Hepatocellular Carcinoma

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The hepatitis B virus (HBV) is a widespread human pathogen that causes liver inflammation, cirrhosis, and hepatocellular carcinoma (HCC). Recent sequencing technologies have refined our knowledge of the genomic landscape and pathogenesis of HCC, but the mechanisms by which HBV exerts its oncogenic role remain controversial. In a prevailing view, inflammation, liver damage, and regeneration may foster the accumulation of genetic and epigenetic defects leading to cancer onset. However, a more direct and specific contribution of the virus is supported by clinical and biological observations. Among genetically heterogeneous HCCs, HBV-related tumors display high genomic instability, which may be attributed to the ability of HBV to integrate its DNA into the host cell genome, provoking chromosomal alterations and insertional mutagenesis of cancer genes. The viral transactivator HBx may also participate in transformation by deregulating diverse cellular machineries. A better understanding of the complex mechanisms linking HBV to HCC will improve prevention and treatment strategies.

Hepatocellular carcinoma (HCC) arises from hepatocytes and represents the most frequent type of primary liver cancer. With annual incidence rates of ~750,000 worldwide, this tumor ranks as the fifth most common cancer in men and the seventh in women (Ferlay et al. 2010). Important geographical differences in HCC incidence have been noted since the late 1970s (Szmuness 1978). The majority of HCC cases (84%) affect developing countries, especially where infection by hepatitis B virus (HBV) is endemic. Owing to national vaccination programs against hepatitis B, the incidence of HCC in these areas has started to decline, but it is increasing in Western countries in relation to the hepatitis C virus (HCV) and nonalcoholic steatohepatitis (NASH) (El-Serag 2012). Because this tumor is often detected at late stages, when it is rapidly fatal, the estimated annual rate of deaths related to liver cancer is similar to the rate of newly diagnosed cases. Therefore, HCC is the second most frequent cause of cancer-related death after lung cancer. Recently, new appraisal of HCC complexity has been made possible by accumulated data generated by massive genome sequencing of hundreds of tumors, pointing to driver genetic alterations and opening the way to personalized therapeutic options for patients suffering from this deadly cancer. In this review, we discuss the oncogenic role of HBV during the multistage process of liver carcinogenesis.

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ETIOLOGICAL FACTORS

Chronic HBV infection currently accounts for about one-half of HCC cases worldwide, especially in Southeast Asia and sub-Saharan Africa, where >70% of HCC patients are positive for the surface antigen HBsAg (Ferlay et al. 2010). In these countries, HBV carriers exposed to aflatoxin have a 60-fold increased risk of developing HCC. In Western countries and Japan, the most common etiological factors are infection with HCV, excessive alcohol intake, diabetes and obesity associated with NASH (El-Serag 2012). Other risk factors include inherited metabolic diseases, such as hemochromatosis, tyrosinemia, and α1-antitrypsin deficiency, which are associated with liver disease, cirrhosis, and eventually HCC (Fig. 1).

Robust epidemiologic evidence has linked chronic hepatitis B to liver cancer development. HCC incidence is higher in areas in which HBV is endemic; liver cancer patients have greater rates of HBV infection than the general population; and the risk of developing HCC is increased by 10- to 100-fold for HBV carriers, with a cancer incidence of ~3%–5% per year in HBV-associated cirrhosis (Beasley et al. 1981; Pagano et al. 2004). Moreover, mammalian hepadnaviruses closely related to HBV, including the woodchuck hepatitis virus (WHV) and the ground squirrel hepatitis virus (GSHV), induce liver tumors in their hosts (Summers et al. 1978; Marion et al. 1980). In HBV patients disease outcome is influenced by various host parameters, with higher risk of HCC associated with liver cirrhosis, male gender, older age, early infection in childhood, and cooperative effects of different risk factors, such as aflatoxin B1, alcohol, diabetes, and obesity. Moreover, the risk is markedly increased by high viral load and hepatitis B e antigen (HBeAg) positivity (Chen et al. 2006). These viral and clinical parameters collected in large cohorts of HBV carriers (REVEAL-HBV study) have led to a proposed predictive score for tumor occurrence (Yang et al. 2011). However, as in the WHV/

