

Early molecular and genetic determinants of primary liver malignancy

Mark A. Feitelson, PhD^{a,b,*}, Jingbo Pan, MD^a,
Zhaorui Lian, MD, PhD^a

^a*Department of Pathology, Anatomy, and Cell Biology, Thomas Jefferson University,
1020 Locust Street, Philadelphia, PA 19107, USA*

^b*Department of Microbiology and Immunology, Kimmel Cancer Center,
Thomas Jefferson University, 1020 Locust Street, Philadelphia, PA 19107, USA*

Hepatocellular carcinoma (HCC) is one of the most frequent tumor types worldwide, with more than an estimated 250,000 new cases annually [1]. There are multiple etiological agents associated with the development of HCC; the most frequent being chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, and long-term exposure to the mycotoxin, aflatoxin B1 (AFB1). HCC is also a common complication of alcoholic cirrhosis, although ethanol appears to not be directly carcinogenic [2]. HCC is also reportedly associated with various genetic conditions, such as hereditary tyrosinemia, α 1-antitrypsin deficiency, and idiopathic hemochromatosis. There is also evidence that HCC may be associated with oral contraceptives and anabolic steroids. Together, these data show that there are many causes of HCC [3,4]. Given that the majority of HCCs are associated with chronic HBV infections, this article presents mechanisms whereby HBV contributes to the development of HCC. It also discusses some of the early genetic changes that contribute importantly to HCC, and how biochemical alterations brought about by the virus and by mutations lead to tumor formation. Elucidation of the changes that cause tumors, in contrast to ones that arise as a consequence of tumor formation (and that contribute to tumor progression), may provide early markers of hepatocarcinogenesis that will be useful in identifying HBV carriers that are progressing toward tumor development. In addition, these early biochemical changes, some of

This work was supported by grants CA48656 and CA66971 to MF from the National Institutes of Health.

* Corresponding author. Room 222, Alumni Hall, Department of Pathology, Anatomy, and Cell Biology, Thomas Jefferson University, 1020 Locust Street, Philadelphia, PA 19107
E-mail address: Mark.Feitelson@mail.tju.edu (M.A. Feitelson).

which are reversible, are likely to provide meaningful targets for drug discovery and intervention before the appearance of multinodular HCC, which is much more difficult to treat than a single tumor. Moreover, elucidation of the early steps in hepatocarcinogenesis will help to decide when and how to treat patients using some of the approaches outlined in the forthcoming articles.

The incidence of HCC is geographically variable, with the highest frequencies observed in sub-Saharan Africa and in the Far East among countries where HBV is endemic, and in regions where aflatoxin-contaminated peanuts are consumed. HCC is also predominantly male associated, with male:female ratios of up to 2:1 [5]. HCC is rapidly fatal, with a life expectancy of about 6 months from time of diagnosis, and a <3% survival rate for untreated cancer over 5 years. Death is often due to liver failure associated with cirrhosis or rapid tumor growth. Although early HCC is cured by surgical resection, the fact that many tumors are asymptomatic means that most patients at risk are not diagnosed in time. These patients often present with inoperable HCC, which usually reflects the presence of large or multiple HCC nodules. Despite the high incidence and mortality, the development of an effective vaccine for HBV, combined with its universal administration to newborns in endemic countries, will significantly lower the incidence of HCC within the next generation. Despite these advances, it will be crucial to identify those among the estimated 350 million carriers of HBV worldwide who are at highest risk for tumor development.

Multistep hepatocarcinogenesis

HCC develops some 30 to 50 years after infection with HBV, suggesting that the pathogenesis of this disease has multiple steps. Some of these steps have a genetic basis, resulting in point mutations, microdeletions, genetic rearrangements, and loss of heterozygosity (LOH) in critical growth regulatory genes. These mutations accumulate over time, in that preneoplastic liver from chronically infected patients have few genetic aberrations, whereas HCC nodules from the same patients have many. Some of these accumulating steps include inactivating mutations in the tumor suppressors p53 and Rb, which normally negatively regulate cell growth [6,7], and other steps involve upregulated expression of the cell cycle protein, cyclin D1, and the oncogene, *c-myc*, which stimulate hepatocellular growth [8,9]. LOH involving multiple chromosomes in single tumors also strongly suggests multistep carcinogenesis [10,11]. The finding that HCC appears within the context of chronic hepatitis or cirrhosis within regions of liver cell dysplasia or adenomatous hyperplasia [12] also suggests that tumors develop from a series of histologically identifiable lesions that precede tumor development. This is not only true of human HCC, but also in mouse models of this tumor [13–15]. HCC also appears to evolve from adenomas in HBV-encoded X antigen (HBxAg) transgenic mice [16], and from similar lesions in *c-myc*,

