Viral pathogenesis of hepatocellular carcinoma

HUBERT E BLUM AND DARIUS MORADPOUR

Department of Medicine II, University of Freiburg, Freiburg, Germany

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common tumors in the world with an estimated 500 000–1000 000 new cases per year. Although less frequent in the USA and Europe, these tumors have an annual incidence of up to 500 cases per 100 000 population in certain regions of Asia and sub-Saharan Africa. The reasons for this high incidence are chronic infections with the hepatitis B virus (HBV) or the hepatitis C virus (HCV) as well as HBV–HCV co-infections. The clinical course of HBV and HCV infection depends in part on molecular characteristics of the viruses, in part on the patients’ HLA haplotype, and in part on other coexisting risk factors. Well-recognized non-viral exogenous agents associated with the pathogenesis of HCC are alcohol and aflatoxins. In the West, alcohol-induced liver injury is a leading cause of liver cirrhosis and the most important HCC risk factor. In southern China and Africa, dietary ingestion of high levels of aflatoxin B1 may represent a special environmental hazard, particularly in chronic HBV carriers. Other exogenous factors have also been incriminated and include dietary iron overload, long-term use of oral contraceptives and high-dose anabolic steroids. The development of hepatic cirrhosis, particularly in association with genetic diseases, such as α-1-antitrypsin deficiency or hemochromatosis, place the individual at a greatly increased risk for the malignant transformation of hepatocytes. In the following, the role of HBV and HCV in the pathogenesis of HCC and strategies aimed at the prevention of HCC will be discussed.

HEPATITIS B VIRUS AND HEPATITIS B VIRUS-INDUCED HEPATOCELLULAR CARCINOMA

Hepatitis B virus is a small DNA virus that belongs to the family of hepadnaviruses which includes the woodchuck hepatitis virus (WHV), ground squirrel hepatitis virus (GSHV), Pekin duck hepatitis B virus (DHBV) and heron hepatitis B virus (HHBV). The viral genome consists of a partially double-stranded DNA molecule of about 3200 nucleotides. The genetic organization of the viral genome and the viral proteins are known in

Correspondence: HE Blum, Department of Medicine II, University of Freiburg, Hugstetter Strasse 55, D-79106 Freiburg, Germany. Email: heblum@ukl.uni-freiburg.de
Presented at the INASL-EASL Meeting, New Delhi, 31 March–2 April, 2002.
© 2002 Blackwell Publishing Asia Pty Ltd
Hepatitis B virus DNA integration

Although HBV and retroviruses share a replication strategy that includes the reverse transcription of a RNA intermediate, viral integration into the host genome is not part of the HBV life cycle but rather occurs as an epiphenomenon of HBV replication. Integrated HBV sequences have been detected in patients with acute and chronic hepatitis B. Epidemiologic studies have convincingly shown that HCC development is closely associated with chronic HBV infection. The incidence of HCC in chronically HBV-infected individuals is approximately 100-fold higher than in the uninfected population and the lifetime HCC risk of men infected at birth is estimated to approach 40%. Importantly, a recent study from Taiwan demonstrated a decline in the incidence of HCC in children after implementation of a universal hepatitis B vaccination program.

A common molecular mechanism for HBV-induced hepatocarcinogenesis has not been discovered thus far, and both viral and host factors have been implicated in the process. On the one hand, most cases of HCC occur after many years of chronic hepatitis, which could provide the mitogenic and mutagenic environment to precipitate random genetic and chromosomal damage and lead to the development of HCC. In this context, in a transgenic mouse model it was recently shown that chronic immune-mediated liver cell injury is sufficient to cause HCC. On the other hand, several lines of evidence support a more direct oncogenic contribution of HBV to HCC development.

**Hepatitis B virus DNA integration**

Great detail. There are five HBV genotypes and numerous HBV mutants as well as HBV quasispecies which have been detected in patients with acute and chronic hepatitis B.

Epidemiologic studies have convincingly shown that HCC development is closely associated with chronic HBV infection. The incidence of HCC in chronically HBV-infected individuals is approximately 100-fold higher than in the uninfected population and the lifetime HCC risk of men infected at birth is estimated to approach 40%. Importantly, a recent study from Taiwan demonstrated a decline in the incidence of HCC in children after implementation of a universal hepatitis B vaccination program. 

A common molecular mechanism for HBV-induced hepatocarcinogenesis has not been discovered thus far, and both viral and host factors have been implicated in the process. On the one hand, most cases of HCC occur after many years of chronic hepatitis, which could provide the mitogenic and mutagenic environment to precipitate random genetic and chromosomal damage and lead to the development of HCC. In this context, in a transgenic mouse model it was recently shown that chronic immune-mediated liver cell injury is sufficient to cause HCC. On the other hand, several lines of evidence support a more direct oncogenic contribution of HBV to HCC development.