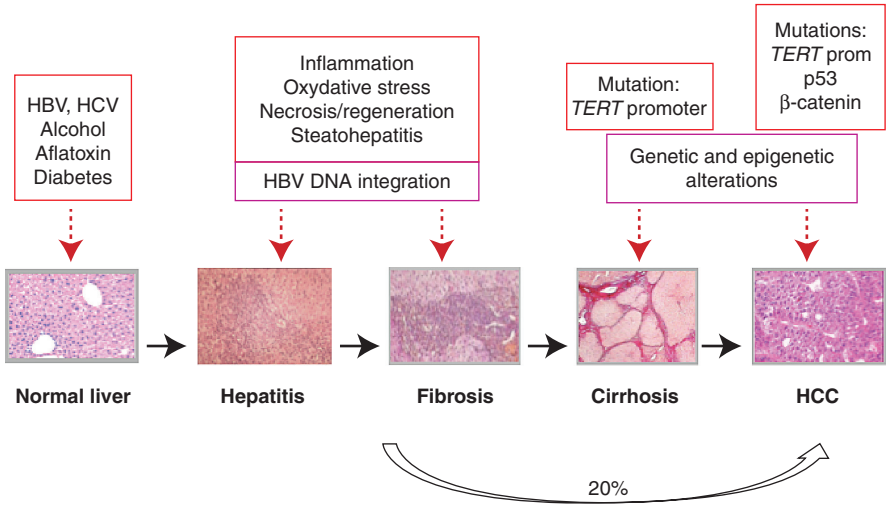

Figure 1. Hepatocellular carcinoma: risk factors and progression of liver disease at preneoplastic and neoplastic stages. As with all cancers, hepatocellular carcinoma (HCC) develops in a stepwise fashion with sequential acquisition of premalignant and malignant characters. During chronic hepatitis, liver injury is caused by persistent inflammation and oxidative stress, which provoke repeated cycles of apoptosis, necrosis, and compensatory regeneration. High-grade cirrhotic nodules (the immediate precursors of HCC) frequently show *TERT* promoter mutations, chromosomal aberrations, and epigenetic silencing of tumor suppressor genes. HCCs can also develop on a background of hepatitis with various degrees of fibrosis in ~20% of cases, mostly in hepatitis B virus (HBV) carriers. HCV, hepatitis C virus. (Courtesy of Catherine Guettier, INSERM U785, Pathology Department, Paul Brousse Hospital.)

woodchuck model, past and resolved HBV infections retain some oncogenic activity, and HCC can occur in patients with "occult" HBV infection (Raimondo et al. 2008). It is worth noting that all HBV genomes might not be endowed with the same tumorigenic potential. In particular, for the two genotypes prevalent in Asian countries, it has been proposed that genotype C takes a more aggressive disease course than genotype B (Yang et al. 2008). Importantly, HBV mutations accumulate at specific positions during chronic hepatitis from healthy carrier state to HCC. Mutations in the PreS region disturb the balance of large, middle, and small envelope proteins, leading to reduced secretion of viral particles associated with accumulation of viral proteins in the endoplasmic reticulum and covalently closed circular (ccc) DNA in the nucleus (Pollicino et al. 2014). In a meta-analysis including >10,000 HBV carriers, it has been shown that PreS mutations combined with the basal core promoter (BCP) mutations C1653T, T1753V, and A1762T/G1764A confer a high probability of HCC development (Liu et al. 2009). These mutations can be detected in plasma several years before HCC diagnosis and represent strong predictive biomarkers.

HCC is a major killer with limited curative options (Forner et al. 2012). It is, therefore, important to implement preventive strategies aimed at preventing viral spread and, for those already infected, reducing viral load. The hepatitis B vaccine has been introduced into national immunization programs for children in most countries. The efficacy of this vaccine in lowering the rate of new infections and the incidence of HCC has been shown fully in countries in which the disease is endemic (Ott et al. 2012). However, the total number of chronic HBV carriers is still slightly increasing, representing 6% of the world population. Current treatments of hepatitis B consist in pegylated interferon-α (IFN-α) and oral nucleoside/nucleotide analogs, mostly lamivudine, adefovir, entecavir, and tenofovir (Scaglione and Lok 2012). Although viral suppression is achieved in most cases, these protocols rarely lead to complete virus eradication (Zoulim 2012), but they prevent further liver damage and they decrease the risk of HCC as well as postsurgery HCC recurrence (Hosaka et al. 2013; Wu et al. 2014). Routine surveillance using ultrasound and serum α-fetoprotein (AFP) levels in patients with cirrhosis allows detection of early tumors that are amenable to surgical resection in about two-thirds of cases, and novel serum biomarkers for early HCC diagnosis are under extensive investigation.

