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Review

Molecular Aspects of Hepatitis B Viral Infection and the Viral Carcinogenesis

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Of many viral causes of human cancer, few are of greater global importance than the hepatitis B virus (HBV). Over 250 million people worldwide are persistently infected with HBV. A significant minority of these develop severe pathologic consequences, including chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). Earlier epidemiological evidence suggested a link between chronic HBV infection and HCC. Further, the existence of related animal viruses that induce acute and chronic infections of the liver, and eventually HCC, confirms the concept that HBV belongs to one of the few human oncogenic viruses. Although it is clear that chronic HBV infections are major risk factors, relatively little is understood about how the viral factors contribute to hepatocarcinogenesis. This review will introduce molecular aspects of the viral infection, and highlight recent findings on the viral contribution to hepatocarcinogenesis.

Keywords: Hepatitis B virus, Hepatocarcinogenesis, Hepatocellular carcinoma

Introduction

In many parts of the world, primary hepatocellular carcinoma (HCC) is one of the most common cancers. In the HBV-endemic area, the majority of HCCs arise in patients who are HBV carriers; even though carriers may represent 5%-10% of the population, they represent 50% to 80% of HCC in those countries (Ganem, 1982). However, stronger evidence for an association comes from careful prospective studies that were done in Taiwan. In this work, large numbers of carriers and non-carriers were followed (Beasley *et al*., 1981). These studies demonstrated that chronic HBV carriers are associated with a 100-fold increase in the risk for HCC development relative to noncarriers. This striking number in cancer risk makes HBV one of the most important environmental risk factors in human cancer epidemiology.

Although earlier epidemiological evidence suggested a link between chronic HBV infection and HCC, how the HBV infection leads to hepatocarcinogenesis was not well established (Buendia, 1992). For many years, studies on viral replication were hampered by a number of experimental limitations. Those limitations were the narrow host range of the virus, which precluded transmission to convenient animal hosts, and the apparent absence of cell lines that supported infection by virus particles. However, progress in understanding the molecular basis of viral replication became possible with the advent of molecular techniques and the discovery of natural animal models of viral infection (Buendia, 1992; Ganem, 1996; Ganem and Varmus, 1987; Nassal, 1996; Nassal and Schaller, 1996). The purpose of this review is to introduce recent molecular discoveries about the hepadnaviral infection and its long-term consequence to its host, HCC.

Hepadnavirus Genomes

The hepadnaviral genome, isolated from infectious virions, is a partial duplex circular genome whose circularity is maintained by 5' cohesive ends (Fig. 1). The genome has an unusual structure in that its two DNA strands are not symmetrical. The viral minus-strand is unit length and has a protein that is covalently linked to its 5' end, which is later identified to be P ORF product, the viral polymerase (Bosch *et al*., 1988; Wang and Seeger, 1992). By contrast, the plus-strand is less than unit length and bears capped oligoribonucleotides at its 5' end. Importantly, the position of the 5' ends of both strands map to the regions of short (11 nucleotide) direct repeats (DRs) in viral DNA. The 5' end of the minus-strand DNA maps within the repeat (termed DR1), while the plus-strand DNA begins within DR2. As will be

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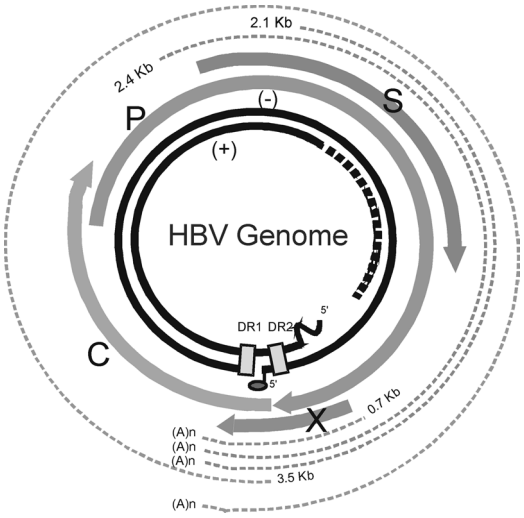

Fig. 1. Genetic organization of hepatitis B virus genome. Inner circle represents virion DNA with dashes denoting the single-stranded gap region. Four open-reading frames are indicated by open arrows: C (core), P (viral polymerase), S (surface glycoprotein), and X (HBx). The outermost dashed lines depict the viral RNAs that are found in the infected cells. DR1 and DR2 are two directly-repeated sequences of 11 bp at the 5' extremities of the minus- and plus-strand DNA.

discussed later, these repeats serve as a donor or acceptor sites in the template switch events during viral genome synthesis (Ganem and Varmus, 1987; Havert and Loeb, 1997).