**Hepatitis B virus DNA integration**

Although HBV and retroviruses share a replication strategy that includes the reverse transcription of a RNA intermediate, viral integration into the host genome is not part of the HBV life cycle but rather occurs as an epiphenomenon of HBV replication. Integrated HBV sequences have been found by Southern blot analysis in the majority of HCC that develop in patients with chronic HBV infection (reviewed in 35,36). Detailed investigation of a number of different HBV integration sites has revealed that integration was random within human chromosomes. Therefore, HBV may act as a non-selective insertionally mutagenic agent. In addition, secondary chromosomal rearrangements associated with HBV DNA integration, such as duplications, translocations, and deletions suggest that a major oncogenic effect of HBV integration may be an increased genomic instability. Deletions as well as translocations may result in the loss of cellular genes with important regulatory functions, thereby increasing the likelihood of cellular transformation.

Only very few examples of HBV integrations within or near known functional cellular genes have been documented. In this regard, characterization of a single HBV integration site in an early HCC revealed that HBV inserted into an exon of the retinoic acid receptor (RAR) alpha gene, thereby resulting in overexpression of an aminoterminally truncated RAR alpha with altered functions (reviewed in 37). Further, it has recently been shown that phosphorylation of RAR alpha inhibits its degradation and enhances cell proliferation. Investigation of another HBV DNA integration site led to the identification of the human cyclin A gene. This integration had occurred within the second intron of the gene, resulting in the production of a spliced HBV-cyclin A fusion transcript. In the chimeric protein, the aminoterminal domain of cyclin A, containing the signals for regulated cyclin degradation, was replaced by viral pre-S2/S sequences, with transcription being initiated from the pre-S2/S promoter, while the carboxyterminal two-thirds of cyclin A, including the evolutionary well-conserved cyclin box, were intact. Constitutive and strong expression of this stabilized cyclin A protein may therefore have led to increased cell proliferation. These and a few additional similarly well characterized cases are notable exceptions, however, because HBV has not been found to commonly integrate in specific domains of known cellular genes.

**Figure 1** Hepatocellular carcinoma risk for chronic liver diseases of different etiologies.

**Figure 2** Hepatocellular carcinoma (HCC) development and strategies aimed at HCC prevention. 1, prevention of liver disease, for example by hygienic measures, prevention of exposure or vaccination against HBV infection, abstinence from alcohol etc. 2, prevention of chronic hepatitis, for example by treatment of acute hepatitis C, abstinence from alcohol, treatment of hereditary liver diseases etc. 3, prevention of liver cirrhosis, for example by antiviral treatment of chronic hepatitis B or C, treatment of other chronic liver diseases. 4, prevention of HCC development in liver cirrhosis, for example by antiviral treatment, inhibition of fibrosis, liver transplantation etc.
Viral pathogenesis of HCC

Hepatitis B virus X gene and truncated surface gene in the pathogenesis of HBV-associated hepatocellular carcinoma

The HBV X gene product, termed HBx, can function as a transcriptional transactivator of various cellular genes associated with growth control and this phenomenon has led to the hypothesis that it may be involved in the development of HBV-associated HCC (reviewed in 40). Alternatively, it was postulated that HBx may promote hepatocarcinogenesis through activation of the Ras-Raf-MAP kinase pathway. In addition, malignant transformation of certain rodent cells, induction of cell cycle progression in quiescent mouse fibroblasts and Chang cells, and interference with cellular DNA repair as well as apoptosis have, among numerous other interactions with host cell functions, been described in different experimental systems. Recently it has been demonstrated that HBx triggers the release of calcium from mitochondria and/or the endoplasmic reticulum, that in turn triggers the calcium-dependent signaling pathway that affects many cellular processes, including transcription, translation, cell cycle control and apoptosis. A role of HBx in HBV-associated hepatocarcinogenesis is further supported by the observation that transgenic mice carrying the X gene under control of its own regulatory elements develop tumors in HBx-transgenic mice. This discrepancy may be explained by differences in the level and duration of X gene expression and the genetic background of the mouse strains used in these studies.

Recent studies suggested that HBx may, similar to gene products of other DNA tumor viruses, interact with p53 and may thereby interfere with the known functions of p53. However, it is presently unclear if such an interaction may have significance at HBx levels expressed in hepatocytes during natural HBV infection. In this context, Puisieux et al. found that HBV replication in Hep G2.2.15 cells did not interfere with p53 functions. Also, none of the various groups of investigators searching for HBx interactions with cellular proteins by the yeast two-hybrid system did, to our knowledge, find an interaction of HBx with p53. Thus, further studies are needed to clarify this intriguing issue.

Another HBV gene product, which has been reported to possess transactivating properties, is a truncated pre-S2/S gene product referred to as truncated middle hepatitis B surface antigen (MHBSs); reviewed in 40).

