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TOPIC HIGHLIGHT

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Molecular mechanism of hepatitis B virus-induced hepatocarcinogenesis

Mirko Tarocchi, Simone Polvani, Giada Marroncini, Andrea Galli

Mirko Tarocchi, Simone Polvani, Andrea Galli, Department of Experimental and Clinical Biomedical Sciences, University of Florence, 50139 Florence, Italy
Giada Marroncini, Andrea Galli, FiorGen Foundation, 50139 Florence, Italy
Author contributions: Tarocchi M made the literature review and wrote the paper; Polvani S critically revised the manuscript; Marroncini G critically revised the manuscript; Galli A supervised the manuscript.
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Correspondence to: Andrea Galli, MD, PhD, Professor, Department of Experimental and Clinical Biomedical Sciences, University of Florence, Viale Pieraccini n°6, 50139 Florence, Italy. andrea.galli@unifi.it
Telephone: +39-55- 4271294 Fax: +39-55-4222409
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Abstract

Hepatitis B virus (HBV) infection is a global public health problem with approximately 2 billion people that have been exposed to the virus. HBV is a member of a family of small, enveloped DNA viruses called hepadnaviruses, and has a preferential tropism for hepatocytes of mammals and birds. Epidemiological studies have proved a strong correlation between chronic hepatitis B virus infection and the development of hepatocellular carcinoma (HCC). HCC is the fifth most common malignancy with about 700000 new cases each year, and more than 50% of them arise in HBV carriers. A large number of studies describe the way in which HBV can contribute to HCC development. Multiple mechanisms have been proposed, including the accumulation of genetic damage due to immune-mediated hepatic inflammation and the induction of oxidative stress. There is evidence of the direct effects of the viral proteins HBx and HBs on the cell biology. Integration of HBV-DNA into the human genome is considered an early event in the carcinogenic process and can induce, through insertional mutagenesis, the alteration of gene expression and chromosomal instability. HBV has also epigenetic effects through the modification of the genomic methylation status. Furthermore, the virus plays an important role in the regulation of microRNA expression. This review will summarize the many mechanisms involved in HBV-related liver carcinogenesis.

Key words: Hepatocellular carcinoma; Hepatocarcinogenesis; Hepatitis B Virus; Chronic hepatitis B infection; Cell biology

Core tip: Hepatitis B virus (HBV) infection is a global health problem. There is evidence that HBV have a causal role in the development of hepatocellular cancer, but the mechanism leading to the transformation of normal hepatocytes into cancer cells is still intricate. This review will summarize the many mechanisms involved in HBV-related liver carcinogenesis.

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HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma is a highly prevalent and lethal neoplasia. It is the fifth most common cancer in men and the ninth in women with respectively about 500000 and 200000 new cases per year; looking in detail the major number comes from Asia (76%), followed by Europe

(8.1%), Africa (7.5%), North America (4.2%), Latin America and Caribbean (3.8%) and Oceania (0.4%)[1]. The global distribution of these new cases is uneven and reflects the differences in the exposition of the local populations to the different etiological factors: in Eastern Asia and Sub-Saharan Africa the dominant risk factor is chronic HBV infection, while in North America, Europe and Japan HCV infection together with excessive alcohol intake are the main risk factor. Furthermore in developed countries the increasing incidence of obesity and consequently of non-alcoholic steatohepatitis is becoming an important risk factor for cirrhosis and HCC as well[2,3]. Other risk factors include type 2 diabetes, hemochromatosis, Wilson`s disease, alpha-1 antitrypsin deficiency, glycogen-storage diseases, autoimmune hepatitis, tyrosinemia and some porphyries[4-6]. Independently from the etiologic factor underlining the presence of chronic liver disease, cirrhosis should be consider major risk factor, in fact 70%-90% of HCC develop from these group of patients.

Even if in the last decades the management of HCC is improved due to the increase diagnostic capacity, the development of evidence-based staging system and the availability of new effective treatments the prognosis is still very poor (overall ratio of mortality to incidence of 0.95)[1] suggesting that a deeper comprehension of the molecular pathway involved in the hepatocarcinogenic process is necessary to improve the outcome of HCC patients.