GENOMIC ALTERATIONS AND ONCOGENIC PATHWAYS IN HBV-RELATED HCC

The cancer cell genome accumulates somatic mutations that reflect the DNA damage and repair processes occurring for long periods. HCCs are genetically heterogeneous tumors, but they have been classified into broad subclasses by studies of gene expression profiles in large tumor sets (Lee et al. 2004). HBV etiology has been associated with overexpression of cell cycle and proliferation genes, overexpression of genes normally expressed in the fetal liver including parentally imprinted genes, and activation of the AKT pathway (Boyault et al. 2007). Analysis of genetic abnormalities has shown that HBV-related tumors display loss of heterozygosity (LOH) on several chromosomes at a higher rate than tumors associated with other etiologies (Marchio et al. 2000). More recently, the development of whole-genome and exome sequencing has produced a burst of information on genetic changes occurring in HCC, such as mutations, deletions, translocations, and copy number variations. The search for major recurrent abnormalities aims at distinguishing functional "driver" mutations from the "passenger" background. The highest mutation rate (59%) was found in the promoter of the telomerase reverse transcriptase (*TERT*) gene and was correlated with strong up-regulation of the gene. Moreover, this mutation occurs at preneoplastic stages in 25% of cirrhotic nodules (Nault et al. 2013). Of note, the frequency of *TERT* promoter mutations is significantly lower in HBV-related HCCs, in which this site is the preferred target of HBV integration (Sung et al. 2012). Confirming earlier studies, other frequently

mutated genes (10%–35% of cases in different studies) are the tumor suppressor *TP53*, mostly in HBV-related tumors; and *CTNNB1*, which encodes the β-catenin oncogene, mostly in HCV-related and nonviral tumors. It is remarkable that these mutations are mutually exclusive, and they define two broad subclasses associated on one side with alteration of cell cycle control and on the other side with activation of Wnt signaling (Boyault et al. 2007).

Importantly, the discovery of a new set of genes with mutation rates between 3% and 20% has shaped global genetic profiles in HCC and the contribution of different etiologies (Table 1) (Li et al. 2011; Fujimoto et al. 2012; Guichard et al. 2012). It has been shown that HCCs harbor highly variable mutation rates (average, ~40 mutations per tumor), with probably few driver mutations (Villanueva and Llovet 2014). Strikingly, novel recurrent mutations were predominantly found in epigenetic regulators, such as chromatin remodelers of the switch/sucrose nonfermenting (SWI/SNF) complex (*ARID1A*, *ARID1B*, *ARID2*, *SMARCA4*, and *PBRM1*), and the histone methyltransferases *MLL*, *MLL2*, *MLL3*, and *MLL4* (Fujimoto et al. 2012). In total, this class of genes is mutated in 50% of HCC cases, and again, *MLL2* and *MLL4* are recurrent targets for HBV integration (Li et al. 2014). The SWI/SNF complex is responsible for regulation of many genes involved in cell cycle control and proliferation. Other mutations have been related to cell cycle regulation and aberrant DNA repair mechanisms and to the activation of signaling pathways, particularly the Janus kinase/signal transducer and activator of transcription (JAK/STAT), RAS/mitogen-activated protein kinase (RAS/MAPK), and AKT pathways (Table 1). Moreover, mutations affecting the antioxidant response have been found in *NFE2L2* (*NRF2*) and *KEAP1* (Guichard et al. 2012). These mutations are known to inhibit KEAP1-mediated ubiquitination and degradation of NRF2, leading to constitutive activation of *NRF2* and up-regulation of target genes involved in reduction of reactive oxygen species (ROS), glutathione synthesis, and xenobiotic metabolism. Interestingly, mutations affecting the oxidative stress pathway are found mostly in tumors with activated Wnt/β-catenin signaling. Finally, mutation and homozygous deletion of the interferon regulatory factor 2 (IRF2), a binding partner of MDM2 that regulates the p53 pathway, were seen mostly in HBV-related HCCs (Guichard et al. 2012).