Chronic hepadnavirus infection and hepatocellular carcinoma

A high rate of HCC has been found in woodchucks infected with WHV (reviewed in 56). Of the 63 experimentally infected chronic WHV carriers followed under controlled conditions at the woodchuck colony at Cornell University, all developed HCC by 3 years after experimental infection, compared to none of the uninfected animals. It is of considerable interest that HCC also occurred in 17 of 63 woodchucks that had serologically recovered from experimental WHV infection (negative for WHsAg, positive for anti-WHc and anti-WHs antibodies). The WHV DNA was detected in a substantial number of these tumors by Southern blot analysis. An increased incidence of HCC in HBsAg-negative individuals with serological evidence of past HBV infection (positive for anti-HBc and anti-HBs antibodies) has also been described. With the use of highly sensitive immunoassays and PCR, HBV has been shown to persist at low levels in a number of these subjects (reviewed in 59).

Investigation of hepadnaviral integration sites to identify cellular oncogenes involved in HCC development was particularly rewarding in the case of HCC associated with chronic WHV infection (reviewed in 36). Activation of myc family oncogenes, presumably resulting from cis- and trans-acting effects of integrated WHV regulatory elements, was found in the majority of these tumors. Recently, it was also found that transgenic mice carrying a mutated c-myc gene and adjacent WHV DNA cloned in original configuration from a woodchuck HCC integration site developed liver tumors, thus underscoring the high oncogenic potential of insertionally activated myc family genes in this animal model. Finally, antisense downregulation of N-myc1 in woodchuck hepatoma cells has recently been shown to reverse the malignant phenotype. Although aflatoxin B1 (AFB1) does not induce HCC in uninfected woodchucks, in chronically WHV-infected animals HCC appears earlier and at a higher incidence, indicating a synergistic carcinogenic effect.

HEPATITIS C VIRUS AND HEPATITIS C VIRUS-INDUCED HEPATOCELLULAR CARCINOMA

Hepatitis C virus is the major agent causing percutaneously transmitted post-transfusion or sporadic non-A, non-B hepatitis (NANB-H). Hepatitis C virus belongs to the Flaviviridae family and has a single-stranded RNA genome of positive polarity and known genetic organization. Infectious HCV cDNA clones have recently been constructed. Similar to HIV infection, in chronic HCV infection approximately $10^{11}$ to $10^{12}$ virions are produced daily. This high level of replication together with the lack of a proof-reading function of the viral RNA polymerase result in the rapid emergence of viral mutants and quasispecies. The HCV sequence analyses revealed substantial heterogeneity of HCV isolates. Depending on the degree of sequence homology, one distinguishes between genotypes (<72%), subtypes (73–84%) and isolates (85–99%). To date, at least six different HCV genotypes and several subtypes have been identified. Different genotypes can occur in a given geographic region albeit with different prevalences; they can even coexist in a given patient. In most chronically infected individuals HCV exists as quasispecies.
Hepatitis C virus naturally infects only humans and experimentally chimpanzees. Apart from hepatocytes, other cells and tissues, in particular cells of the hematopoietic system, can probably also be infected by HCV. The clinical, immunological and virological factors affecting the natural course of HCV infection are not fully elucidated. Major histocompatibility complex (MHC) class I-dependent HCV-specific cytotoxic CD8-positive T lymphocytes, as well as MHC class II-dependent HCV-specific CD4-positive T lymphocytes, are clearly very important.

Epidemiologic studies revealed the presence of anti-HCV antibodies as a marker of chronic hepatitis C in 15–80% of patients with HCC, depending upon the patient population studied (reviewed in 74, 75). For example, HCV is a major cause of HCC in Japan, Italy, and Spain whereas it may play a less important role in South Africa and Taiwan. Similar to HBV, HCC associated with HCV infection evolves after many years of chronic infection and is generally preceded by the development of liver cirrhosis.

Hepatitis C virus may be associated with HCC via chronic liver injury, as described in the previous section for HBV. However, recent clinical and experimental evidence raises the possibility that HCV might operate also through other pathways in promoting malignant transformation of hepatocytes. Hepatocellular carcinomas have been found in a series of chronically HCV-infected patients without liver cirrhosis. Moreover, a transforming potential of the aminoterminal portion of the NS3 protein and of the core protein have been described. In this context, the core protein has been reported to influence various cellular functions, including, among others, enhancement or inhibition of apoptosis and repression of p53, as well as p21WAF1 promoter activity. Most of these studies, however, were performed in experimental overexpression systems, and their relevance to natural HCV infection will need to be further addressed. Finally, HCC has recently been reported to develop in certain HCV core-transgenic mice.

