BJMG 10/2 (2007) 15-22
10.2478/v10034-008-0002-4

REVIEW ARTICLE

MOLECULAR PATHOGENESIS OF HEPATOCELLULAR CARCINOMA

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ABSTRACT

The most important risk factors for the development of human hepatocellular carcinoma (HCC) are chronic infection with hepatitis B virus (HBV) and/or hepatitis C virus (HCV), high dietary exposure to hepatic carcinogen aflatoxin B1 and alcohol abuse. Hepatitis B virus exerts its effects through integration of the viral DNA into the hepatocyte genome, or through acting as transcriptional regulator for several cellular proto-oncogenes and tumor-suppressor genes. Hepatitis C virus may affect hepatocytes via the transcriptional regulation activity of the HCV core protein or via the HCV non structural proteins NS5A, NS5B and NS2, interfering with the regulation of cell cycle and apoptosis. Environmental exposure to aflatoxin B1 can cause a specific missense mutation in codon 249 of the p53 tumor-suppressor gene. Habitual alcohol consumption leads to production of reactive oxygen species and peroxidation damage to DNA. The objective of this review is to make you acquainted with the most common risk factors and the most frequent genetic aberrations associated with the development of HCC.

Key words: Aflatoxin; Alcohol; Hepatitis; Hepatocellular carcinoma (HCC)

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world. It ranks third in mortality rate and is responsible for approximately 600,000 to 1,000,000 deaths annually [1]. Hepatocellular carcinoma typically develops in close association with pre-existing cirrhosis. The liver with cirrhosis may contain pre-neoplastic lesions that are in an intermediate stage between non neoplastic regenerating nodules and malignant HCC. These nodular lesions are designated as dysplastic nodules and are further divided into low-grade dysplastic nodules (LGDNs) and high-grade dysplastic nodules (HGDNs), depending on their histological examination. Progression of HCC proceeds from LGDNs and HGDNs to advanced HCC [2].

Hepatocellular carcinomas show a wide variability in incidence and in principal risk factors in different regions. Globally, chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) and prolonged dietary exposure to aflatoxin are responsible for about 80% of all HCC in humans [3]. It is most frequent in Eastern and Southeastern Asia and sub-Saharan Africa, regions of high dietary aflatoxin exposure and endemic HBV infection. Recently, HCC has become more common in Japan and the United States in parallel with an increasing incidence of HCV and HBV infections leading to the development of chronic active hepatitis. In Northern Europe and the United States it is often related to alcoholic

liver disease [1]. In areas with an intermediate incidence such as Southern Europe, HCV infection is considered to be the predominant cause [3].

HEPATITIS B VIRUS-MEDIATED HEPATOCARCINOGENESIS

Hepatitis B virus infection is one of the most common diseases, with an estimated 350 million chronically infected carriers worldwide. Chronic HBV can lead to cirrhosis, which is considered to be a principal factor predisposing to the development of HCC [1].

In patients with chronic hepatitis some, but not all, of the infected cells are destroyed due to relatively inefficient T-cell response. This leads to a cycle of liver cell destruction and regeneration in the context of continuous intrahepatic inflammation that often terminates in HCC. A model of chronic immune-mediated liver disease using transgenic mice provided evidence that HBV-specific chronic immune-mediated liver cell injury is sufficient to initiate and sustain the process of hepatocarcinogenesis [4].