Molecular cloning of HBV DNA that is extracted from Dane particles, infectious virion particles, revealed a coding organization that is highly compact (i.e., every nucleotide in the genome is within a coding region.) As shown in Fig. 1, 4-open-reading frames (ORF) are present in the genome (Ganem, 1996). The viral DNA polymerase that is encoded by the P ORF has reverse transcriptase activity. The C (core) ORF encodes the structural protein of the nucleocapsid, while ORF S encodes the viral surface glycoproteins. Lastly, ORF X encodes a viral regulatory protein, HBx, which is best characterized as a transcriptional transactivator (Murakami, 2001).

Infection Cycle

The peculiar asymmetries of virion DNA provided early clues that a mechanism other than semi-conservative DNA synthesis is involved in the hepadnaviral genome replication. In 1982, Summers and Mason reported a seminal discovery that viral DNA replication proceeds by reverse transcription of an RNA intermediate (Summers and Mason, 1982). Current understanding of the infection cycle follows. Following receptor binding, virions deliver their nucleocapsids to the cytoplasm. These then translocate to the nucleus, where their

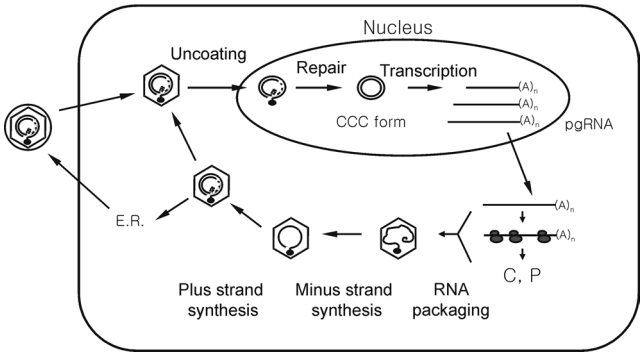

Fig. 2. Infection cycle of hepadnaviruses. See text for details.

genomic DNA is matured to the covalently-closed circular (ccc) DNA form. Then, cccDNA becomes a template for transcription of the pregenomic RNA (pgRNA) and subgenomic RNAs by the host RNA polymerase II. The pgRNA serves as a template for reverse transcription, as well as for the translation of the C and P proteins (Fig. 2). Viral pgRNAs are selectively encapsidated within the core particles in the cytoplasm, together with the P gene product (Junker-Niepmann *et al*., 1990; Hirsch *et al*., 1991; Jeong *et al*., 2000). Within this structure, viral DNA synthesis is initiated; following minus-strand synthesis, plus-strand DNA synthesis occurs. Although they have a DNA genome, hepadnaviruses replicate through reverse transcription of RNA intermediate, resulting in a relaxed circular DNA genome (Summers and Mason, 1982). Upon completion of genomic DNA synthesis, progeny cores bud into intracellular membranes to acquire their glycoprotein envelope. Enveloped virions are then secreted via the constitutive pathway of the vesicular transport.

Hepadnavirus Family and Animal Models

It is now clear that HBV is one member of a family of related viruses that are now known as hepadnaviruses (for *hepa*totropic *DNA* viruses). The first of the nonhuman hepadnaviruses to be discovered was the woodchuck hepatitis virus (WHV). A series of similar viruses have now been discovered from a variety of animal species, including the Pekin duck (duck hepatitis B virus or DHBV) (Ganem and Varmus, 1987) and Beechey ground squirrels (ground squirrel hepatitis virus or GSHV) (Ganem *et al*., 1982). The host range of the animal hepadnaviruses is narrow. In most cases, the transmission of virus to hosts that are closely related to the natural host species has been demonstrated. Considerable interest has been paid to WHV. This is because: (i) genome shares approximately a 60% nucleotide sequence identity with its human counterpart, and (ii) all of the WHV carrier woodchucks succumb to HCC after 2-4 years (Popper *et al*., 1981; Popper *et al*., 1987).