### MOLECULAR HEPATOCARCINONEGENESIS

Central to the concept of molecular carcinogenesis are mutations of oncogenes and tumor suppressor genes as well as genetic instability of cellular DNA, including mismatch repair deficiency and impaired chromosomal segregation. In hepatocarcinogenesis, these genetic events occur in the setting of liver cell injury and necrosis associated with an increased rate of hepatocyte regeneration and mitosis. Any exogenous agent, viral or other, that contributes to chronic low-grade liver cell damage and mitosis potentially increases the risk of HCC development, rendering liver cell DNA susceptible to additional genetic alterations. Overall, there is a variety of molecular mechanisms by which environmental and viral carcinogens may play a role in HCC development.

### Oncogenes

Activated cellular oncogenes, particularly those of the ras family, have been found in a number of experimental hepatocarcinogenesis models. In human hepatocarcinogenesis, however, no consistent pattern of protooncogene activation has emerged so far for HCC. It is also of interest that no structural or functional changes of a large panel of oncogenes have been found in a transgenic mouse model that is believed to resemble the process of human hepatocarcinogenesis.

### Tumor suppressor genes

Restriction fragment length polymorphism studies of paired HCC and non-tumorous liver samples have revealed relatively frequent (>20% in ≥10 informative cases) chromosomal allelic losses (loss of heterozygosity, LOH) in HCC on chromosomes 4, 5q, 8p, 10q, 11p, 13q, 16, 16p and 16q, 17p and 22q, suggesting that these sites may harbor tumor suppressor genes involved in the pathogenesis of HCC. In general, these genetic alterations appear to occur at later stages of HCC development.

Interestingly, a G to T mutation at the third base position of codon 249 of the p53 gene, leading to a substitution of arginine to serine, was found in a significant number of HCC in patients from southern Africa and the Qidong area in China. It was suggested that this ‘hot spot’ mutation was associated with high AFB1 intake in food and may have contributed to the high incidence of HCC in these areas. This finding was supported by in vitro studies indicating that the third base of codon 249 of p53 was preferentially targeted to form adducts with AFB1.

### DNA mismatch repair genes

In addition to oncogenes and tumor suppressor genes, DNA mismatch repair genes have recently been identified as a new class of susceptibility genes involved in the pathogenesis of inherited and sporadic human tumors, most notably hereditary non-polyposis colorectal cancer (HNPPC). Defective DNA mismatch repair can lead to the accumulation of mutations and microsatellite instability in the cellular genome and thus increase the chance of malignant transformation. The role of DNA mismatch repair defects in HCC development is currently unknown. Recent observations, however, suggest that HBx may interfere with components of the DNA repair machinery.

### Telomerase activation

The progressive shortening of chromosome ends, or telomeres, accompanies normal cell division and may contribute to cellular aging and serve as a control mechanism against unregulated cellular proliferation.
Recently, a remarkable correlation between certain types of cancer and the expression of telomerase, a ribonucleoprotein enzyme preventing the shortening of telomeres, was found. Indeed, expression of telomerase may be a common pathway leading to cancer. In this regard, in a recent study telomerase activity was found in 85% of HCC tissues.

**Growth factors**

As in most other forms of cancer, the unregulated expression of growth factors and of components of their signaling pathways may play an important role in hepatic oncogenesis. Indeed, overexpression of certain growth factors was found in HCC, including insulin-like growth factor II, transforming growth factor, and insulin receptor substrate 1. Surprisingly, hepatocyte growth factor (HGF) has been shown to inhibit the growth of a number of hepatoma cell lines and no neoplasms developed in transgenic mice expressing HGF in the liver under control of the albumin promoter. Moreover, in a recent study in c-myc-HGF double-transgenic mice it was found that coexpression of HGF markedly reduced c-myc-induced neoplastic changes. The knowledge regarding growth factors in HCC, however, is still incomplete and additional factors are likely to emerge as potentially important candidates involved in hepatocarcinogenesis.

Using oligonucleotide or cDNA microarray expression profiling, suppression subtractive hybridization and other methods should allow further elucidation of the genetic events underlying HCC pathogenesis and identification of novel diagnostic markers as well as therapeutic targets.1-93

**SUMMARY AND PERSPECTIVES**

Hepatocellular carcinoma is one of the most common malignant tumors worldwide. The major risk factors for HCC development are now well defined and some of the multiple steps involved in hepatocarcinogenesis have been elucidated in recent years. However, no clear picture of how and in what sequence these factors interact at the molecular level has emerged. Malignant transformation of hepatocytes may occur as a consequence of various etiologies, such as chronic viral hepatitis, alcohol, and metabolic disorders, in the context of increased cellular turnover induced by chronic liver injury, regeneration and cirrhosis. Activation of cellular oncogenes, inactivation of tumor suppressor genes, overexpression of certain growth factors, and possibly telomerase activation and DNA mismatch repair defects may contribute to the development of HCC. Finally, aflatoxins have been shown to induce specific mutations of the p53 tumor suppressor gene, thus pointing to the contribution of environmental factors to tumor development at the molecular level.