Hepatitis B virus is a single-stranded DNA virus that becomes integrated into the host genome [1]. The HBV genome consists of four overlapping open reading frames that encode DNA polymerase (P), HBV surface antigen (HBsAg), HBV core protein (HBcAg) and a regulatory X protein (HBx) [5]. The HBV contributes to hepatocarcinogenesis *via* three mechanisms. First, HBV viral DNA can integrate into the host genome and induce chromosomal instability. Integration of HBV DNA into the host genome can induce large inverted duplications, deletions, amplifications, or chromosomal translocations [6]. Second, it may also activate cellular proto-oncogenes or suppress growth-regulating genes *in cis* [1]. A recent study has shown that integration is located upstream to the promoter of human telomerase reverse transcriptase (hTERT) gene, which codes for the catalytic subunit of telomerase ribonucleoprotein, and that HBV enhancer can activate the transcription of the hTERT gene in hepatocarcinoma cell lines [7]. The third mechanism by which HBV contributes to carcinogenesis is through expression of viral proteins, in particular, HBx. Intracellular localization studies have demonstrated that the protein HBx functions as a transcriptional regulator in both cytoplasm and nucleus [7]. In the nucleus it regulates the promoters of different genes including proto-oncogenes such as v-myc and c-myc [myelocytomatosis viral oncogene homolog (avian)] and c-myb and v-myb [myeloblastosis viral oncogene homolog (avian)] [1], c-jun oncogene [3] and v-fos and c-fos (Finkel-Biskis-Jinkins murine osteogenic sarcoma viral oncogene homolog) [8], tumor suppressor genes including adenomatous polyposis coli (APC), p53, cyclin-dependent kinase inhibitor 1A ($p21^{waf1/cip1}$), Wilms tumor 1 (WT1) gene [1] and retinoblastoma (Rb) gene [9], and transcriptional factors such as nuclear factor-kappa B (NFκB), activating protein 2 (AP-2) [10] and cAMP response element-binding/activating transcription factor-1 proteins (ATF/CREB) [3]. It increases the expression of the epidermal growth factor receptor in HCC cell lines and potentiates transforming growth factor α (TGFα) [8]. On the other hand, HBx protein upregulates the expression of TGFα1 and promotes TGFα1 signaling [9]. Transfection of the human hepatocellular liver carcinoma cell line (HepG2) with an HBx expression vector increased insulin-like growth factor (IGF-II) gene expression which was mediated by protein kinase C (PKC) and p44/p42 mitogen-activated protein MAP kinases (p44/p42MAPK) [11]. The HBx protein exerts its effects on the cell cycle through upregulation of cyclin-dependent kinase 2 (CDK2) and cell division cycle 2 (CDC2), and enhances their active association with cyclin E/cyclin A and cyclin B. It also activates the cyclin A promoter and promotes cycling of growth-arrested cells into a G_1 phase of the cell cycle [7]. Transcription of $p21^{waf1/cip1}$, a key regulatory protein in cell cycle progression, is activated by HBx in the presence of functional p53 [7] and also in a p53-independent manner [3]. The HBx protein can inhibit apoptosis by binding to p53 which leads to inactivation of the sequence specific DNA binding and transcriptional activating properties of p53 [3]. The HBx protein also downregulates TGFβ-induced apoptosis in hepatocytes by stimulating phosphoinositide 3-kinase (PI3K) activity [7]. It has been demonstrated that HBV achieves protection from apoptotic death through activating the HBx-PI3K-Akt-Bad pathway [Akt is also known as protein kinases B (PKB), Bad: B-cell CLL/lymphoma 2 (BCL-2)-antagonist of cell death] [12].

Cytoplasmic HBx was detected either as punctate granular staining or in dispersed, finely granular patterns. Detailed analysis of cytoplasmic compartmentalization of HBx showed no association