Hepadnaviral Infection and HCC

Besides epidemiological evidence, further support for the oncogenic potential of hepadnavirus infection came with the discovery of the animal hepadnaviruses. In retrospect, animal models of hepadnaviruses, including WHV and GSHV, have been invaluable to further substantiate the link between the HBV infection and HCC (Buendia, 1992). Studies of the WHV infection showed that this virus is even more potent than HBV as a hepatic carcinogen. Nearly 100% of woodchucks that are infected from birth with WHV will develop HCC, starting at around 18 to 24 months of age (Popper *et al*., 1981; Popper *et al*., 1987). Further, ground squirrels that are infected with GSHV will also display an increased prevalence of HCC, though the tumors are less frequent and arise later in life (Marion *et al*., 1986).

An obvious question that is raised by these observations concerns the role of the virus in liver cancer. The first clue came from the discovery of integrated viral DNA in HCC and HCC-derived cell lines (Brechot *et al*., 1980; Marion *et al*., 1980). The HBV integrants are usually highly rearranged with deletions, inversions, and sequence reiterations that are all commonly observed. In addition, alterations of host DNA often accompany these integrations (Rogler *et al*., 1985; Yaginuma *et al*., 1985). However, unlike retroviruses, chromosomal integration is not an obligatory part of the hepadnaviral infection cycle. Nonetheless, by analogy to the retroviral counterpart, it was speculated that the viral integration might lead to insertional mutagenesis. Importantly, tumors are clonal with respect to these integrants. This implies that the integration event(s) accompanied or preceded the clonal expansion of the cells, which suggests that the viral integration is not the "cause", but the "consequence" (Brechot *et al*., 2000).

In contrast, the results that were obtained from the woodchuck model supported the insertional mutagenesis hypothesis. It was found that WHV sequences are present at or near N-myc2, a psuedogene of N-myc, in almost every liver tumor that arose in WHV-infected animals. In almost all of the cases that have been examined, the integration of WHV results in the activation of the transcription of the N-myc2 locus (Fourel *et al*., 1990; Fourel *et al*., 1994). Similar efforts with a tumor that was obtained from HBV-infected patients revealed no common cellular target for HBV integration. Therefore, the insertional mutagenesis that was observed with woodchuck models cannot be generalized beyond the woodchuck models.

Oncogenic Properties of Viral Proteins: HBx

Despite compelling epidemiological evidence, to what extent the viral gene products contribute to hepatocarcinogenesis is still in question. Among the viral gene products, considerable attention was given to HBx (Yen, 1996; Murakami, 2001). It was noted that three mammalian hepadnaviruses share a regulatory gene (termed X); whereas, no counterpart of this gene is found in the distantly-related nononcogenic duck hepatitis B virus (DHBV) (Ganem and Varmus, 1987). HBx, a 154 amino acid gene product of the X gene, is known as a promiscuous transcriptional transactivator, since it is capable of transactivating the gene expression by acting on a wide range of viral and cellular regulatory elements (Yen, 1996). It was, therefore, suggested that HBx might play a role in liver carcinogenesis. Until recently, its role during the infection cycle remained elusive, since HBx has been shown to be dispensable for viral genome replication in tissue cultures (Blum *et al*., 1992). Subsequently, the requirement of the HBx gene product for productive viral infection has been firmly established in the woodchuck model (Chen *et al*., 1993; Zoulim *et al*., 1994). Therefore, HBx is an essential viral protein during the viral infection cycle *in vivo*, although its specific function pertaining to the infection cycle is unknown.