c-myc/transforming growth factor alpha (TGF α), and *c-myc*/TGF β 1 transgenic mice [17,18]. A major problem in the interpretation of these and other events is identifying whether they occur in sequence during the pathogenesis of disease. In addition, it is not known which events are associated with the cause or consequences of tumor formation, and which steps are virus dependent or independent [19]. As outlined below, there are an increasing number of studies in which dozens to hundreds of genes are differentially expressed in HCC compared with nontumor or uninfected liver. Although the results of these investigations are provocative, they present pieces of the puzzle that remain to be assembled into a comprehensive picture of what constitutes HCC at the cellular and molecular levels.

Steps leading to tumor formation

The appearance of HCC in children suggests that there is a genetic predisposition to this tumor type [20], although the inability to identify most of the predisposing genes has made it difficult to identify the rate-limiting steps in hepatocarcinogenesis. There are several molecular changes, however, that occur in high frequency before tumor appearance and that likely represent early changes in tumor development. For example, mice that are deficient in poly(ADP-ribose) polymerase (PARP) and heterozygous for Ku80 (a subunit of the DNA-protein kinase complex), develop HCC [21,22]. Both PARP and Ku80 are tumor suppressors that maintain chromosome stability by promoting DNA repair. The fact that HCC is observed only when deficiencies are observed in both genes suggests that the inactivation of multiple negative growth regulatory (tumor suppressor) pathways are required for tumor formation, and that any major compromise in DNA repair may be a major risk factor for tumor development. Although it is not clear whether the loci containing these genes are lost in human HCC, LOH has been detected in chromosomes 1q (where PARP-1 is found) or 2q (where Ku80 is found) in some HCCs, suggesting that these may be cancer susceptibility genes [23–26]. Another cancer susceptibility gene is *Lkb1*, where germline mutations are associated with an increased risk for cancer development [27]. In this case, mice that are heterozygous for the *Lkb1* gene, which induces angiogenesis, suppresses growth, and triggers apoptosis [28,29], develop HCC at greater than 50 weeks of age [15]. Ongoing work with mouse models of HCC will help to identify additional genetic changes that predispose to tumor development, and if these changes also occur in populations at high risk for HCC, they will undoubtedly become targets in studies aimed at the chemoprevention of this tumor type.

Contribution of HBxAg to early stages of tumor formation

The HBV encoded X antigen, HBxAg, which contributes importantly to liver cell transformation in vitro and to the development of HCC [30,31],

binds to many cellular proteins or alters the expression of many cellular genes that are likely to contribute to hepatocarcinogenesis. For example, HBxAg binds to the DDB1 subunit of a UV-damaged DNA binding protein [32,33], the latter of which appears to be important for maintaining the integrity of DNA repair [34,35]. Initially, a partial correlation was observed between the reduction of repair activity in cells expressing HBxAg and the ability of HBxAg to bind to DDB1 [33]. HBxAg has also been shown to bind to and functionally inactivate p53 [16,36,37], and it is thought that this also disrupts p53/ERCC3 complexes, which are important in mediating transcription-coupled repair [38,39]. The finding that the frequency of spontaneous mutations is not any higher in X transgenic compared with control mice [40] suggests that HBxAg does not promote the development of mutations in the absence of chronic liver disease (CLD) and hepatocellular turnover. The fact that CLD is immune mediated [41], however, and thus accompanied by the generation of cytotoxic cytokines and reactive oxygen intermediates (ROI), suggests that if HBxAg inhibits DNA repair *in vivo*, it may permit the accumulation of mutations. Oxidative stress also precedes the development of HCC in transgenic mice that overproduce and accumulate intracellular HBxAg [42,43], and in genetic diseases characterized by intrahepatic accumulation of a cellular protein (such as α_1 -antitrypsin deficiency). In fact, the central role of ROI in the initiation and progression of multistage carcinogenesis [44,45] may be one of the ways whereby HBxAg contributes to this process. In this context, ROI stimulates HBxAg *trans*-activation activity [46], which may promote alterations in host gene expression that contribute to hepatocarcinogenesis. If this is true, it may help explain why CLD in HBV carriers is a major risk factor for the development of HCC [5].