with the endoplasmic reticulum, plasma membrane or lysosomes but a substantial association of HBx with mitochondria [13]. In mitochondria, HBx may induce mitochondria-dependent cell death via indirect interaction with pro-caspase-9 but it has also been reported to efficiently block caspase 3 (CPP32) activity and apoptosis in hepatoma cells [7]. The HBx protein has been demonstrated to activate many signal transduction pathways in the cytoplasm such as the Janus kinases (JAKs) and signal transducers and activators of transcription (STATs) pathway, which causes activation of STAT-regulated genes [3]. In HBx stable expressing cells, the tyrosine phosphorylation of STAT3 and STAT5 and *in vitro* kinase activity of JAK1, are upregulated [7]. Constitutive activation of JAK/STAT by Hbx protein may contribute to the development of HCC by stimulating cell growth by cross-talk through v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homogog (avian) src- and/or the v-ras oncogene homolog ras-associated signal transduction. HBxAg constitutively activates ras and src kinase. Activated ras leads to activation of MAPK and PI3K signaling pathways which cooperate with other oncogenes, such as c-myc, in tumorigenesis [9]. Activation of signaling cascades involving Ras small GTPase (encoded by ras oncogene) raf protooncogene serine-threonine protein kinase (Raf) and MAP kinases (Ras/Raf/MAPK) can activate the transcription factors AP-1 and NFκB which contribute to the deregulation of cell cycle checkpoint controls [3]. The HBx protein interacts with pathway involving wnt and catenin (cadherin-associated protein) β1 oncogenes (Wnt/β-catenin) through association with Wnt protein, through activation of elk-related tyrosine kinase (ERK) or *via* hypermethylation of E-cadherin promoter, all of which lead to elevated levels of activated β-catenin [9].

HEPATITIS C VIRUS-MEDIATED HEPATOCARCINOGENESIS

It is estimated that 170 million people are seropositive for anti-HCV, of which 127 million are chronically infected [1]. After acute viral infection, over half of the patients develop chronic HCV, which causes more frequent cirrhosis than chronic HBV (70 *versus* 50%) [8].

Hepatitis C virus is a single-stranded RNA virus that does not integrate into the host genome [1]. Host-viral protein interactions are the major pathways of hepatocarcinogenesis. The HCV genome is translated into a polyprotein which is processed into at least 10 polypeptides, including structural (Core, E1, E2, and p7) and non structural (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) proteins [14].

Analysis of HCV-associated HCC tumor tissues revealed a high incidence of chromosome instability. The overexpression of the NS5A protein leads to an unscheduled delay in mitotic exit and to multi-polar spindles [15]. The expression of this protein may lead to a reduced synthesis (S) phase and an increase in the postsynthetic gap 2/mitotic (G2/M) phase [16]. The NS5B protein also triggers cell cycle arrest in the G2 phase, and its functional interaction with NS5A suggests a possible cooperation of these proteins in HCV-induced mitotic impairments [15]. The NS5A expression may alter the levels of intracellular calcium and reactive oxygen species which activate STAT3 and NFκB [16]. The latter induces the expression of anti-apoptotic factors, such as inhibitors of apoptosis (IAP) and Bcl-2 in chronic HCV patients [15]. The NS5A protein can protect against TNFα-mediated apoptotic cell death, interact with the newly identified tumor suppressor bridging integrator 1 (Bin1), and inhibit apoptosis. The NS5A protein also inhibits p53-mediated apoptosis by sequestering p53 in cytoplasm and forms a complex with PI3K in the epidermal growth factor (EGF) signaling pathway, enhances the PI3K-AKT pathway and contributes to cell survival in virus-infected cells [16].

The NS2 protein acts as an apoptosis inhibitor by interaction with the liver-specific pro-apoptotic protein cell death-inducing DFFA-like affector B (CIDE-B). The expression of NS2 may inhibit the human TNFα promoter and the expression of cyclin A that leads to cell cycle arrest in the S phase [14].

Hepatitis C virus core protein is found in various subcellular compartments, including cytosol, endoplasmic reticulum/Golgi apparatus, mitochondria, and nuclei [17]. It has transcriptional regulating functions on diverse cellular genes including activation of the c-myc promoter and suppression of the c-fos oncogene promoter [18]. It could promote both apoptosis and cell proliferation through its interaction with p53 [3]. The core protein can modulate the expression of the cyclin-dependent inhibitor $p21^{Waf1}$, and an increased level of p21 corresponds to a decrease in CDK2 kinase activity with hepato-