Although further work is necessary for better cataloging of major mutations and pathways in HCC, genomic studies undoubtedly improve our knowledge of the oncogenic role of HBV and open the way to personalized drug treatments, notably those targeting the AKT/mTOR and JAK/STAT pathways.

Table 1. Altered pathways and mutated genes in HCC

Pathway/function	Gene	Rate[a] (%)
Telomere stability	*hTERT* (promoter)	59[b]
Genome integrity and cell cycle	*TP53*	10–40
	IRF2	5
	ATM	5
WNT pathway	*CTNNB1*	10–35
	AXIN1	4–9
	APC	2
Epigenetic modifiers	*ARID1A*	10–16
Chromatin remodeling	*ARID1B*, *ARID2*	5–7 1–7[b]
Histone methyltransferases	*MLL* family genes	
Oxidative stress	*NFE2L2*	6–10
	KEAP1	3–8
JAK/STAT pathway	*JAK1*	9
	IL6ST	3
Growth factor signaling	*EGFR*	<5
RAS/MAPK pathway	*BRAF*, *KRAS*, *NRAS*	<5 10
	RPS6KA3	
AKT pathway	*PIK3CA*, *PTEN*	<5

[a]Range indicates different rates in several publications.
[b]Genes that are targeted by HBV DNA integration (see Fig. 2).

HBV DNA INTEGRATION

Although the life cycle of HBV does not depend on integration of viral DNA into the host genome, integration of HBV DNA sequences into chromosomes has been observed in a majority of HBV-related HCCs as well as in HBV-in-

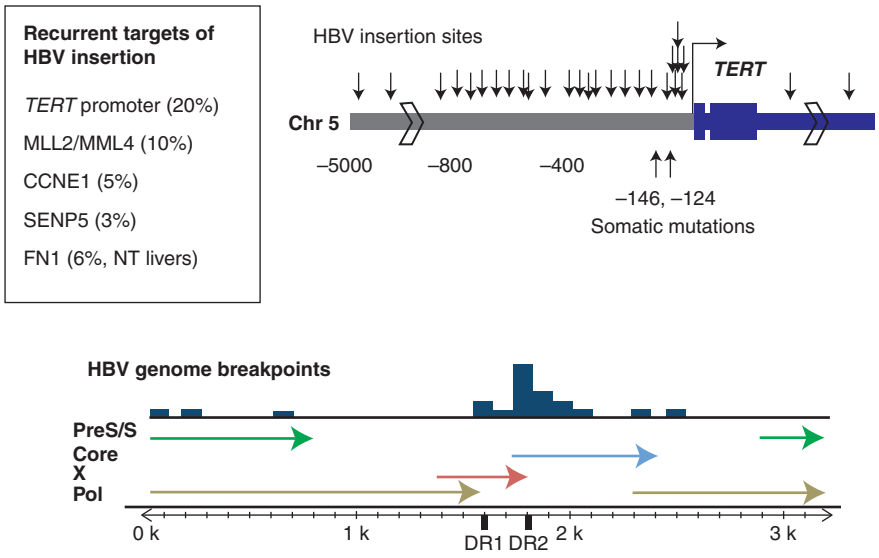

Figure 2. HBV DNA integration in human HCC: recurrent cellular targets and viral breakpoints. Data were deduced from published whole-genome sequencing studies covering ~200 tumors and matched nontumor liver tissues. (*Top*) Integrations into *TERT* promoter and introns (Fujimoto et al. 2012; Jiang et al. 2012; Sung et al. 2012; Toh et al. 2013; Li et al. 2014). Hot-spot somatic mutations in the *TERT* promoter are shown with arrows below the figure (Nault et al. 2013). (*Bottom*) Distribution on the HBV genome of viral sites found frequently at the virus–host junctions, showing preferential integration near the DR1 repeat within the 3′ end of the *X* gene. The arrows represent the viral genes, and positions are numbered from the EcoR1 site. Chr, chromosome.

