromatosis also generate reactive oxygen/nitrogen species that both damage DNA and mutate cancer - related genes such as tumour suppressor gene p53.[9] The p53 biological network is a key responder to this oxidative and nitrosative stress. Depending on the extent of the DNA damage, p53 regulate transcription of protective antioxidant genes and the extent of DNA damage that ultimately trans-activates pro-oxidant genes which eventually contribute to apoptosis. The x gene of HBV (HBx) is the most common open reading frame integrated into the host genome in HCC and the integrated HBx is frequently mutated. Mutant HBx proteins still retain their ability to bind to p53 and attenuate DNA repair and p53-mediated apoptosis. Hence, both viruses and chemicals (especially vinyl chloride) are implicated in the etiology of p53 mutation during the molecular pathogenesis of HCC.

HCC is a major cause of cancer morbidity and mortality in many parts of the world, including Asia and Sub Saharan Africa, where there are >500,000 new cases each year and >200,000 deaths annually in the People's Republic of China (P.R.C) alone.[10] The major etiological factors associated with development of HCC in these regions are infection with HBV and or HCV and long time exposure to high levels of AFBI in the diet.[11-12]

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Mechanisms underlying Hepatocarcinogenesis

The biology, mode of transmission, and epidemiology of HBV continue to be actively investigated and have been recently reviewed.[13] A mutation in the HBV genome can alter the expression of multiple proteins. In many cases of HCC in China and Africa, a double mutation in the HBV genome, an adenine-to-thymine transversion at nucleotide 1762 and a guanine-to-adenine transition at nucleotide 1764 ($1762^{T}/1764^{A}$) has been found in tumours.[14-15]

This segment of the HBV genome contains an overlapping sequence for the base core promoter region and the HBx gene; therefore, the double mutation in codon 130 and 131 of the HBx gene reported in human HCC is identical to the 1762 and 1764 nucleotide changes.[16] The onset of these mutations was shown to be associated with the increasing severity of the HBV infection and cirrhosis.[14-15] HBx in transformed hepatocyte has been demonstrated to inhibit the repair of damaged hepatocyte DNA. This effect may be mediated by interaction with p53 or through binding to the damaged DNA binding protein (DDB), which plays an accessory role in nucleotide excision repair.[13] In addition, HBx activates cell signalling cascades involving mitogen-activated protein kinase (MAPK) and Janus family tyrosine kinases (JAK)/signal transducer and activators of transcription (STAT) pathways.[13] The process by which tumour DNA is released into circulating blood is unclear but may result from accelerated necrosis,apoptosis, or other processes.[17] A specific codon 249 p53 mutation detectable in plasma samples at the time of HCC diagnosis, can be measured in some individual at least 5 years before diagnosis.[18]

Heterogeneity in etiological factors of HCC

The frequency of HCC is particularly high in Asia and Africa due to the high frequency of viral hepatitis infections and to Aflatoxin B1 exposure (AFB1). Over the last 10 years, the incidence of HCC has noticeably increased in United Kingdom, France and United States. This is probably linked to viral hepatitis C infections. Etiological factors that are associated with the development of hepatic tumours are well known in these regions. They include infection with the hepatitis B virus (HBV) or hepatitis C virus (HCV), heavy alcohol intake, prolonged dietary exposure to AFBl or vinyl chloride and primary hemochromatosis. In 90% of the HCC cases, at least one of these risk factors can be identified either alone or in combination with another factor. The presence of each risk factor among patients varies according to the geographical origin of the patients. Globally, exposure to HCV, HBV and AFBI are responsible for about 80% of all HCC in humans' worldwide but the principal risk factor varies between countries. In Japan almost all HCC are linked to HCV infection, whereas in Africa HBV infections are predominant.[19] In France, HBV and HCV infections and alcohol intake are identified with approximately equal frequency. Exposure to AFBI is commonly found in sub-tropical countries where humid heat can lead to the development" of *Aspergillus flavus* in improperly stored foods such as cereals and peanuts. This mycotoxin is strongly hepatocarcinogenic in experimental animal models and acts synergistically with HBV infection to increase the risk of HCC.[20] Tobacco exposure is the leading carcinogen associated with multiple solid tumours[21]. Several investigators have previously reported an association between tobacco and HCC with odds ratios ranging from 1.5 to 6.8.[22-23] However, other studies found no association between tobacco and HCC.[24]

Hepatocarcinogenesis

The different risk factors of HCC include chronic lesions in the liver with associated inflammation, necrosis of hepatocytes and fibrosis. Overall, HCC development is closely associated with cirrhosis and more than 80% of the tumours are found in a chronic hepatitis or a cirrhotic background.[25] Dysplastic nodules and macroregenerative nodules have long been considered to be the likely precursors of HCC because of their frequent association with the HCC occurrence.[26] Chromosome aberrations occur in HCC and these may already contain genetic aberrations. However, in rare cases (less than 10% of the cases), HCC are observed in non-cirrhotic liver and even without inflammatory lesions. The HCC which develop in an otherwise normal liver are usually found in patients without well-established risk factors. Some of these cases may correspond to the malignant transformation of liver adenoma that are rare benign hepatocellulartumours sometimes found in young women taking oral contraceptives.[27]

HBV infection

The incidence of HCC has been shown to vary widely worldwide. [28] Among males, the highest incidence rates are found in eastern Asia, particularly in China where HCC was reported to be the third most common cause of cancer death[29]. Chronic infection with the HBV has been reported by various authors as the strongest risk factor for HCC worldwide. [28, 30-32] However, populations with similar prevalence of HBV infection have different incidence of HCC, suggesting the presence of other important risk factors. Aflatoxins, a group of mycotoxins produced by the common fungi *Aspergillus flavus* and *Aspergillus parasiticus*, are established human hepatocarcinogens and are well-known HCC risk factors when present in foodstuffs.[32-35] Some epidemiological and animal

studies have found evidence for an HBV-aflatoxin interaction in hepatocarcinogenesis. [35-41]