HBV BACKGROUND

Hepatitis B virus (HBV) infection is a major global public health problem with approximately 2 billion people that present evidence of contact with the virus. Considering that more than 350 million individuals in the global population are chronic HBV carriers, this virus stands as one of the most common human pathogens[7]. The risk of developing chronic hepatitis B (CHB) infection after an exposure seems to depend on the age at which the virus is first contracted[8]. Infection within the first year of life has a 90% chance in developing a CHB; infections in childhood represent a risk of 20%-30%; less than 1% of the exposure in adults go on to develop CHB[8]. Chronic infection may progress to cirrhosis and ultimately hepatocellular carcinoma (HCC). CHB infection remains the major etiological factor of HCC worldwide with more than one half of HCC patients being HBV infected[9]. Several observations indicate an etiologic association between CHB and the development of HCC including a high prevalence of HBV surface antigen (HBsAg) among HCC patients. CHB increases up to a 20-fold the risk of developing HCC also in the absence of cirrhosis. The REVEAL-HBV study found that serum HBV DNA levels and HCC risk correlate in a linear relationship[10]. Furthermore the presence of HBV-DNA integration within the hepatic cells increases about 100 times the relative risk for HCC among HBsAg carriers compared with negative individuals[11].

Specific viral and host factors can contribute to an increased risk of HCC among patients with CHB: increasing age, male gender, and longer duration of infection, all increase the risk of HCC and the presence of cirrhosis is the single most-important risk factor for HCC[12].

Host genetic background is known to have an influence in the story of the disease; in fact belonging to a specific ethnical group increases the susceptibility of HBV carrier to develop HCC. Among all Africans have the worst outcome, followed by Asians[13]. In these two geographic areas more than 70% of HCC develop in patients with HBV infection[14]. In Asia annual incidence among HBV carriers is more than 0.2% with a risk of HCC development before the cirrhotic stage[15]. In Africa of great importance is the potential aflatoxin exposure that increases highly the risk, obliging to start HCC surveillance in youth. The synergic effect of HBV infection and aflatoxin exposure increases the risk to develop HCC by 60 time compared to healthy individuals[16].

Several meta-analysis tried to associate genetic polymorphisms with an increased risk of HCC in CHB carriers; contradictory evidences are present in literature. While more studies are needed to unveil the mechanisms connecting some of these genetic changes to HCC, current results suggest a positive correlation of TNFα and GSTT1 polymorphisms with HCC[17]. On the viral side, for a long time the attention has been focused on high viral load as predictive for HCC development, but other viral risk factors have been found including the viral genotype. Until now 11 HBV genotypes (A–J) have been identified, based on differences in their genome sequence[18]. HBV genotypes have distinct geographical and ethnic distributions: genotype A is pandemic but most prevalent in northern Europe, North America and central Africa; genotypes B and C are found in eastern Asia, Korea, China, Japan, Polynesia and Vietnam; genotype D is also pandemic but is predominant in the Mediterranean area, the Middle East and India; genotype E is typical for Africa; genotype F is found in Native Americans and in Polynesia; genotype G is present in western Europe and North America and genotype H is found predominantly in Central America. The epidemiological evidences indicate that the genotype C has a higher risk of causing HCC than B, and D has a higher than A[19]. Furthermore the presence of enhancer II/basal core promoter mutations (A1762T/G1764A), and mutants with preS deletions are associated as well with increased risk of HCC[20,21]. External cofactors that can also promote HCC development in CHB carriers are concomitant infection with human immunodeficiency virus (HIV), hepatitis C or D virus, cigarette smoking, environmental pollution, chronic alcohol consumption, aflatoxin exposition, and metabolic syndrome[12,17,22]. HCC is the third leading cause of cancer-related death worldwide[23]. Recent epidemiological data have demonstrated that liver cancer incidence is continuously rising and will continue to do so for more than a decade, not only in Asia and Africa but also in North America and Europe[9]. Despite the avail-

ability of an efficacious and safe hepatitis B vaccine[24] about 600000 people die worldwide every year due to either the acute or chronic effects of the virus, with a high proportion dying of HCC. In this context, advances in our understanding of the molecular basis of HCC are urgently needed to develop early tumour markers and novel targeted agents with improved therapeutic efficiency[25]. Here we review the molecular mechanisms linking CHB to malignant transformation of liver cells.