On the other hand, the transcriptional transactivator properties of HBx have been demonstrated on a variety of viral and cellular regulatory elements (Yen, 1996). HBx does not directly bind to DNA, and may stimulate transcription by interacting with transcription factors or with the basal transcription machinery of host RNA polymerase II and III (Murakami, 2001). Besides its nuclear function as a transcriptional transactivator, several studies indicated that HBx influences the cellular signaling pathways in the cytoplasm as well, which is a function that is consistent with the predominant cytoplasmic localization of HBx *in vivo* and in most experimental systems (Benn and Schneider, 1994; Natoli *et al*., 1994). Several studies indicated that HBx influences cellular signaling pathways. It was reported that HBx activates src (Klein and Schneider, 1997) and the ras/raf/ERK pathway, which leads to transcriptional transactivation and the stimulation of proliferation in quiescent cells (Natoli *et al*., 1994; Benn *et al*., 1996). In addition, HBx activates NF-kB (Lucito and Schneider, 1992; Chirillo *et al*., 1996; Su and Schneider, 1996). Recently, Bouchard *et al.* reported that HBx enhances HBV replication 5- to 10-fold in transfected HepG2 cells by triggering the cytosolic Ca^{2+} release, which in turn activated the proline-rich tyrosine kinase 2 (Pyk2) (Bouchard *et al*., 2001). Then, the activated Pyk2 activates Src kinase. They provided compelling evidence that indicated the role of HBx in context of the viral genome replication. It should be interest to identify the vial targets that respond to the Pyk2 and Src activation.

Although the pleiotropic functions of HBx were illustrated, it is unclear which functions directly contribute to the viral carcinogenesis. It is relevant that HBx has been implicated in both the deregulation of the cell cycle control or cell proliferation (Koike *et al*., 1994b; Benn and Schneider, 1995), as well as the apoptosis (Chirillo *et al*., 1997; Kim *et al*., 1998). Apoptosis normally eliminates the cells with damaged DNA; that is, the ones that are most likely to engender a neoplastic clone. The proapoptotic activity of HBx may

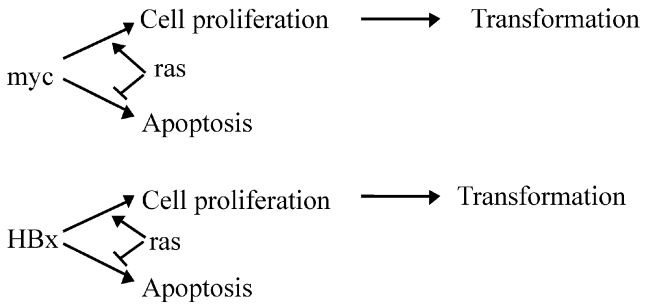

Fig. 3. Our working hypothesis to explain the role of HBx in multistep oncogenesis (Kim *et al*., 2001).

contribute to viral oncogenesis by exerting a selective pressure that favors the emergence of mutated cells. Collectively, HBx has pleiotropic activities that might involve in viral carcinogenesis.

Numerous attempts have been made to examine the oncogenic potential of HBx in cell cultures (Shirakata *et al*., 1989; Hohne *et al*., 1990; Oguey *et al*., 1996; Tarn *et al*., 1999). However, its transforming ability was barely measurable, only when the cells were immortalized by other oncogenes, such as the SV40 T-antigen (Hohne *et al*., 1990). Further, most of the transgenic mice that harbored the HBx gene had no serious liver diseases or tumors (Koike *et al*., 1994a; Guidotti *et al*., 1995). Only in certain transgenic lineages of the CD-1 strain, where HBx was expressed at high levels, did HBx weakly promote tumorigenesis (Kim *et al*., 1991). In addition, HBx was shown to potentate c-myc induced liver oncogenesis in transgenic mice (Terradillos *et al*., 1997). Data obtained from these transgenic studies suggested that HBx had no acute transforming activity, but its overexpression in a certain genetic background might induce tumor formation, possibly in collaboration with activated cellular oncogene(s) in the multistage transformation. Although it is generally believed that HBx is a cofactor in hepatocarcinogenesis, there is still no direct evidence for the oncogenic contribution of HBx. Recently, we demonstrated that H-ras collaborates with HBx to transform cells by suppressing the HBx-mediated apoptosis (Kim *et al*., 2001). We hypothesized that HBx could contribute to the neoplastic transformation in collaboration with other oncogenes, such as H-ras, that renders cells to counteract the HBx-mediated apoptosis (Fig. 3). This report not only firmly establishes the oncogenic activity of HBx, but also defines its role during the multistage carcinogenesis.