fected liver tissues in the early 1980s (Bréchot et al. 1980; Shafritz et al. 1981). This discovery was rapidly followed by analysis of integrated sequences through cloning of large genomic DNA fragments into phage libraries, which revealed incomplete and frequently rearranged viral genomes that cannot support HBV replication. Widespread insertions were detected over all chromosomes with no preferential cellular site (Nagaya et al. 1987). Interestingly, an HBV sequence encompassing the "cohesive-ends" region located between the viral repeats DR1 and DR2 was identified at virus–host junctions in one-half of cases (Fig. 2). Preferential breakpoint hot spot in this region that contains initiation sites of HBV replication suggests that the relaxed circular DNA or minus-strand replicative intermediates are the substrates for integration into chromosomal DNA (Hino et al. 1989). Furthermore, such breakpoints coincide with the 3′ end of the *X* gene, allowing the production of chimeric virus–host transcripts carrying carboxy-terminally truncated X sequences fused to flanking host DNA, which retain transactivation function (Takada and Koike 1990).

Initial studies of the chromosomal sites of viral integration described repetitive elements and fragile sites, but failed to identify recognized oncogenes or tumor suppressors. However, in several cases, HBV sequences were inserted into genes, such as the retinoic acid receptor RAR-β and cyclin A2, giving rise to the production of chimeric proteins with altered functions and potential roles in tumorigenesis (Dejean et al. 1984; Wang et al. 1990). Further studies of larger tumor sets using polymerase chain reaction (PCR)-based approaches showed frequent insertions near or within coding regions, with recurrent targeting of the human telomerase (*TERT*) and *MLL2* genes, suggesting that HBV integrations are involved in clonal evolution of the tumors by conferring selective advantage (Murakami et al. 2005; Tamori et al. 2005).

Recently whole-genome sequencing of hundreds of samples has provided a detailed catalog

of HBV integration sites in HCCs as well as in nontumorous livers (Fujimoto et al. 2012; Jiang et al. 2012; Sung et al. 2012; Toh et al. 2013; Li et al. 2014). These studies confirmed the prevalence of viral junctions near DR1 and DR2 and widespread distribution of insertion events across all chromosomes. They also showed that HBV DNA integration preferentially targets promoters and coding regions. Accordingly, long lists (>1000) of cellular genes at HBV insertion sites are now available. Strikingly, through compilation of recent studies, evidence has been obtained that most HBV integrations targeted a unique gene with a recurrence rate of only ~10% (Li et al. 2014). The most frequent reiterative HBV integrations in HCC were found in the *TERT* (seen in almost 20% of HCCs), *MLL4* (~10%), and *CCNE1* (~5%) gene loci (Fig. 2). Moreover, transcriptomic and RNA sequencing analysis has shown that HBV integration is frequently associated with up-regulation of target gene expression and with chimeric virus–host transcripts, confirming early studies (Jiang et al. 2012; Sung et al. 2012). Overall, genes targeted by HBV integration in tumors are associated with cellular processes, such as development, differentiation, and positive regulation of transcription. In HBV-infected livers, HBV insertions also occur at a high rate, but with significant differences in the cellular insertion sites, with frequent insertions in metabolic genes, notably the fibronectin (*FN1*) gene, and preferential targeting of introns and intergenic regions (Sung et al. 2012). Thus, large-scale studies have provided evidence for a direct, *cis*-acting effect of HBV integration and reassured the *trans*-acting effects in a significant percentage of HBV-related tumors.

An important aspect is the long-lasting association of HBV integration with genomic instability. Large chromosomal alterations (deletions, duplications, and translocations) have been seen frequently at virus–host junctions, and recent genome sequencing studies have detected increased copy number variation (CNV) events near HBV integration sites (Jiang et al. 2012; Sung et al. 2012). Importantly, evaluation of the clinical correlations of HBV integration has revealed that multiple HBV integrations in the same tumor ($n \geq 3$) are associated with aggressive tumor features, such as higher levels of serum AFP, tumor occurrence at younger age, and unfavorable prognosis (Sung et al. 2012).

THE WOODCHUCK MODEL OF HEPADNAVIRUS-INDUCED TUMORIGENESIS

Hepadnaviruses infecting rodents in northern America, namely, WHV and related viruses infecting different squirrel species (GSHV, Richardson squirrel hepatitis virus (RSHV), and arctic squirrel hepatitis virus (ASHV); see Mason 2015) are closely related to HBV. They induce acute and chronic hepatitis and liver cancer in their hosts. In particular, WHV-infected woodchucks have been used as a model of viral carcinogenesis and for evaluating antiviral drugs in the management of HBV infection (Tennant et al. 2004).