cyte arrest in the gap 0/gap 1 (G0/G1) phase of the cell cycle. Cyclin E is elevated in cells that express the core protein. The decreased expression of the hepatic pro-growth factors, hepatocyte growth factor (HGFR) and α-fetoprotein (AFP) in HepG2 cells shows that the Core protein expression suppresses cell proliferation [18]. The core protein stimulates HCC cell proliferation at least partly through upregulation of Wnt-1 at the transcriptional level [3]. The core protein expression can lead to increased expression of the inhibitor of kappa B (IκB) α subunit of the IκB complex which enhances inactivation of the NFκB transcription complex [18]. The core protein may contribute to transformation *via* mechanisms that involve oxidative stress which stimulates JAK/STAT signaling [9]. It also upregulates STAT3 and activates downstream signaling molecule Bcl-2-like 1 protein (Bcl-xL) [17]. It can inhibit multiple activators of apoptosis, including TNF receptor superfamily member 6 (Fas) and TNFα, possibly through constitutive activation of a MAPK/ERK signaling cascade [1]. It may downregulate the expression of the suppressor of cytokine signaling (SOCS-1) gene, and thus contribute to the pathogenesis in HCV infection including hepatocarcinogenesis [17].

AFLATOXIN-MEDIATED CARCINOGENESIS

The aflatoxins are among the most potent genotoxic agents known [19]. Aflatoxin B1 (AFB1) is produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*, and contaminates improperly stored rice, corn, and peanuts [8]. It induces various chromosomal aberrations, unscheduled DNA synthesis and chromosomal strand breaks in human cells [19]. Aflatoxin B1 is metabolized by hepatic cytochrome p450 to exo-8,9-epoxide, which is mutagenic [1]. After conversion, AFB1-8,9-epoxide can react with guanine nucleotides in the hepatocyte DNA to form a number of adducts capable of forming subsequent repair-resistant adducts, depurination, or of leading to error-prone DNA repair that results in single-strand breaks, base pair substitutions, or frame shift mutations. The most prevalent aflatoxin-nucleic acid adduct is AFB-N7-guanine, and mispairing of the adduct may induce both transversion and transition mutations [19].

Environmental exposure to hepatic carcinogen AFB1 may cause a specific missense mutation in codon 249 of the p53 tumor-suppressor gene. The most prevalent mutation induced by aflatoxin in the p53 gene is a GC>TA transversion (arginine→serine alteration in the p53 protein) in the third nucleotide of codon 249 [1]. The frequency of codon 249 mutations parallels the level of aflatoxin exposure [20]. Very importantly, AFB1 has synergistic effects with the HBV that may sensitize hepatocytes to the carcinogenic effects of aflatoxin [21]. Due to the similar frequency of mutations in p53 in regions with a high incidence of HBV and varying aflatoxin exposure, it has been suggested that alterations in p53 independent of the codon 249 mutation may play an important role in HBV-associated HCC. It has been demonstrated that codon 249 is not the major site of adduction by AFB1; adducts were detected in codons in exons 7 and 8. The G>T transversions at the third base of codon 249 were preferentially induced *in vitro* in human hepatocarcinoma cells incubated with AFB1, although G>T and C>A transversions were also observed in adjacent codons [20].

The electrophilic intermediates (epoxides), resulting from conversion of AFB1, are detoxified mainly by glutathione-S-transferase (GST) that catalyze the conjugation of reduced glutathione to electrophilic centers of the substrates. Activity of the pathways that detoxify the mutagenic AFB1-8,9-exo-epoxide can be decreased by polymorphisms of the GST gene [22].

It has been suggested that interaction among the genotypes of three enzymes associated with AFB1 metabolism and DNA repair [GSTM1 null genetic polymorphism, megakaryocyte-associated tyrosine kinase (HYL1*2) genotype YH/HH) and X-ray repair complementing defective repair in Chinese hamster cells (XRCC1) genotype AG/GG] and AFB1 intake may have functional significance in the repair of AFB1-induced genetic lesions [23].