Perspectives

During the past two decades, the major principles of hepadnavirus infections have been resolved (Seeger and Mason, 2000). The transfection system that is capable of producing the infectious viral particle has allowed investigators to examine the mechanism of viral genome replication. In addition, the course of viral infections *in vivo* has been characterized by using animal models. In spite of these accomplishments, many important questions are still unanswered

On the level of viral replication, two major issues are still unknown. First, the viral receptor has not been identified. It is generally believed that the viral receptor is a major determinant for the observed tissue tropism of hepadnaviruses. Using the biochemical approach, a novel carboxypeptidase was identified as a potential DHV receptor (Kuroki *et al*., 1994; Kuroki *et al*., 1995). However, the observation that the ectopic expression of the carboxypeptidase did not render cells susceptible to the DHBV infection suggested that additional factors control the viral infection (Tong *et al*., 1995; Urban *et al*., 1998). Secondly, the cis-acting sequences for the viral genome replication are not fully characterized. Although the donor and acceptor sites that are used for the template switches during the hepadnaviral DNA synthesis have been identified (i.e., epsilon, DR1 and DR2), it is largely unknown whether other cis-acting sequences (apart from the donor and acceptor sites) might be involved in the template switches during the hepadnaviral DNA synthesis. Recently, three additional cis-acting elements (named 3E, M, and 5E) were identified in DHBV, which when deleted, abrogates the plus-strand DNA synthesis (Havert and Loeb, 1997). In contrast, it is unknown whether such cis-acting elements exist for HBV genome replication. Complete mapping of the cis-acting element will facilitate in designing a novel HBV-derived liver-targeting vector. Such recombinant viral vectors have been essential for the unambiguous identification of retroviral receptors, including those of the Moloney murine leukemia virus, avail leukosis virus, and human immunodeficiency virus (Dalgleish *et al*., 1984; Albritton *et al*., 1989; Young *et al*., 1993). Further, the HBV vector will be a novel hepatotropic gene therapy vector to treat genetic disease, as well as liver diseases (Protzer *et al*., 1999).

Regarding viral carcinogenesis, the specific contribution of HBx to oncogenesis is still largely unknown. Recent evidence for HBx's role in triggering the cytosolic Ca^{2+} release needs to be further explored in context to oncogenesis as follows (Bouchard *et al*., 2001): (i) By what mechanism does HBx trigger the Ca^{2+} release? (ii) What are the consequences of the Ca^{2+} release to the host cell? In addition, the genetic dissection of the HBx polypeptide will take us one step closer to closure of the X files (Ganem, 2001; Nassal, 2002). A molecular analysis of these unresolved issues should provide insights into which new therapeutic targets could be identified to cure the enormous number of patients with chronic HBV infections.

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References

Albritton, L. M., Tseng, L., Scadden, D. and Cunningham, J. M. (1989) A putative murine ecotropic retrovirus receptor gene encodes a multiple membrane-spanning protein and confers susceptibility to virus infection. *Cell* **57**, 659-666.

Beasley, R. P., Hwang, L. Y., Lin, C. C. and Chien, C. S. (1981) Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22 707 men in Taiwan. *Lancet* **2**, 1129-1133.

Benn, J. and Schneider, R. J. (1994) Hepatitis B virus HBx protein activates Ras-GTP complex formation and establishes a Ras, Raf, MAP kinase signaling cascade. *Proc. Natl. Acad. Sci. USA* **91**, 10350-10354.

Benn, J. and Schneider, R. J. (1995) Hepatitis B virus HBx protein deregulates cell cycle checkpoint controls. *Proc. Natl. Acad. Sci. USA* **92**, 11215-11219.

Benn, J., Su, F., Doria, M. and Schneider, R. J. (1996) Hepatitis B virus HBx protein induces transcription factor AP-1 by activation of extracellular signal-regulated and c-Jun N-terminal mitogen-activated protein kinases. *J. Virol.* **70**, 4978-4985.

Blum, H. E., Zhang, Z. S., Galun, E., von Weizsacker, F., Garner, B., Liang, T. J. and Wands, J. R. (1992) Hepatitis B virus X protein is not central to the viral life cycle *in vitro*. *J. Virol.* **66**, 1223-1227.

Bosch, V., Bartenschlager, R., Radziwill, G. and Schaller, H. (1988) The duck hepatitis B virus P-gene codes for protein strongly associated with the 5'-end of the viral DNA minus strand. *Virology* **166**, 475-485.