The tumorigenic process in WHV-infected woodchucks appears to be quite similar to HBV-related hepatocarcinogenesis in humans, despite the absence of cirrhosis in the rodent model. When infected shortly after birth, virtually all woodchucks that become chronic WHV carriers develop HCCs, whereas no liver tumor has been detected in noninfected woodchucks (Tennant et al. 2004). Moreover, HCCs carrying integrated viral sequences occur in 17% of woodchucks that recover from acute infection (Korba et al. 1989). At preneoplastic stages, infected hepatocytes support WHV replication at high levels and carry frequent WHV DNA integrations in host chromosomes (Rogler and Summers 1984; Mason et al. 2005). Almost all woodchuck HCCs carry one or several integrated viral sequences, and the structure of WHV inserts is similar to that of integrated HBV sequences. They are made of subgenomic fragments, either linear or rearranged, with no complete genome. Chromosomal alterations are also seen in flanking cellular DNA.

Analysis of the cellular sites of WHV insertion has revealed a high rate of integrations in the Myc family genes c-*myc* and N-*myc*, resulting in overexpression of the normal Myc proteins. The predominant target sites were the

N-*myc*2 intronless retroposon and two nearby loci called *win* and *b3n* (Fig. 3) (Fourel et al. 1990, 1994; Wei et al. 1992; Bruni et al. 1999). Integrations into the N-*myc*2 gene were found preferentially in large, advanced-stage tumors, highlighting the strong oncogenic activity of N-*myc*2 (Jacob et al. 2004). The patterns of WHV DNA insertion in c-*myc* are similar to those of Moloney murine leukemia virus (MLV) in murine T-cell lymphomas (Hsu et al. 1988). *myc* is an efficient oncogene in the mouse liver and a master regulator of the early steps of human liver tumorigenesis (Kaposi-Novak et al. 2009). The direct role of Myc in woodchuck HCC was shown by the systematic development of liver tumors in transgenic mice carrying either c-*myc* or N-*myc*2 and nearby integrated WHV sequences from woodchuck tumors (Etiemble et al. 1994; Renard et al. 2000). Thus, integration of WHV DNA into *myc* genes confers a selective growth advantage on target hepatocytes, inducing the emergence of neoplastic nodules and providing a decisive step in tumor progression. Curiously, neither HBV nor GSHV integration appears to target the *myc* genes. The reasons for this specificity of WHV are unknown, but it might be responsible for the stronger oncogenicity of WHV compared with other hepadnaviruses (Popper et al. 1987).

The woodchuck transcriptome has been recently sequenced and annotated, and the expression profiles of livers and tumors from WHV-infected animals have been analyzed by custom microarrays (Fletcher et al. 2012). Interestingly, viral infection was associated with major immune-related changes indicating liver invasion by neutrophils, plasma cells, myeloid lineage cells, B and T cells, as well as markers of interferon response, T-cell exhaustion, and inhibition of cytokine signaling. These data, together with down-regulation of metabolic pathways, such as bile acid and steroid hormone metabolism, emphasize the role of inflammation and hepatic dysfunction at preneoplastic stages and suggest that similar mechanisms are involved in woodchuck and human hepatocarcinogenesis. At the tumor stage the woodchuck transcriptomic signature is highly similar to that of the "poor survival subclass" identified in human liver tumors (Lee et al. 2004), characterized by strong cell proliferation and expression of antiapoptotic genes. It is also related to the human S2 subclass (Hoshida et al. 2009), in which activation of MYC and AKT signaling was associated with elevated levels of the hepatic progenitor markers AFP and epithelial cell adhesion molecule (EpCAM) and relative suppression of IFN target genes. These data provide clues for further evaluation of HBV-related HCC and clearly establish the translational value of the woodchuck model.