ALCOHOL-MEDIATED HEPATOCARCINOGENESIS

Alcohol use is common in the Americas and Western Europe and is increasing in the UK, Taiwan, Japan and Korea [24]. Chronic ethanol-induced hepatic disease is characterized by progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis and HCC [25]. However, HCC has been iden-

tified in patients with alcohol-induced liver disease but without evident cirrhosis. Chronic, heavy ethanol consumption was associated with a 5- to 7-fold increased risk for HCC development when ethanol use exceeded 80 g/day for more than 10 years. Habitual alcohol consumption acts synergistically with HBV infection: there is 3- to 4-fold elevated risk for development of HCC in patients with chronic HBV who habitually consume alcohol. Relatively, there is an approximately 2-fold increased risk for HCC in patients with HCV and habitual alcohol consumption [24]. In both viral and alcohol mediated liver damage, there is consistent evidence of enhanced production of free radicals and/or significant decrease of antioxidant defense. This leads to marked perturbation of the cellular redox status, with consequent oxidative stress [26].

Hepatocytes represent the major site of ethanol metabolism through three main pathways: alcohol dehydrogenase [(ADH) located in the cytosol], the microsomal ethanol oxidizing system (MEOS), located in endoplasmic reticulum and comprised mainly of inducible cytochrome P450 2E1 (CYP2E1), and catalase (located in peroxisomes) [27]. The reactions of the ADH-dependent ethanol metabolism lead to production of reduced nicotinamide adenine dinucleotide (NADH), and results in increased oxygen use and in synthesis of reactive oxygen species (ROS) that cause peroxidation damage to DNA, lipids and proteins [1]. The intermediate acetaldehyde can cause additional ROS generation [1] and formation of DNA and protein adducts [1,28].

Microsomal CYP2E1 oxidizes carcinogens present in alcoholic beverages, tobacco smoke and the diet, thus enhancing their activation. The c2 allelic polymorphism of CYP2E1 gene is associated with increased CYP2E1 gene expression in patients with a history of alcohol use compared with control HCC patients [24]. In addition, the induction of CYP2E1 can change immune system responses which leads to increased susceptibility to viral infections (*e.g.,* HBV and HCV) [1]. Chronic alcohol abuse causes chronic immune system activation, which is the mechanism underlying alcohol-related liver disease [29]. Ethanol can cause several types of cell injury including cell death. It is probably responsible for auto-activation of the normally quiescent zymogen proform of caspase-8, which further activates caspase-3, the final step in apoptotic cell death. It has been demonstrated that alcohol sensitizes primary hepatocytes and HepG2 cells to TNFα-mediated toxicity [25]. It also alters the activity of phospholipase C, phospholipase D, adenylate cyclase, PKC, JNK, p42/p44 MAPK, STAT and NFκB in hepatic tissues [27]. There is support for the possible role of ethanol in DNA methylation abnormalities in HCC *via* a decreased level of the hepatic methyladenosyltransferase II that results in decreased production of S-adenosylmethionine, the methyl donor for DNA methylation reactions [24].

GENETIC ALTERATIONS IN HEPATOCELLULAR CARCINOMA

Loss of heterozygosity (LOH) in a cell represents the loss of one parent's contribution to part of the cell's genome. Normally, there are slight differences between the two copies of a gene, since one comes from each parent. When this heterozygosity is lost, it signifies that a mutation likely exists. The LOH can arise *via* several pathways, including deletion, gene conversion and chromosome loss. Loss of heterozygosity of polymorphic loci in a chromosomal fragment implies the presence of putative tumor suppressor genes. Loss of heterozygosity can be identified in cancers by noting the presence of heterozygosity at a genetic locus in an organism's germline DNA, and the absence of heterozygosity at that locus in the cancer cells. This is often done using polymorphic markers, such as microsatellites or single nucleotide polymorphisms for which the two parents contributed different alleles. A genome-wide scan for LOH in a diverse collection of HCC samples from Europe and Asia has shown the highest percentages in loci at chromosomes 8p23, 4q22-24, 4q35, 17p13, 16q23-24, 6q27, 1p36 and 9p12-14 [30]. The most frequently affected chromosome arm is 1q, with rates of amplification ranging between 58 and 78% in HCC [31]. Other chromosome arms commonly altered with gains include 6p, 8q and 17q, and allelic losses are found at 1p, 4q, 6q, 8p, 9p, 13q, 16q and 17p [32].