HEPATITIS B VIRUS

HBV is a member of a family of small enveloped DNA viruses called hepadnaviruses, which infect a restricted number of mammals and birds. These viruses share a narrow host range and preferential tropism for hepatocytes. The genome of hepadnaviruses is a circular, partially double-stranded DNA genome that replicates via an RNA intermediate[26]. The HBV genome consists of two asymmetric DNA strands, which forms a partially double-stranded relaxed circular DNA structure that is around 3.2 kb in length. The HBV genome is organized into 4 overlapping open-reading frames (ORFs) that give rise to 5 messenger RNAs (mRNA). The largest one is a 3.5 kb mRNA strand that is composed of two subspecies: a 3.5 kb precore/core mRNA that is translated into the e antigen (HBeAg), and a second 3.5 kb strand termed the pregenomic mRNA that is translated into the core and polymerase proteins. The remaining mRNAs include the 2.4 kb large and 2.1 kb small surface mRNAs, which encode the three viral surface proteins. The 0.7 kb small mRNA gives rise to the hepatitis B X (HBx) protein that is essential for virus replication and appears to play an important role in HBV-induced HCC. The HBV genome encodes also a 2.2 kb singly spliced pre-genomic RNA producing the newly discovered hepatitis B spliced protein (HBSP) involved in proliferation and viability of HBV-infected cells[27,28].

The HBV enters the hepatocytes and releases, by disintegration of the nucleocapsid, the relaxed circular DNA (rcDNA) that can be transported to the nucleus, where it is converted into covalently closed circular DNA (cccDNA). It is present in the nucleus of infected hepatocytes bound to both histone and nonhistone proteins, in a stable freestanding episomal conformation. cccDNA serves as the template for transcription of all viral mRNAs and is unaffected by all current nucleotide analog antivirals because they inhibit DNA replication, which occurs after cccDNA formation. In fact cccDNA persists during therapy even after the clearance of HBsAg, and this is the reason why disease recurrence is possible even after successful treatment[29,30].

MECHANISMS OF HBV-RELATED HCC INDUCTION

HBV can promote HCC in many ways. There is a large amount of data describing the multiple pathways involved in this process, including the accumulation of genetic damage due to immune-mediated hepatic inflammation, the induction of oxidative stress, a virus-specific mechanisms involving the viral proteins HBx and HBs, the insertional mutagenesis with integration of HBV DNA into the host genome that alters the expression of endogenous genes or induces chromosomal instability, epigenetic modification through the modification of the genomic methylation status and also the regulation of microRNA (miRNA) expression.

Immune and inflammatory factors

In many different tissues chronic inflammation is known to play a vital role in cancer development. In the liver, repeated cycles of inflammation induced apoptosis and hepatocyte regeneration, increasing the risk of hepatocarcinogenesis. T cell dysfunction, cytokine production, and inflammation-mediated alteration of specific signaling pathways play important role in the development of HCC. During inflammation, the activation and interaction between STAT3 and nuclear factor (NF)-κB play vital roles in controlling the communication between cancer cells and inflammatory cells. NF-κB and STAT3 are two major factors that keep in check the ability of preneoplastic and malignant cells to resist apoptosis-based tumor-surveillance and regulate tumor angiogenesis and invasiveness. HBV infection and inflammation induced NF-κB activation, that in turn promoted immune escapes, facilitating the development of HCC. STAT3 activation induced by interleukin (IL)-6, IL-6 cytokine family, and IL-22, also promoting the development of HCC[31,32].