Bouchard, M. J., Wang, L. H. and Schneider, R. J. (2001) Calcium signaling by HBx protein in hepatitis B virus DNA replication. *Science* **294**, 2376-2378.

Brechot, C., Gozuacik, D., Murakami, Y. and Paterlini-Brechot, P. (2000) Molecular bases for the development of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC). *Semin. Cancer Biol.* **10**, 211-231.

Brechot, C., Pourcel, C., Louise, A., Rain, B. and Tiollais, P. (1980) Presence of integrated hepatitis B virus DNA sequences in cellular DNA of human hepatocellular carcinoma. *Nature* **286**, 533-535.

Buendia, M. A. (1992) Hepatitis B viruses and hepatocellular carcinoma. *Adv. Cancer Res.* **59**, 167-226.

Chen, H. S., Kaneko, S., Girones, R., Anderson, R. W., Hornbuckle, W. E., Tennant, B. C., Cote, P. J., Gerin, J. L., Purcell, R. H. and Miller, R. H. (1993) The woodchuck hepatitis virus X gene is important for establishment of virus infection in woodchucks. *J. Virol.* **67**, 1218-1226.

Chirillo, P., Falco, M., Puri, P. L., Artini, M., Balsano, C., Levrero, M. and Natoli, G. (1996) Hepatitis B virus pX activates NF-kappa B-dependent transcription through a Raf-independent pathway. *J. Virol.* **70**, 641-646.

Chirillo, P., Pagano, S., Natoli, G., Puri, P. L., Burgio, V. L., Balsano, C. and Levrero, M. (1997) The hepatitis B virus X gene induces p53-mediated programmed cell death. *Proc. Natl. Acad. Sci. USA* **94**, 8162-8167.

Dalgleish, A. G., Beverley, P. C., Clapham, P. R., Crawford, D. H., Greaves, M. F. and Weiss, R. A. (1984) The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature* **312**, 763-767.

Fourel, G., Couturier, J., Wei, Y., Apiou, F., Tiollais, P. and Buendia, M. A. (1994) Evidence for long-range oncogene activation by hepadnavirus insertion. *EMBO J.* **13**, 2526-2534.

Fourel, G., Trepo, C., Bougueleret, L., Henglein, B., Ponzetto, A., Tiollais, P. and Buendia, M. A. (1990) Frequent activation of N-myc genes by hepadnavirus insertion in woodchuck liver tumors. *Nature* **347**, 294-298.

Ganem, D. (1982) Persistent infection of humans with hepatitis B virus: mechanisms and consequences. *Rev. Infect. Dis.* **4**, 1026-1047.

Ganem, D. *Hepadnaviridae and Their Replication*, 3rd ed. Lippincott-Raven Publishers, Philadelphia.

Ganem, D. (2001) Virology. The X files--one step closer to closure. *Science* **294**, 2299-2300.

Ganem, D., Greenbaum, L. and Varmus, H. E. (1982) Virion DNA of ground squirrel hepatitis virus: structural analysis and molecular cloning. *J. Virol.* **44**, 374-383.

Ganem, D. and Varmus, H. E. (1987) The molecular biology of the hepatitis B viruses. *Annu. Rev. Biochem.* **56**, 651-693.

Guidotti, L. G., Matzke, B., Schaller, H. and Chisari, F. V. (1995) High-level hepatitis B virus replication in transgenic mice. *J. Virol.* **69**, 6158-6169.

Havert, M. B. and Loeb, D. D. (1997) cis-Acting sequences in addition to donor and acceptor sites are required for template switching during synthesis of plus-strand DNA for duck hepatitis B virus. *J. Virol.* **71**, 5336-5344.

Hirsch, R. C., Loeb, D. D., Pollack, J. R. and Ganem, D. (1991) cis-acting sequences required for encapsidation of duck hepatitis B virus pregenomic RNA. *J. Virol.* **65**, 3309-3316.

Hohne, M., Schaefer, S., Seifer, M., Feitelson, M. A., Paul, D. and Gerlich, W. H. (1990) Malignant transformation of immortalized transgenic hepatocytes after transfection with hepatitis B virus DNA. *EMBO J.* **9**, 1137-1145.