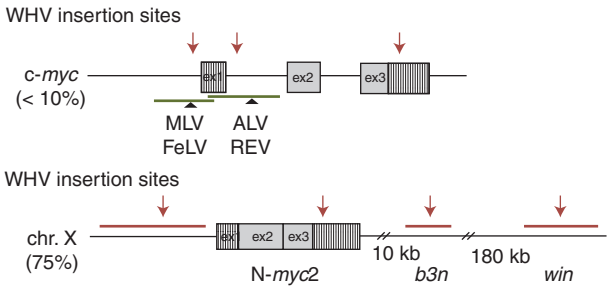

Figure 3. Insertional activation of Myc family genes in woodchuck hepatitis virus (WHV)-induced woodchuck HCCs. WHV insertion sites into the c-*myc* and N-*myc*2 loci are shown with arrows, and preferred integration sites of retroviruses including murine leukemia virus (MLV), feline leukemia virus (FeLV), avian leukemia virus (ALV), and avian reticuloendotheliosis virus (REV) are mapped under c-*myc*. The *b3n* and *win* loci are located 10 and 180 kb downstream from N-*myc*2 on the woodchuck X chromosome, and viral integration in these loci leads to N-*myc*2 activation. Percentages of WHV integration at each locus in a panel of 70 woodchuck tumors analyzed are shown on the *left*. chr., chromosome.

ONCOGENIC POTENTIAL OF THE HBx TRANSACTIVATOR

Among HBV proteins, HBx has been termed "viral oncoprotein" because of its pleiotropic activities on cell cycle regulation, signaling pathways, and DNA repair (Bouchard and Schneider 2004; Tang et al. 2006; Benhenda et al. 2009; Slagle and Bouchard 2015). Evidence for the oncogenic potential of HBx has been obtained in transgenic mice. Liver tumors were described in a transgenic mouse line with high-level expression of HBx in the liver, generated in the outbred CD-1 background (Kim et al. 1991) and in HBx knockin transgenic lines generated by homologous recombination into the p21 locus (Wang et al. 2004). In other transgenic mice, expression of HBx by itself did not lead to HCC development, but it cooperated with diethyl nitrosamine (DEN) and with c-Myc or insulin receptor substrate-1 (IRS-1) transgenes by increasing premalignant liver lesions and accelerating tumor onset (Slagle et al. 1996; Terradillos et al. 1997; Longato et al. 2009). Thus, in these models HBx does not appear to behave as a dominant oncogene but rather as a cofactor during hepatocarcinogenesis.

An essential role of HBx in the viral life cycle is to activate HBV transcription. This activity involves multiple interactions with cellular partners and might be responsible for the wide range of functions attributed to HBx, as for other viral transactivators (Neuveut et al. 2010). The transactivation of cellular oncogenes and growth factors leading to aberrant oncogenic signaling and the deregulation of cell cycle progression are two mechanisms that might account for the weak oncogenicity of HBx. In particular, the interactions of HBx with DNA damage-binding protein 1 (DDB1), HBX-interacting protein (HBXIP), or Polo-like kinase 1 (Plk-1) have been implicated in disruption of the mitotic cell cycle or DNA repair mechanisms, thereby affecting genetic stability (Martin-Lluesma et al. 2008; Wen et al. 2008; Studach et al. 2010). Taken together, these studies and many others provide a strong link between HBx expression and chromosomal instability in HBV-related carcinogenesis.

CONCLUSIONS

Like other human tumor viruses, HBV does not seem to be fully oncogenic. It provides a subset of the necessary oncogenic steps during the tumorigenic process, whereas other steps are provided by host and environmental cofactors, such as chronic inflammation, oxidative liver damage, tumor microenvironment, cirrhosis, gut microbiota, and exposure to dietary hepatocarcinogens (Hernandez-Gea et al. 2013; Shlomai et al. 2014). The combination of direct and indirect oncogenic effects of HBV is far from being understood. A wealth of information produced recently by new sequencing technologies has started to unveil the complex pattern of genetic alterations in HCC and provided preliminary evidence for specific abnormalities and pathways in HCCs with different disease etiologies. In particular, with a widely expanding list of cellular target sites for viral insertion, HBV DNA integration has been directly involved in malignant transformation in a large number of cases. Similarly, a variety of functions have been assigned to the viral transactivator HBx, which does not transform infected cells but appears to hijack several cellular control processes important for cancer development. Deciphering the complex regulation of the HBV life cycle in infected hepatocytes will provide new targets for preventing cancer development in chronically infected patients and tumor recurrence after surgical resection of HBV-related HCCs. Finally, data collected from genomic studies hold promise for translational medicine.

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