In the immune system, $CD4^+$Th1 and $CD8^+$ T cells play an important role in the growth inhibition and the death of cancer cells. This ability seems to be related to their capability to secrete INF-γ, but other cytokines may be involved; in several experimental studies have been proved that the depletion of these population of T cells impaired the immune response against the cancers and the rejection of the implanted tumor cells suggesting theirs involvement in the immune response against cancer[33]. Differently the regulatory T cells (Treg) are a subpopulation of T cells ($CD4^+$, $CD25^+$and $Foxp3^+$) which maintain tolerance to self-antigens, and abrogate autoimmune disease by suppressing immune responses of other cells. Treg cells have been reported to play a key role in the immune impairment involved in HBV-related HCC. Elevated TGF-β activity, associated with the persistent presence of HBV in the liver tissue, suppresses the expression of miRNA-34a, leading to enhanced production of chemokine CCL22, which recruits Treg cells[34]. HBV infection was found to increase the immunomodulatory activity of Treg by up-regulating the expression of forehead box P3 transcriptional regulator (FoxP3), cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) and glucocorticoid-induced tumor necrosis factor (TNF) receptor family gene (GITR). The expansion of Treg cells and the enhancement of their suppressor function prevent the anti-tumor immune response against HCC tumor antigens and inhibit tumor immune surveillance against HCC[35]. Finally, Li *et al*[36] were able to investigate Treg

cells in the circulatory blood and tumor tissues of patients with HBV-related HCC and their data indicate that an increased amount of Treg cells either in peripheral blood or in the tumor tissue correlates to poor prognosis.

HBV and oxidative stress

In literature, there are many indications that HBV can induce a pro-oxidative status. In fact, increased level of oxidative stress, sulfhydryl and lipid peroxidation were found in CHB patients[37]. Additionally, typical products of reactive oxygen species (ROS) are enhanced in carriers with high HBV DNA titers[38].

Oxidative stress is a disturbance in the cellular equilibrium that can result from a lack of antioxidant deference capacity or by an increased production of ROS. The excess of ROS can damage lipids, proteins or DNA, and consequently alter different cellular pathways and influence gene expression, cell adhesion, cell metabolism, cell cycle and cell death. ROS-induced oxidative DNA damage can increase chromosomal aberrations associated with cell transformation[39]. ROS may also activate cellular signaling pathways, such as those mediated by mitogen-activated protein kinase (MAPK), NF-κB, phosphatidylinositol 3-kinase (PI3K), p53, FOXO, β-catenin/Wnt, COUTFII and others associated with angiogenesis[40-42].

Because of the role of these pathways in mutagenesis, tumor promotion, and progression, ROS are considered potential carcinogens[43].

In vivo and *in vitro* experiments have shown that HBV infection is able to induce oxidative stress by reproducing the same increase found in CHB patients. Furthermore, to demonstrate that oxidative stress plays a critical role in hepatic injury, several studies showed that the total peroxide levels, a parameter of oxidative stress, as well as alanine aminotransferase (ALT) levels are significantly higher in patients with chronic hepatitis compared to asymptomatic carriers[44].

Interestingly, transgenic mice that produce and accumulate HBsAg inside the hepatocytes have increased levels of inflammation and oxidative stress before the development of dysplastic foci and HCC[45,46]. Moreover, transgenic mice that express HBx protein[47] have high levels of ROS, suggesting that the viral induction of oxidative stress occurs through many mechanisms.

Mitochondria are a major source of ROS inside the cells, which can be produced through electron leakage from the mitochondrial respiratory chain[48]. HBx targets mitochondria binding to the voltage-dependent anion-selective channel protein 3 (VDAC3), and alters the mitochondrial membrane potential and increases endogenous ROS levels[49-51]. HBx also induces oxidative stress through cytosolic calcium signaling, resulting in Ca++ accumulation into mitochondria, consequent increased levels of ROS and activation of cellular kinases (PYK2 and SRC kinases), leading to the activation of transcription factors NF-κB and STAT3 which promote HBV replication and early steps of HCC[52-54]. The increased level of Ca++ in presence of ROS can trigger endoplasmic reticulum (ER) stress and the unfolded protein response (UPR). In fact, if the ER stress remains constantly elevated, UPR signaling cannot be maintained and the cell activates the autophagic process to restore ER integrity, which is important for viral replication[55]. HBx alone in the context of whole viral genome transfections caused the mitochondrial translocation of mitogen-activated protein kinase Raf-1. This event induced by oxidative stress involves the Src- and the PAK-mediated phosphorylation of Raf-1, leading to its activation[56]. HBx also influences the lipid peroxidation *via* downregulation of SeP expression, resulting in increased expression of TNF-α as shown in an *in vitro* study in the human hepatoblastoma cell line HepG2[57].