Tumor-Suppressor Genes. Tumor-suppressor genes normally function to inhibit cellular proliferation. They are considered to be 'recessive,' since loss of function of both alleles is necessary to generate the mutant phenotype. A significant percentage of HCC patients carry mutations in the TP53 tumor-suppres-

sor gene encoding the p53 protein [33]. In HCC, the rate of mutations of p53 ranges from 0% (in 129 HCC samples from Spain) to 67% (in 15 HCC samples from Senegal). There is a remarkable differential mutation rate according to geographic area: higher rates of mutation were documented in West Africa and Southeast Asia and lower rates in western countries. The rate of mutations of p53 in 107 geographically and ethnically diverse HCC samples was 25 and 12% in high- and low-aflatoxin exposure regions, respectively [31]. In most cases, loss of p53 function occurs through allelic deletions at chromosome 17p13 or due to missense mutations within the specific DNA-binding domain. The R249S mutation (G/C>T/A transversion) in p53 is frequent in some regions with high aflatoxin exposure [30]. Although the p53 gene promoter does not contain a CpG island, an increase in promoter methylation of the p53 that led to reduced gene expression in human HCC has been described [34].

Mutations have been found in the gene encoding the axis inhibition protein 1 (AXIN1) gene (16p13.3) in 7-10% of patients with HCC which led to aberrant activation of Wnt signaling [33]. The canonical Wnt pathway describes a cascade that involves translocation of β-catenin from the cell membrane into the nucleus where it regulates specific target genes, including c-myc and cyclin D. The AXIN protein is part of a protein complex involved in the degradation of β-catenin [31]. Mutations in the AXIN1 in the absence of mutation in the catenin (cadherin-associated protein) β1 (CTNNB1) gene (codes for β-catenin) could prevent degradation and lead to nuclear accumulation of β-catenin. In addition, over expression of wild-type AXIN1 can sequester both normal and mutated β-catenin in the cytoplasm, which leads to apoptosis [30].

Oncogenes: the β-Catenin Pathway. The most frequently mutated oncogene in human HCC is CTNNB1 (located at 3p21) encoding β-catenin protein. The β-catenin is involved in cell-cell adhesion by association with E-cadherin and also in transmission of the proliferative/ survival signal during embryonic development [30]. In the absence of Wnt signaling, β-catenin is phosphorylated by functional interactions with glycogen synthase kinase (GSK)-3β, AXIN, conductin (AXIN2) and the APC protein, leading to its degradation by the ubiquitin-proteasome system [35]. The activation of the Wnt signal induces β-catenin stabilization through inhibition of GSK-3β activity. After translocation to the nucleus, β-catenin is able to activate a number of genes including c-myc, cyclin D1, WNT1 inducible signaling pathway protein 2 (WISP), fibronectin and matrix metalloproteinase genes. The CTNNB1 mutations in human HCC include mostly missense mutations as well as interstitial deletions of the CTNNB1 exon 3 with a prevalence of 18-41% [30]. Another gene possibly involved in hapatocarcinogenesis is Frizzled (FZD) which codes for a transmembrane receptor in the Wnt-signaling cascade. The FZD signals to β-catenin to escape its association with E-cadherin, thus influencing cell-cell adhesion [31].

CONCLUSIONS

The identification of the principal risk factors involved in the molecular pathogenesis of primary HCC remains poorly understood. Further studies are needed in order to elucidate the complex underlying mechanisms of this common malignancy. The elucidation of new molecular and/or genetic targets should lead to the development of more efficacious treatment for patients with HCC.

REFERENCES

1. McKillop IH, Moran DM, Jin X, Koniaris LG. Molecular pathogenesis of hepatocellular carcinoma. J Surg Res 2006; 136(1): 125-135.