Jeong, J. K., Yoon, G. S. and Ryu, W. S. (2000) Evidence that the 5'-end cap structure is essential for encapsidation of hepatitis B virus pregenomic RNA. *J. Virol.* **74**, 5502-5508.

Junker-Niepmann, M., Bartenschlager, R. and Schaller, H. (1990) A short cis-acting sequence is required for hepatitis B virus pregenome encapsidation and sufficient for packaging of foreign RNA. *EMBO J.* **9**, 3389-3396.

Kim, C. M., Koike, K., Saito, I., Miyamura, T. and Jay, G. (1991) HBx gene of hepatitis B virus induces liver cancer in transgenic mice. *Nature* **351**, 317-320.

Kim, H., Lee, H. and Yun, Y. (1998) X-gene product of hepatitis B virus induces apoptosis in liver cells. *J. Biol. Chem.* **273**, 381-385.

Kim, Y. C., Song, K. S., Yoon, G., Nam, M. J. and Ryu, W. S. (2001) Activated ras oncogene collaborates with HBx gene of hepatitis B virus to transform cells by suppressing HBx-mediated apoptosis. *Oncogene* **20**, 16-23.

Klein, N. P. and Schneider, R. J. (1997) Activation of Src family kinases by hepatitis B virus HBx protein and coupled signaling to Ras. *Mol. Cell. Biol.* **17**, 6427-6436.

Koike, K., Moriya, K., Iino, S., Yotsuyanagi, H., Endo, Y., Miyamura, T. and Kurokawa, K. (1994a) High-level expression of hepatitis B virus HBx gene and hepatocarcinogenesis in transgenic mice. *Hepatology* **19**, 810-819.

Koike, K., Moriya, K., Yotsuyanagi, H., Iino, S. and Kurokawa, K. (1994b) Induction of cell cycle progression by hepatitis B virus HBx gene expression in quiescent mouse fibroblasts. *J. Clin. Invest.* **94**, 44-49.

Kuroki, K., Cheung, R., Marion, P. L. and Ganem, D. (1994) A

cell surface protein that binds avian hepatitis B virus particles. *J. Virol.* **68**, 2091-2096.

Kuroki, K., Eng, F., Ishikawa, T., Turck, C., Harada, F. and Ganem, D. (1995) gp180, a host cell glycoprotein that binds duck hepatitis B virus particles, is encoded by a member of the carboxypeptidase gene family. *J. Biol. Chem.* **270**, 15022-15028.

Lucito, R. and Schneider, R. J. (1992) Hepatitis B virus X protein activates transcription factor NF-kappa B without a requirement for protein kinase C. *J Virol.* **66**, 983-991.

Marion, P. L., Salazar, F. H., Alexander, J. J. and Robinson, W. S. (1980) State of hepatitis B viral DNA in a human hepatoma cell line. *J. Virol.* **33**, 795-806.

Marion, P. L., Van Davelaar, M. J., Knight, S. S., Salazar, F. H., Garcia, G., Popper, H. and Robinson, W. S. (1986) Hepatocellular carcinoma in ground squirrels persistently infected with ground squirrel hepatitis virus. *Proc. Natl. Acad. Sci. USA* **83**, 4543-4546.

Murakami, S. (2001) Hepatitis B virus X protein: a multifunctional viral regulator. *J Gastroenterol.* **36**, 651-660.

Nassal, M. (1996) Hepatitis B virus morphogenesis. *Curr. Top. Microbiol. Immunol.* **214**, 297-337.

Nassal, M. (2002) Ca^{2+}: the clue to hepatitis B virus X protein function? *Hepatology* **36**, 755-757.

Nassal, M. and Schaller, H. (1996) Hepatitis B virus replication--an update. *J. Viral. Hepat.* **3**, 217-226.

Natoli, G., Avantaggiati, M. L., Chirillo, P., Puri, P. L., Ianni, A., Balsano, C. and Levrero, M. (1994) Ras- and Raf-dependent activation of c-jun transcriptional activity by the hepatitis B virus transactivator pX. *Oncogene* **9**, 2837-2843.

Oguey, D., Dumenco, L. L., Pierce, R. H. and Fausto, N. (1996) Analysis of the tumorigenicity of the X gene of hepatitis B virus in a non-transformed hepatocyte cell line and the effects of co-transfection with a murine p53 mutant equivalent to human codon 249. *Hepatology* **24**, 1024-1033.