Other HCC cell lines (human HuH7 and murine ML1-4a), stably transfected with the pre-S mutants (truncated forms of preS/S polypeptide), exhibited enhanced levels of ROS through endoplasmic reticulum (ER) stress pathways. The oxidative DNA damage has also been confirmed in the livers of transgenic mice carrying the pre-S mutant[58].

HBV-DNA Integration

Another proposed mechanism of induction of HCC by HBV is through its integration in the host genome. HBV-DNA integration into human host chromosomes occurs in the infected liver since early stages of natural acute infections. Multiple integrations have been detected in chronic hepatitis tissues, and integrated HBV sequences have been seen in 80%-90% of HBV-related HCCs[59]. HBV insertions have been associated with major genetic alterations within the cell genome, including generalized genomic instability, gene and chromosomal deletions and translocations, amplification of cellular DNA, and generation of fusion transcripts[60-61]. These alterations in the host genome may alter the expression of miRNAs, oncogenes, and tumor-suppressor genes, events that could lead to the development of HCC.

Early studies suggested that viral integration into the host gene occurred randomly, but in the last decades with the introduction of whole genome sequencing, several groups were able to identify some preferred site for HBV integration, typically close to or inside of certain target genes, such as TERT (telomerase reverse transcriptase), *FN1* (Fibronectin 1), SMAD5 (SMAD family member 5), *MLL4* (Myeloid/lymphoid or mixed-lineage leukemia 4), ARHGEF12 (Rho guanine nucleotide exchange factor GEF 12), CYP2C8 (Cytochrome P450, family 2, subfamily C, polypeptide 8), PHACTR4 (Phosphatase and actin regulator 4), PLXNA4 (Plexin A4), RBFOX1 (RNA binding protein, fox-1 homolog), ADH1B (Alcohol dehydrogenase 1B), CPS1 (Carbamoyl-phosphate synthetase 1), ESRRG (Estrogen-related receptor gamma), LRFN2 (Leucine rich repeat and fibronectin type III domain containing 2), MYOM1 (Myomesin 1), RAI1 (Retinoic acid induced 1), CTDSPL2 (CTD small phosphatase like 2), LRP1B (Low density lipoprotein-related protein 1B), SENP5 (SUMO1/sentrin specific peptidase 5), ROCK1

(Rho-associated, coiled-coil containing protein kinase 1), PDGF receptor and CCNE1 (cyclin E1)[59,62-64]. The identification of these and other recurrent sites of viral integration inside or in the proximity of genes controlling cellular proliferation, survival, differentiation and immortalization, suggests that this process may indeed be involved directly in hepatocarcinogenesis.

However, evidence for fusion proteins came from a direct promoter insertion mechanism that was first provided in HBV-related HCC where insertion targeted either the retinoic acid receptor-β (RAR-β) gene or the human cyclin A gene, resulting in tumour-specific chimeric proteins endowed with novel, pro-carcinogenic functions.

Finally, although integrated viral sequences are defective for replication, they might also contribute to tumorigenesis through the production of truncated and mutated HBx or preS2/S proteins. These proteins may act on HCC development by disrupting the control of cellular gene expression or by activating oncogenic signaling pathways[65,66].

DNA methylation

DNA methylation is one of the most intensely studied epigenetic modifications in mammals. Aberrant DNA methylation patterns - hypermethylation and hypomethylation compared to normal tissue - have been associated with a large number of human cancers. Hypermethylation typically occurs at CpG islands in the promoter region and is associated with the inactivation of certain tumor-suppressor genes. The enzymes responsible for the maintenance of methylation patterns are the DNA methyltransferases (DNMT) and a large body of evidence shows that HBx upregulates DNMT1, DNMT3A1 and DNMT3A2[67]. Conversely, global hypomethylation has also been reported to induce genomic instability and contribute to cell transformation and progression of cancer.