2. Nam SW, Park JY, Ramasamy A, Shevade S, Islam A, Long PM, Park CK, Park SE, Kim SY, Lee SH, Park WS, Yoo NJ, Liu ET, Miller LD, Lee JY. Molecular changes from dysplastic nodule to hepatocellular carcinoma through gene expression profiling. Hepatology 2005; 42(4): 809-818.

3. Panga R, Tsea E, Poon RTP. Molecular pathways in hepatocellular carcinoma. Cancer Lett 2006; 240(2): 157-169.

4. Nakamoto Y, Guidotti LG, Kuhlen CV, Fowler P, Chisari FV. Immune pathogenesis of hepatocellular carcinoma. J Exp Med 1998; 188(2): 341-350.

5. Li B, Gao B, Ye L, Han X, Wang W, Kong L, Fang X, Zeng Y, Zheng H, Li S, Wu Z, Ye L. Hepatitis B virus X protein (HBx) activates ATF6 and IRE1-XBP1 pathways of unfolded protein response. Virus Res 2007; 124(1-2): 44-49.

6. Cougot D, Neuveut C, Buendia MA. HBV-induced carcinogenesis. J Clin Virol 2005; 34(Suppl 1): S75-S78.

7. Zhang X, Zhang H, Ye L. Effects of hepatitis B virus X protein on the development of liver cancer. J Lab Clin Med 2006; 147(2): 58-66.

8. Cha C, DeMatteo RP. Molecular mechanisms in hepatocellular carcinoma development. Best Pract Res Clin Gastroenterol 2005; 19(1): 25-37.

9. Feitelson MA. Parallel epigenetic and genetic changes in the pathogenesis of hepatitis virus-associated hepatocellular carcinoma. Cancer Lett 2006; 239(1): 10-20.

10. Wei W, Huang W, Pan Y, Zhu F, Wu J. Functional switch of viral protein HBx on cell apoptosis, transformation, and tumorigenesis in association with oncoprotein Ras. Cancer Lett 2006; 244(1): 119-128.

11. Kang-Park S, Lee JH, Shin JH, Lee YI. Activation of the IGF-II gene by HBV-X protein requires PKC and p44/p42 map kinase signalings. Biochem Biophys Res Commun 2001; 283(2): 303-307.

12. Kang-Park S, Im JH, Lee JH, Lee YI. PTEN modulates hepatitis B virus-X protein induced survival signaling in Chang liver cells. Virus Res 2006; 122(1-2): 53-60.

13. Henkler F, Hoare J, Waseem N, Goldin RD, McGarvey MJ, Koshy R, King IA. Intracellular localization of the hepatitis B virus HBx protein. J Gen Virol 2001; 82: 871-882.

14. Kim KM, Kwon S-N, Kang J-I, Lee SH, Jang SK, Ahn B-Y, Kim YK. Hepatitis C virus NS2 protein activates cellular cyclic AMP-dependent pathways. Biochem Biophys Res Commun 2007; 356(4): 948–954.

15. Baek K-H, Park H-Y, Kang C-M, Kim S-J, Jeong S-J, Hong E-K, Park J-W, Sung Y-C, Suzuki T, Kim C-M, Lee C-W. Overexpression of hepatitis C birus NS5A protein induces chromosome instability via mitotic cell cycle dysregulation. J Mol Biol 2006; 359(1): 22-34.

16. Wang J, Tong W, Zhang X, Chen L, Yi Z, Pan T, Hu Y, Xiang L, Yuan Z. Hepatitis C virus nonstructural protein NS5A interacts with FKBP38 and inhibits apoptosis in Huh7 hepatoma cells. FEBS Lett 2006; 580(18): 4392-4400.

17. Basu A, Meyer K, Lai KK, Saito K, Di Bisceglie AM, Grosso LE, Ray RB, Ray R. Microarray analyses and molecular profiling of Stat3 signaling pathway induced by hepatitis C virus core protein in human hepatocytes. Virology 2006; 349(2): 347-358.

18. Nguyen H, Sankaran S, Dandekar S. Hepatitis C virus core protein induces expression of genes regulating immune evasion and anti-apoptosis in hepatocytes. Virology 2006; 354(1): 58-68.