Popper, H., Roth, L., Purcell, R. H., Tennant, B. C. and Gerin, J. L. (1987) Hepatocarcinogenicity of the woodchuck hepatitis virus. *Proc. Natl. Acad. Sci. USA* **84**, 866-870.

Popper, H., Shih, J. W., Gerin, J. L., Wong, D. C., Hoyer, B. H., London, W. T., Sly, D. L. and Purcell, R. H. (1981) Woodchuck hepatitis and hepatocellular carcinoma: correlation of histologic with virologic observations. *Hepatology* **1**, 91-98.

Protzer, U., Nassal, M., Chiang, P. W., Kirschfink, M. and Schaller, H. (1999) Interferon gene transfer by a hepatitis B virus vector efficiently suppresses wild-type virus infection. *Proc. Natl. Acad. Sci. USA* **96**, 10818-10823.

Rogler, C. E., Sherman, M., Su, C. Y., Shafritz, D. A., Summers, J., Shows, T. B., Henderson, A. and Kew, M. (1985) Deletion in chromosome 11p associated with a hepatitis B integration site in hepatocellular carcinoma. *Science* **230**, 319-322.

Seeger, C. and Mason, W. S. (2000) Hepatitis B virus biology. *Microbiol. Mol. Biol. Rev.* **64**, 51-68.

Shirakata, Y., Kawada, M., Fujiki, Y., Sano, H., Oda, M., Yaginuma, K., Kobayashi, M. and Koike, K. (1989) The X gene of hepatitis B virus induced growth stimulation and tumorigenic transformation of mouse NIH3T3 cells. *Jpn. J. Cancer. Res.* **80**, 617-621.

Su, F. and Schneider, R. J. (1996) Hepatitis B virus HBx protein activates transcription factor NF-kappaB by acting on multiple cytoplasmic inhibitors of rel-related proteins. *J. Virol.* **70**, 4558-4566.

Summers, J. and Mason, W. S. (1982) Replication of the genome of a hepatitis B--like virus by reverse transcription of an RNA intermediate. *Cell* **29**, 403-415.

Tarn, C., Bilodeau, M. L., Hullinger, R. L. and Andrisani, O. M. (1999) Differential immediate early gene expression in conditional hepatitis B virus pX-transforming versus nontransforming hepatocyte cell lines. *J. Biol. Chem.* **274**, 2327-2336.

Terradillos, O., Billet, O., Renard, C. A., Levy, R., Molina, T., Briand, P. and Buendia, M. A. (1997) The hepatitis B virus X gene potentates c-myc-induced liver oncogenesis in transgenic mice. *Oncogene* **14**, 395-404.

Tong, S., Li, J. and Wands, J. R. (1995) Interaction between duck hepatitis B virus and a 170-kilodalton cellular protein is mediated through a neutralizing epitope of the pre- S region and occurs during viral infection. *J. Virol.* **69**, 7106-7112.

Urban, S., Breiner, K. M., Fehler, F., Klingmuller, U. and Schaller, H. (1998) Avian hepatitis B virus infection is initiated by the interaction of a distinct pre-S subdomain with the cellular receptor gp180. *J. Virol.* **72**, 8089-8097.

Wang, G. H. and Seeger, C. (1992) The reverse transcriptase of hepatitis B virus acts as a protein primer for viral DNA synthesis. *Cell* **71**, 663-670.

Yaginuma, K., Kobayashi, M., Yoshida, E. and Koike, K. (1985) Hepatitis B virus integration in hepatocellular carcinoma DNA: duplication of cellular flanking sequences at the integration site. *Proc. Natl. Acad. Sci. USA* **82**, 4458-4462.

Yen, T. S. B. (1996) Hepadnaviral X protein: review of recent progress. *J. Biomed. Sci.* **3**, 20-30.

Young, J. A., Bates, P. and Varmus, H. E. (1993) Isolation of a chicken gene that confers susceptibility to infection by subgroup A avian leukosis and sarcoma viruses. *J. Virol.* **67**, 1811-1816.

Zoulim, F., Saputelli, J. and Seeger, C. (1994) Woodchuck hepatitis virus X protein is required for viral infection *in vivo*. *J. Virol.* **68**, 2026-2030.