Hepatocyte transformation occurs in the setting of chronic liver injury, regeneration, hyperplasia, cirrhosis and loss of growth control that eventually leads to the development of HCC and is commonly preceded by genetic mutations and rearrangements. There are many etiologic factors that affect various steps in the process and some may act synergistically. Chronic viral hepatitis, alcohol, and certain metabolic disorders may act predominantly through a pathway of chronic liver injury. Some evidence suggests that HBV may in addition play a more direct role in the molecular pathogenesis of HCC. Understanding hepatocyte growth regulation at the molecular level may eventually lead not only to a better understanding of the cellular events involved in hepatocyte transformation, but in all likelihood also to innovative preventive and therapeutic strategies.

Apart from improving HCC therapy, the refinement and implementation of existing as well as the discovery and development of novel strategies aimed at HCC prevention are most important. Primary prevention has been shown to reduce HCC development in some patient groups at risk. For example, hepatitis B vaccination of children in Taiwan has actually resulted in a decline of the HCC incidence. Further, antiviral therapy of patients with chronic hepatitis B or C should contribute to HCC prevention. Public health measures to reduce food contamination with aflatoxins and eliminate excessive alcohol use should also reduce the incidence of chronic liver disease, cirrhosis and thereby HCC. Apart from primary HCC prevention, interventions aimed at secondary prevention after successful HCC treatment are also a very active area of basic and clinical research. In the future therefore preventive measures should have a major impact on reducing the incidence of HCC, one of the most common and devastating malignancies in the world.