In HCC, alteration of DNA methylation occurs in the early stage of cancer development, and in these patients the increased risk of cancer development was due both to genomic hypomethylation with related increased chromosome instability and localized hypermethylation with decreased tumor suppressor gene expression[67].

Lately, DNA hypermethylation in the promoter region of specific oncosuppressor genes was found in HBV-related HCC[68-70]: RASSF1A (Ras association domain family member 1), p16INK4A and P21WAF1/CIP1 are involved in cell cycle maintenance and their altered expression is an early event in the development of HCC; CDH1 (E-cadherin) is involved in cell adhension and metastatization; GSTP1 downregulation exposes cells to oxidation damage and electrophilic carcinogens; ASPP1 and ASPP2 have an important role in apoptosis. Alterations were found also in the promoter region of hTERT, maintaining its expression and increasing proliferative capacity of the cell, and in the promoter region of COX-2 leading to a more proinflammatory state.

HBx protein

The viral protein HBx is a 154 amino acids long protein acting as a pleiotropic transactivator; it does not bind directly to DNA but rather acts on cellular promoters by protein-protein interactions and modulating cytoplasmic signaling pathways.

Interestingly HBx is expressed at low levels during acute and chronic hepatitis and can induce a humoral and cellular immune response[71,72]. Thanks to viral DNA integration into the host genome, the HBx gene is maintained and transcribed in human HCC tumor cells even if complete HBV replication is absent[73,74].

In the cytoplasm, it activates mitogenic signaling cascades while in the nucleus it modulates gene expression via interaction with numerous transcription factors. The large body of evidences suggests its central function in a large number of signaling pathways involved in oncogenesis, proliferation, apoptosis, inflammation and immune response. Furthermore, HBx may act as a paracrine factor and activates stellate cells[75,76].

HBx play a role in chromosomal instability by targeting centrosome dynamics and mitotic spindle formation through its binding with different cellular partners implicated in centrosome formation. HBx has been suggested to induce multipolar spindle formation, chromosome segregation defects, and appearance of multinucleate cells by inducing aberrant centrosome duplication; these biological actions might be due to sequestration of the nuclear transport receptor Crm1 in the cytoplasm[77], and/or HBx binding to the hepatitis HBx interacting protein (HBXIP), a regulator of centrosome duplication[78] or to the UV-damaged DNA binding protein 1 (DDB1)[79] and induction of lagging chromosomes by binding to BubR1[80].

HBx may increase the expression of matrix metalloproteinase and facilitated cellular migration[81-83].

HBx transactivates a number of cellular promoters and enhancers containing binding site for NF-κB, activator protein 1 (AP-1), AP-2, CCAAT-enhancer-binding protein (c-EBP), RNA polymerase and nuclear factor of activated T-cells (NF-AT), cellular promoter of genes associated with cell proliferation as IL-8, TNF, transforming growth factor (TGF) beta and epidermal growth factor receptor (EGRF) and cytosolic signal transduction pathways as Ras/Raf mitogen-activated protein kinase, Src kinases, cJun Nterminal kinase, Jak1/STAT and protein kinase (PK)[84,85], which have overlapping effects on cell proliferation and viability.

HBx interacts with the acetyltransferases CBP/p300, and this interaction induces the activation of CREB-dependent transcription. The activation of CREB/ATF trans-activation function by HBx appears redundant since HBx has been shown to increase CREB/ATF DNA-binding affinity as well as to enhance the recruitment of CBP/p300 to CREB/ATF bound to cellular DNA[86,87]. The modulation of CREB/ATF plays an essential role in liver metabolism and proliferation, and CREB has been implicated in hepatocarcinogenesis[88].

Important targets of HBx are p53 and p53 family[89]. In fact, the viral protein directly binds to p53 and impairs its function. HBx, through the interaction with p53, can alter p53-mediated apoptosis, transactivation properties of p53[90], cell cycle regulation[91], DNA repair genes[92,93], and tumor suppressor genes[71].

In the mitochondria HBx interacts with the heat shock protein 60 and 70[94] and the voltage dependent anion channel (VDAC) isoform VDAC3[50].