19. Wang J-S, Groopman JD. DNA damage by mycotoxins. Mutat Res 1999; 424(1-2): 167-181.

20. Jackson PE, Groopman JD. Aflatoxin and liver cancer. Bailliere's Clin Gastroenterol 1999; 13(4): 545-555.

21. Ming L, Thorgeirsson SS, Gail MH, Lu P, Harris CC, Wang N, Shao Y, Wu Z, Liu G, Wang X, Sun Z. Dominant Role of Hepatitis B virus and cofactor role of aflatoxin in hepatocarcinogenesis in Qidong, China. Hepatology 2002; 36(5): 1214-1220.

22. Asare GA, Bronz M, Naidoo V, Kew MC. Interactions between aflatoxin B1 and dietary iron overload in hepatic mutagenesis. Toxicology 2007; 234(3): 157-166.

23. Long XD, Ma Y, Wei YP, Deng ZL. The polymorphisms of GSTM1, GSTT1, HYL1*2, and XRCC1, and aflatoxin B1-related hepatocellular carcinoma in Guangxi population, China. Hepatol Res 2006; 36(1): 48-55.

24. Morgan TR, Mandayam S, Jamal MM. Alcohol and hepatocellular carcinoma. Gastroenterology 2004; 127(5): S87-S96.

25. Balasubramaniyan V, Shukla R, Murugaiyan G, Bhonde RR, Nalini N. Mouse recombinant leptin protects human hepatoma HepG2 against apoptosis, TNF-α response and oxidative stress induced by the hepatotoxin-ethanol. Biochim Biophys Acta 2007; 1770(8): 1136-1144.

26. Loguercio C, Federico A. Oxidative stress in viral and alcoholic hepatitis. Free Radic Biol Med 2003; 34(1): 1-10.

27. McKillop IH, Schrum LW. Alcohol and liver cancer. Alcohol 2005; 35(3): 195-203.

28. Hsiang C-Y, Wu S-L, Chen J-C, Lo H-Y, Li C-C, Chiang S-Y, Wu H-C, Ho T-Y. Acetaldehyde induces matrix metalloproteinase-9 gene expression via nuclear factor-κB and activator protein 1 signaling pathways in human hepatocellular carcinoma cells: association with the invasive potential. Toxicol Lett 2007; 171(1-2): 78-86.

29. Djordjević D, Nikolić J, Stefanović V. Ethanol interactions with other cytochrome P450 substrates including drugs, xenobiotics, and carcinogens. Pathol Biol 1998; 46(10): 760-70.

30. Buendia MA. Genetics of hepatocellular carcinoma. Semin Cancer Biol 2000; 10(3):185-200.

31. Villanueva A, Newell P, Chiang DY, Friedman SL, Llovet JM. Genomics and signaling pathways in hepatocellular carcinoma. Semin Liver Dis 2007; 27(1): 55-76.

32. Nishimura T, Nishida N, Komeda T, Fukuda Y, Ikai I, Yamaoka Y, Nakao K. Genome-wide semiquantitative microsatellite analysis of human hepatocellular carcinoma: discrete mapping of smallest region of overlap of recurrent chromosomal gains and losses. Cancer Genet Cytogenet 2006; 167(1): 57-65.

33. Buendia, MA. Tumor suppressors in hepatocellular carcinoma: many are called, but few are chosen. J Hepatol 2007; 46(4): 546-548.

34. Pogribny IP, James SJ. Reduction of p53 gene expression in human primary hepatocellular carcinoma is associated with promoter region methylation without coding region mutation. Cancer Lett 2002; 176(2): 169-174.

35. Calvisi DF, Conner EA, Ladu S, Lemmer ER, Factor VM, Thogeirsson SS. Activation of the canonical Wnt/β-catenin pathway confers growth advantages in c-Myc/E2F1 transgenic mouse model of liver cancer. J Hepatol 2005; 42(6):842-849.