**REFERENCES**

1. Schafer DF, Sorrell MF. Hepatocellular carcinoma. *Lancet* 1999; 353: 1253–7.
2. Lee WM. Hepatitis B virus infection. *N. Engl. J. Med.* 1997; 337: 1733–45.
3. Di Bisceglie AM. Hepatitis C (see comments). *Lancet* 1998; 351: 351–5.
4. Kew MC, Yu MC, Kedda M-A, Coppin A, Sarkin A, Hodkinson J. The relative roles of hepatitis B and C viruses in the etiology of hepatocellular carcinoma in southern African blacks. *Gastroenterology* 1997; 112: 184–7.
5. Donato F, Boffetta P, Puoti M. A meta-analysis of epidemiological studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma. *Int. J. Cancer* 1998; 75: 347–54.
6. Okuda K. Hepatocellular carcinomas associated with hepatitis B and C virus infections: Are they any different? *Hepatology* 1995; 22: 1883–5.
7. Lauer GM, Walker BD. Hepatitis C virus infection. *N. Engl. J. Med.* 2001; 345: 41–52.
8. Di Bisceglie AM. Natural history of hepatitis C: Its impact on clinical management. *Hepatology* 2000; 31: 1014–18.
9. Liang TJ, Rehermann B, Szeff LB, Hoofnagle JH. Pathogenesis, natural history, treatment, and prevention of hepatitis C. *Ann. Intern. Med.* 2000; 132: 296–305.
10 Blum HE. Variants of hepatitis B, C and D viruses: Molecular biology and clinical significance. *Digestion* 1995; 56: 85–95.
11 Thursz MR, Kwiatkowski D, Allsopp CE, Greenwood BM, Thomas HC, Hill AV. Association between an MHC class II allele and clearance of hepatitis B virus in the Gambia. *N. Engl. J. Med.* 1995; 332: 1065–9.
12 Congia M, Clemente MG, Dessi C et al. HLA class II genes in chronic hepatitis C virus-infection and associated immunological disorders. *Hepatology* 1996; 24: 1338–41.
13 Tibbs C, Donaldson P, Underhill J, Thomson L, Manabe K, Williams R. Evidence that the HLA DQA1*03 allele confers protection from chronic HCV-infection in northern European caucasoids. *Hepatology* 1996; 24: 1342–5.
14 Donato F, Tagger A, Chiesa R et al. Oral contraceptives and primary liver cancer in England and Wales. *Br. J. Cancer* 1997; 75: 963–5.
15 Benn J, Schneider RJ. Hepatitis B virus HBx protein and hepatocellular carcinoma: A case-control study in Italy. *Hepatology* 1997; 26: 579–84.
16 Eaton DL, Gallagher EP. Mechanism of aflatoxin carcinogenesis. *Annu. Rev. Pharmacol. Toxicol.* 1994; 34: 135–72.
17 Chen C-J, Wang L-Y, Lu S-N et al. Elevated aflatoxin exposure and increased risk of hepatocellular carcinoma. *Hepatology* 1996; 24: 38–42.
18 Aguilar F, Hussain SP, Cerutti P. Aflatoxin B1 induces transversion of G to T in codon 249 of the p53 tumor suppressor gene in human hepatocytes. *Proc. Natl Acad. Sci. USA* 1993; 90: 8586–90.
19 Bailey EA, Iyer RS, Stone MP, Harris TM, Essigmann JM. Mutational properties of the primary aflatoxin B1-DNA adduct. *Proc. Natl Acad. Sci. USA* 1996; 93: 1535–9.
20 Mandishona E, MacPhail AP, Gordeux VR et al. Dietary iron overload as a risk factor for hepatocellular carcinoma in black Africans. *Hepatology* 1998; 27: 1563–6.
21 Mant JWF, Vessey MP. Trends in mortality from primary liver cancer in England and Wales. *Br. J. Cancer* 1995; 72: 800–3.
22 Waetjen LE, Grimes DA. Oral contraceptives and primary liver cancer: Temporal trends in 3 countries. *Obstet. Gynecol.* 1996; 88: 945–9.
23 Poynard T, Bedossa P, Opolon P et al. Natural history of liver fibrosis progression in patients with chronic hepatitis C. *Lancet* 1997; 349: 825–32.
24 Deuffic S, Poynard T, Buffat L, Valleron A-J. Trends in primary liver cancer. *Lancet* 1998; 351: 214–15.
25 Taylor-Robinson SD, Foster GR, Arora S, Hargravens S, Thomas HC. Increase in primary liver cancer in the UK, 1979–94. *Lancet* 1997; 350: 1142–3.
26 De Vos Irvine H, Goldberg D, Hole DJ, McMenamin J. Trends in primary liver cancer. *Lancet* 1998; 351: 215–16.
27 El-Serag HB, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. *N. Engl. J. Med.* 1999; 340: 745–50.
28 Yuen MF, Cheng CC, Launder IJ, Lam SK, Ooi CG, Lai CL. Early detection of hepatocellular carcinoma increases the chance of treatment: Hong Kong experience. *Hepatology* 2000; 31: 330–5.
29 Villa E, Moles A, Ferretti I et al. Natural history of inoperable hepatocellular carcinoma: Estrogen receptors' status in the tumor is the strongest prognostic factor for survival. *Hepatology* 2000; 32: 233–8.
30 El-Serag HB, Mason AC, Key C. Trends in survival of patients with hepatocellular carcinoma between 1977 and 1996 in the United States. *Hepatology* 2001; 33: 62–5.
31 Cho KJ, Lee SS, Kang SK. Histiocytic necrotizing lymphadenitis. A clinicopathologic study of 45 cases with in situ hybridization for Epstein–Barr virus and hepatitis B virus. *J. Korean Med. Sci.* 1996; 11: 409–14.
32 Beasley RP, Hwang LY, Lin CC, Chien CS. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22707 men in Taiwan. *Lancet* 1981; 2: 1129–33.
33 Chang MH, Chen CJ, Lai MS et al. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group (see comments). *N. Engl. J. Med.* 1997; 336: 1835–9.
34 Nakamoto Y, Guidotti L, Kuhlen C, Fowler P, Chisari F. Immune pathogenesis of hepatocellular carcinoma. *J. Exp. Med.* 1998; 188: 341–50.
35 Matsubara K, Tókito T. Integration of hepatitis B virus DNA and its implications for hepatocarcinogenesis. *Mol. Biol. Med.* 1990; 7: 243–60.
36 Buendia MA. Hepatitis B viruses and hepatocellular carcinoma. *Adv. Cancer Res.* 1992; 59: 167–226.
37 Dejean A, de The H. Hepatitis B virus as an insertion mutagen in a human hepatocellular carcinoma. *Mol. Biol. Med.* 1990; 7: 213–22.
38 Adachi S, Okuno M, Matsushima-Nishiwaki R et al. Phosphorylation of retinoid X receptor suppresses its ubiquitination in human hepatocellular carcinoma. *Hepatology* 2002; 35: 332–40.
39 Wang C, Siddiqui A. Structure and function of the hepatitis C virus internal ribosome entry site. *Curr. Top. Microbiol. Immunol.* 1995; 203: 99–115.
40 Caselmann WH. Trans-activation of cellular genes by hepatitis B virus proteins: a possible mechanism of hepatocarcinogenesis. *Adv. Virus Res.* 1996; 47: 253–202.
41 Bena J, Schneider RJ. Hepatitis B virus HBx protein activates Ras-GTP complex formation and establishes a Ras, Raf, MAP kinase signaling cascade. *Proc. Natl Acad. Sci. USA* 1994; 91: 10 350–4.
42 Hohne M, Schaefer S, Seifer M, Feitelson MA, Paul D, Gerlich WH. Malignant transformation of immortalized transgenic hepatocytes after transfection with hepatitis B virus DNA. *EMBO J.* 1990; 9: 1137–45.
43 Koike K, Moriya K, Iino S et al. High-level expression of hepatitis B virus HBx gene and hepatocarcinogenesis in transgenic mice. *Hepatology* 1994; 19: 810–19.
44 Benn J, Schneider RJ. Hepatitis B virus HBx protein deregulates cell cycle checkpoint controls. *Proc. Natl Acad. Sci. USA* 1995; 92: 11 215–19.
45 Becker S, Lee T-H, Butel J, Slagle B. Hepatitis B virus X protein interferes with cellular DNA repair. *J. Virol.* 1998; 72: 266–72.
46 Su F, Schneider R. Hepatitis B virus HBx protein sensitizes cells to apoptotic killing by tumor necrosis factor alpha. *Proc. Natl Acad. Sci. USA* 1997; 94: 8744–9.
Viral pathogenesis of HCC