HBx seems to regulate the angiogenic process in HCC[95-98]. Indeed, HBx expression induces transcriptional up-regulation of the vascular endothelial growth factor (VEGF) and the proangiogenic growth factor angiopoietin 2 (ANG2). It also plays an important role in the hypoxia inducible factor HIF-1 cellular level. In fact HBx binds to and stabilizes HIF1α and at the same time stimulates HIF1α transcription, thus promoting angiogenesis.

HBx can activate Wnt/beta-catenin signaling in two different ways: by up-regulating cytoplasmic beta-catenin[99] or alternatively by hypermethylating E-cadherin promoter and consequently repressing its transcription[100].

HBV preS/S proteins

The preS/S ORF encodes three different, structurally related envelope proteins refered to as the large (L), middle (M), and small (S) proteins that are synthesized from alternative initiation codons. These three proteins share the same carboxy-terminus part but have different amino-terminal extensions. In particular, the S protein corresponding to the HBV surface antigen (HBsAg) consists of only 226 amino acids (aa), the M protein contains an extra N-terminal extension of 55 aa, and the L protein has a further N-terminal sequence of 108-119 aa compared with the M protein. Until now, few mechanisms of action of preS/S encoded proteins have been known to be involved in the hepatocarcinogenic process. During HBV replicative cycle, HBsAg can accumulate into the ER, induce ER stress and consequently increase the cellular level of oxidative stress. This process happens also in presence of pre-S2 mutants in which the viral proteins amass in the ER. The induced ER stress upregulates the cytoplasmic Cyclin A, increasing infected cell proliferation; at the same time, Cyclin A upregulation can promote, through centrosome over-duplication, chromosome instability which is a well-known mechanism in HBV-related hepatocarcinogenesis[101].

Additionally, HBsAg seems to have an effect on the mitochondrial function: on one hand it binds to enoyl coenzyme A hydratase short chain 1 (ECHS1) and can induce cell apoptosis by decreasing the mitochondrial membrane potential (MMP)[102], while on the other hand HBsAg could inhibit JTB (jumping translocation breakpoint), leading to increased cell motility and decreased apoptosis[103]. Interestingly, pre-S2 mutant protein in type Ⅱ ground glass hepatocytes (GGHs) could directly interact with the c-Jun activation domain-binding protein 1 (JAB1), inducing an hyperphosphorylation of the tumor-suppressor retinoblastoma (RB) and its inactivation[104].

Futhermore, pre-S2 protein can act as a transactivator and, interacting with the hTERT promoter[105], increase telomerase activity, a key step in the development of HCC and other cancers. Interestingly, HBV is also able to upregulate of telomerase activity by HBV DNA integration in proximity of the promoter[106] or by HBx capacity to increase the SP1 binding to hTERT promoter and induce its transcription[107]. Despite hTERT activation, telomere in HCC cells remain shorter than in normal somatic cells, predisposing to occasional telomere and chromosomal instability, and polyploidy[108]

miRNAs

MiRNAs are small non-coding RNA molecules (19-25 nucleotides in length) that regulategene expression at transcriptional and post-transcriptional levels, usually resulting in gene silencing via translational repression or target degradation of gene mRNA. Since their discovery in the early 1990s, over 1000 miRNAs have been characterized in human cells. In the last decade, many groups have investigated the role of this biological entity in patho-physiological processes; several publications have unveiled the function of miRNAs in the development, progression and metastatization of HCC. Specifically, a growing number of studies were able to identify specific miRNAs modulated in CHB patients; some of them are regulated by HBV infection and have a role in hepatocarcinogenic process.