Several mechanisms underlying this principle have been proposed to explain the interaction between HBV and aflatoxin. The increase in cellular proliferation induced by HBV could increase the probability for clonal expansion of an existing aflatoxin induced-*p53* 249^{ser} mutation[42]. An increase in levels of aflatoxin metabolism enzymes (*e.g.,* P450 enzymes in which its activity is associated with increased hepatotoxicity of aflatoxin) has been described for HBV transgenic mice and has been postulated as a mechanism for interaction.[43] The HBx protein, which is encoded by HBV interferes with the nucleotide excision repair pathway, a major repair pathway which cells use to repair damaged DNA.[44] However, the presence of mutant HBx protein could increase the frequency of aflatoxin-induced mutations.[44] Also, HBV infection was reported to increase oxidative stress, which could lead to an increase in *p53* mutations.[45]

Mechanisms of HBV-mediated hepatocarcinogenesis

HBV infection can promote carcinogenesis by at least 3 different mechanisms. First, integration of the viral DNA in the host genome can induce chromosome instability. Second, insertional mutations of HBV are known to activate endogenous genes of retinoic acid â-receptor, cyclin A and mevalonate kinase which are involved in cell cycle control, cellular proliferation and differentiation. The second mechanism is associated with specific intracellular receptors. Recently, 15 new genes were found to be altered by HBV integration in tumors suggesting that viral integration in the vicinity of genes controlling cell proliferation, viability and differentiation is a mechanism frequently involvedin HBV hepatocarcinogenesis. The third mechanism of carcinogenesis linked to HBV infection is based on the expression of viral protein, in particular HBx, to modulate cell proliferation and viability. Moreover, HBx binds to p53 and inactivates p53-dependent activities, including p53-mediated apoptosis. Recently, the association between hepatitis B virus and Hepatocellular carcinoma and the molecular mechanism of action that is involved in the hepatocarcinogenesis has been extensively described.[46,47]

Interaction of AFB1 with DNA and chromatin proteins (histone)

After an exposure to AFB1, accumulations of damaged DNA are found in the liver, as a result of conversion of the AFBI to its active metabolites. AFBI is a very potent mutagen and the AFBI epoxides (active metabolites of aflatoxin) react with guanine in DNA, leading to genetic changes. The most frequent mutation induced is the (guanine-cytosine to thymine-adenine) GC to TA transversion. However, quantitative determination of AFB1 in human aflatoxin albumin adducts has been ellucidated.[48] The mutational pattern of p53 gene in HCC from regions where AFBI exposure level is high, revealed (guanine to thymine) G to T transversion at codon 249 in more than 50% of the cases. A more detail study revealed that AFB1 binds preferentially to lysyl amino acid residues in histone proteins.[10] The binding of AFB1 to histone proteins has significant functional implications because histone has been reported to be the packaging material for DNA and histone H1 is the most external of the histone proteins wrapped around DNA.[10,49] Because of the high content of basic amino acids in histones, it is conjectured that there is a strong electrostatic interaction between them and DNA and that (addition of acetyl group) acetylation of the lysyl sites which is involved in this type of interaction, reduces the net positive charge of the histone and loosens the bonds between histone and DNA. Acetylation is reported to occur at the amino group of lysly amino acid residues which is the same binding site of AFBl.[50, 10]

The effect of AFBl binding to histone is therefore likely to be similar to reaction elicited by Acetylation (a post transcriptional modification), which is the partial loosening of the histone-DNA bond and the consequent degradation of the histone by specific proteases.[51]It is generally accepted that such a partial loosening of the histone DNA bonds always precedes gene expression. This means that it is most likely that it is the binding of AFBl to lysly amino acid residues in histone with the consequent loosening of the histone-DNA bond that makes p53 accessible for damage. It is also likely that the binding of AFB1 to histones with the consequent loosening of histone is primary to its binding to the DNA of p53 genes even though the binding to DNA subsequently exceeds its binding to histone. Taken together, the binding of AFBI to DNA is responsible for the inhibition of RNA Synthesis, which is involved in gene expression.

The implication of the above is that it is the binding to chromatin proteins (histone) may be involved in the expression of the mutated p53 gene resulting from the interaction of AFBI with DNA and chromatin proteins. The p53 gene is reported to be mutated in HCC after exposure to aflatoxin.[52] Recently, some authors have extensively discussed the association between AFB1 and the associated risk factors involved in HCC.[53-55]

Conclusion

The nexus between hepatocellular carcinoma and the associated risk factors cannot be overemphasized. The interaction between aflatoxin and HBV or HCV in hepatocarcinogenesis and multi-stage carcinogenesis is grossly elucidated. Characterization of the genetic alterations associated with HCC tumors is an essential step to increase our knowledge of hepatocarcinogenesis. Systematic search for these alterations in series of tumors including tumour grades, stages, etiologies and the associated pre-neoplastic lesions is therefore necessary to find and identify the pattern of accumulation of the genetic alterations during tumour progression. Microarray analysis and metagenomics may also contribute significantly to identifying new carcinogenetic pathways altered in these tumors. New insight should therefore be geared towards getting a better clinical application, to identity tumour markers that are useful for early detection of tumors, to predict prognosis, or to find new therapeutic targets with their underlying molecular mechanism of action.

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