47 Terradillos O, Polliccino T, Leeceur H et al. p53-independent apoptotic effects of the hepatitis B virus HBx protein in vivo and in vitro. Oncogene 1998; 17: 2115–23.

48 Bouchard MJ, Wang LH, Schneider RJ. Calcium signaling by HBx protein in hepatitis B virus DNA replication. Science 2001; 294: 2376–8.

49 Gannem D. Virology. The X files: One step closer to closure. Science 2001; 294: 2299–300.

50 Kim CM, Koike K, Saito I, Miyamura T, Jay G. HBx gene of hepatitis B virus induces liver cancer in transgenic mice. Nature 1991; 351: 317–20.

51 Lee HS, Kim ST, Kim CY. Identification of integrated hepatitis B virus DNA sequences in human hepatocellular carcinomas in Korea. J. Korean Med. Sci. 1990; 5: 145–58.

52 Feitelson MA, Zhu M, Duan LX, London WT. Hepatitis B virus x antigen and p53 are associated in vitro and in liver tissues from patients with primary hepatocellular carcinoma. Oncogene 1993; 8: 1109–17.

53 Wang XW, Forrester K, Yeh H, Feitelson MA, Gu JR, Ueda H, Ullrich SJ, Gangemi JD. Hepatitis B virus X protein inhibits p53 sequence-specific DNA binding, transcriptional activity, and association with transcription factor ERCC3. Proc. Natl Acad. Sci. USA 1994; 91: 2230–4.

54 Ueda H, Ulrich SJ, Gangemi JD et al. Functional inactivation but not structural mutation of p53 causes liver cancer. Nat. Genet. 1995; 9: 41–7.

55 Puisieux A, Ji J, Guillot C et al. p53-mediated cellular response to DNA damage in cells with replicative hepatitis B virus. Proc. Natl Acad. Sci. USA 1995; 92: 1342–6.

56 Tennant BC. Hepatocarcinogenesis in experimental woodchuck hepatitis virus infection. In: Sirica AE, ed. The Role of Cell Types in Hepatocarcinogenesis. Boca Raton, FL: CRC Press, 1992: 323–49.

57 Gerin JL. Antiviral agents for hepatitis B (editorial). Hepatology 1991; 14: 198–9.

58 Korba BE, Cote PJ, Wells VF et al. Natural history of woodchuck hepatitis virus infections during the course of experimental viral infection: Molecular virologic features of the liver and lymphoid tissues. J. Virol. 1989; 63: 1360–70.

59 Wands JR, Liang TJ, Blum HE, Shafritz DA. Molecular pathogenesis of liver disease during persistent hepatitis B virus infection. Semin. Liver Dis. 1992; 12: 252–64.

60 Etienoble J, Degott C, Renard CA et al. Liver-specific expression and high oncogenic efficiency of a c-myc transgene activated by woodchuck hepatitis virus insert. Oncogene 1994; 9: 727–37.

61 Wang H-P, Zhang L, Dandi M, Rogler C. Antisense downregulation of N-myc1 in woodchuck hepatoma cells reverses the malignant phenotype. J. Virol. 1998; 72: 2192–8.

62 Bannasch P, Koshikhou NI, Hacker HJ et al. Synergistic hepatocarcinogenic effect of hepadnaviral infection and dietary aflatoxin B1 in woodchucks. Cancer Res. 1995; 55: 3318–30.

63 Choo QL, Kuo G, Weiner AJ, Overby LR, Bradley DW, Houghton M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. Science 1989; 244: 359–62.

64 Kuo G, Choo QL, Alter HJ et al. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science 1989; 244: 362–4.

65 Kolykhlov AA, Agapov EV, Bligbt KJ, Mihalik K, Feinestone SM, Rice CM. Transmission of hepatitis C by intraperative inoculation with transcribed RNA. Science 1997; 277: 570–4.