miR-143, miR-34, and miR-19 have been found to be upregulated in HBV-related HCC and to promote a more aggressive cancer phenotype, while Let-7a downregulation by HBx increased cell proliferation[109]. HBx also downregulates miR-152 with the consequent upregulation of DNMT1, which methylates the promoters of many tumor suppressor genes. Interestingly, other publications reported additional miRNAs that are involved in HBV-related HCC[110]: miR-221, which is downregulated in acute HBV infection, normally expressed in chronic HBV infection and upregulated in HCC; miR-101, which is constantly downregulated in HBV infection and in HCC tissues, has been associated with HCC development; miR-18a/miR-18b and miR-106a may be key effectors for the progression of HCC. Very recent data suggest other possible miRNAs involved in HCC development in CHB patients: miR-224 that is inversely correlated to autophagy in HBV-related HCC specimens[111], miR-122 that is regulated by HBx through PPARg[112], miR-15a and miR-16-1 directly downregulated by HBx[113], miR-132 regulated by the hypermethylation status induced by HBx[114], or miR-148 that is also downregulated by HBx through the suppression of p53-mediated activation[115]. Recently, Xu *et al*[116] reviewed the HBV-HCC correlation and reported miR-602, miR-143, miR-29a, miR-148a, miR-373, miR-101, miR-152, miR-16, and miR-661 as miRNAs possibly implicated in the carcinogenic process. In the future, more extensive analysis will be able to create a precise profile of HBV modulation of miRNAs and

their function in the different phases of the hepatocarcinogenetic process.

FUTURE THERAPEUTIC STRATEGIES

Up today HCC remain a devastating cancer, so the safest strategy remain the prevention, through the control of the risk factors. Prevention of HBV infection among them can be accomplished by large scale vaccination or in patients with preexistent CHB a continued suppression of HBV replication with antiviral (*e.g.*, entecavir and adefovir dipivoxil) can prevent complications of HBV-related liver disease and decrease the risk of HBV-related HCC development[117]. In fact the REVEAL-HBV study suggests that the degree of HBV viremia predicts HCC risk independently of cirrhosis, HBeAg+ or ALT levels, introducing the concept that virologic suppression through antiviral therapy may have a major impact on the prevention of liver cancer. After the rise of HCC then the treatment selection follows the Barcelona Clinic Liver Cancer (BCLC) staging system that assigns the prognosis and proposes the therapeutic strategies for each stage. In the proposed strategies only one systemic chemotherapeutic molecule (Sorafenib) is approved as first line of treatment. Since the emergence of Sorafenib as the new standard for the systemic treatment of HCC, the idea of targeting specific molecular pathways implicated in the pathogenesis and progression of HCC, has promoted a revolutionary change in the treatment of this disease. Genetic modifications and alteration of critical molecular signaling pathways have been identified as contributing to the tumor development and progression. Among the numerous signaling pathways implicated in the development and growth of HCC are worth to be noted Ras/Raf/MAPK, Wnt-b-catenin, EGFR, insulin-like growth factor receptor, VEGFR, NF-KB, AKT-mTOR, Notch and Hedgehog. Thanks to the acquired knowledge on altered pathways in HCC several promising novel anticancer agents are currently under evaluation (56 drugs)[118] including tyrosine kinase inhibitors, monoclonal antibodies and oligonucleotide antisense. Furthermore monotherapy can be very effective in vitro but targeting one pathway may result in the activation of other pathways in HCC cells suggesting that the most attractive strategy for the future should be the combination of different targeted agents to improve the efficacy of these molecules[119,120]. Research will permit a more comprehensive understanding of hepatocarcinogenic process and to identify new molecular targets for therapeutic intervention.

CONCLUSION

Among the different etiologic agents, HBV is the major risk factor for developing HCC. In the future, the global neonatal vaccination program will greatly reduce the burden of HBV and ultimately of HCC, but more than 700000 new HCC cases are still identified each year. In the last decades, progresses have been made in understanding the multifactorial process through which HBV infection can promote hepatocarcinogenesis. On one hand, HBV infection is able to induce a chronic inflammatory status in the patient, to increase the oxidative stress and to modulate the host immune response against infected hepatocytes. On the other hand, HBV DNA integration may lead to chromosomal instability and alteration of gene expression. Furthermore, HBV products directly disrupt normal cellular signal pathways, contributing to induce HCC. Other factors like genomic methylation and miRNA expression have been identified to play a role in HBV-related development of HCC.

A deeper knowledge of the mechanisms that mediate the HBV carcinogenic process is essential for developing novel strategies to prevent and treat liver cancer in chronic HBV carriers.

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