66 Yanagi M, Purcell RH, Emerson SU, Bukh J. Transcripts from a single full-length cDNA clone of hepatitis C virus are infectious when directly transfected into the liver of a chimpanzee. Proc. Natl Acad. Sci. USA 1997; 94: 8738–43.

67 Zeuzem S, Franke A, Lee J-H, Herrman GR, Ruster B, Roth WK. Phylogenetic analysis of hepatitis C virus isolates and their correlation to viremia, liver function tests, and histology. Hepatology 1996; 24: 1003–9.

68 Neumann A, Lam N, Dahari H et al. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-alpha therapy. Science 1998; 282: 103–7.

69 Bukh J, Miller RH, Purcell RH. Genetic heterogeneity of hepatitis C virus: Quasispecies and genotypes. Semin. Liver Dis. 1995; 15: 41–63.

70 Simmonds P. Variability of hepatitis C virus genome. Curr. Stud. Hematol. Blood Transfus. 1994; 61: 12–35.

71 Lerat H, Berfy F, Trabaud O, Major M, Trépo C. Specific detection of hepatitis C virus minus strand RNA in hematopoietic cells. J. Clin. Invest. 1996; 97: 845–51.

72 Cerny A, Ferrari C, Chisari FV. The class I-restricted cytoxic T lymphocyte response to predetermined epitopes in the hepatitis B and C viruses. Curr. Top. Microbiol. Immunol. 1994; 189: 169–86.

73 Ferrari C, Vali A, Galati L et al. T-cell response to structural and nonstructural hepatitis C virus antigens in persistent and self-limited hepatitis C virus infections. Hepatology 1994; 19: 286–95.

74 Blum HE. Does hepatitis C virus cause hepatocellular carcinoma? Hepatology 1994; 19: 251–5.

75 Di Bisciglia AM. Hepatitis C and hepatocellular carcinoma. Semin. Liver Dis. 1995; 15: 64–9.

76 Kiyosawa K, Tanaka E, Sodeyama T et al. Hepatitis C virus infection is associated with liver cancer without cirrhosis. J. Hepatol. 1997; 27: 2376–82.

77 De Mitri MS, Poussin K, Baccarini P et al. HCV-associated liver cancer without cirrhosis. Lancet 1995; 345: 413–15.

78 Sakamuro D, Furukawa T, Takegami T. Hepatitis C virus nonstructural protein NS3 transforms NIH 3T3 cells. J. Virol. 1995; 69: 3893–6.

79 Ray R, Lagger L, Meyer K, Ray R. Hepatitis C virus core protein cooperates with ras and transforms primary rat embryo fibroblasts to tumorigenic phenotype. J. Virol. 1996; 70: 4438–43.

80 Ruggieri A, Harada T, Matsuura Y, Miyamura T. Sensitization to Fas-mediated apoptosis by hepatitis C virus core protein. Virol. 1997; 229: 68–76.

81 Ray RB, Steele R, Meyer K, Ray R. Transcriptional repression of p53 promoter by hepatitis C virus core protein. J. Biol. Chem. 1997; 272: 10983–6.

82 Moriya K, Fujie H, Shintani Y et al. The core protein of hepatitis C virus induces hepatocellular carcinoma in transgenic mice. Nat. Med. 1998; 4: 1065–7.
83 Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990; 61: 759–67.
84 Liu B, Nicolaides NC, Markowitz S et al. Mismatch repair gene defects in sporadic colorectal cancers with microsatellite instability. Nat. Genet. 1995; 9: 48–55.
85 Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. Cell 1996; 87: 159–70.
86 Fearon ER. Human cancer syndromes: Clues to the origin and nature of cancer. Science 1997; 278: 1043–50.
87 Kinzler KW, Vogelstein B. Gatekeepers and caretakers. Nature 1997; 386: 761–3.
88 Moradpour D, Wands JR. The molecular pathogenesis of hepatocellular carcinoma. J. Viral Hepatol. 1994; 1: 17–31.
89 Moradpour D, Wands JR, Blum HE. Molecular biology of hepatitis B and C virus and hepatocellular carcinoma. Mol. Cancer Biol. 1996; 3: 875–904.
90 Ozturk M. Genetic aspects of hepatocellular carcinogenesis. Semin. Liver Dis. 1999; 19: 235–42.
91 Miyasaka Y, Enomoto N, Nagayama K et al. Analysis of differentially expressed genes in human hepatocellular carcinoma using suppression subtractive hybridization. Br. J. Cancer 2001; 85: 228–34.
92 Shirota Y, Kaneko S, Honda M, Kawai HF, Kobayashi K. Identification of differentially expressed genes in hepatocellular carcinoma with cDNA microarrays. Hepatology 2001; 33: 832–40.
93 Graveel CR, Jatkoe T, Madore SJ, Holt AL, Farnham PJ. Expression profiling and identification of novel genes in hepatocellular carcinomas. Oncogene 2001; 20: 2704–12.