A central feature of chronic infection is the integration of HBV DNA fragments into the host DNA. It turns out that HBV DNA integration into HepG2 cells or into the host chromosomes of transgenic mice results in chromosomal instability [47,48], which may contribute to LOH at many sites during chronic infection. This is consistent with observations of widespread LOH in tumors derived from HBV carriers compared with HBV negative patients [49]. Integration of HBV DNA into chromosomal DNA has also been shown to be in or near the cyclin A gene [50], the retinoic acid receptor gene [51], and other genes that are known to regulate cell viability and growth [52] in a few cases, suggesting that HBV may act as an insertional mutagen in a small fraction of patients with HCC [53]. The great majority of HBV DNA integration events, however, are random with respect to the sites within host DNA. Many of these events result in the integration of the X gene, which is positioned at the end of the virus genome where recombination with host DNA occurs [54]. This usually results in the abundant production of X mRNA [55,56] and protein [57–59] in the livers of patients with chronic liver disease. Closer examination of the HBxAg polypeptide made by these integrants has shown that it is often truncated at the carboxy-terminus [60]. Some of these truncated mutants behave differently from full-length HBxAg

in their abilities to mediate *trans*-activation, regulate apoptosis, stimulate cell growth, and promote tumorigenesis [61–64]. Hence, the place within host DNA that HBV integrates may not be nearly as important for transformation as the products of the integrated viral DNA fragments and their levels of expression.

In chronic HBV infection, HBxAg blocks a number of negative growth regulatory, tumor suppressor, or senescence related pathways that may contribute importantly to the early stages of hepatocarcinogenesis. For example, HBxAg binds to and functionally inactivates p53 [16,36,37,65] and the senescence related factor, p55^{sen}, by cytoplasmic sequestration [66,67]. In addition, there is a direct correlation between cytoplasmic sequestration of wild type p53 and the development of altered foci, adenomas, and HCC in HBxAg transgenic mice [16]. HBxAg also transcriptionally downregulates the expression of the translation initiation factor, *suil*, as well as the senescence factor and cyclin dependent kinase inhibitor, p21^{WAF1/CIP1/SDI1}, both of which inhibit hepatocellular growth [66]. Functional inactivation of the retinoblastoma (Rb) tumor suppressor by hyperphosphorylation has also been observed in HbxAg-positive cells, resulting in the activation of E2F1 and stimulation of the cell cycle [63]. The finding that these steps occur in nontumor liver cells implies that the inactivation of these pathways represent important early steps in hepatocarcinogenesis.

HBxAg also activates growth stimulatory pathways. For example, HBxAg upregulates the expression of insulin-like growth factor 2 (IGF2) in premalignant proliferative nodules [68] and IGF_{R1} in hepatoma cell lines [6], implying that HBxAg may set up an autocrine loop [69] that enhances cell growth independent of other serum growth factors. Further, HBxAg stimulated cell growth is associated with constitutive activation of the *ras/raf/mitogen activated protein kinase (MAPK)* and nuclear factor kappa- β (NF κ -B) signal transduction pathways [70,71]. In this context, the finding of upregulated dynein expression in HCC [72], whose antiapoptotic effects may be associated with its binding to and inactivation of the NF κ -B inhibitor, I κ B, highlights a possible role for NF κ -B signal transduction in tumor development. In addition, upregulated expression of rhoB has been reported in some HCCs [72]. RhoB is in the *ras* gene family, is associated with cell transformation, and may be a common denominator to both viral and nonviral hepatocarcinogenesis. HBxAg activation of NF κ -B also results in the stimulated expression of a unique, upregulated gene 7 (URG7) that mediates the resistance of HBxAg expressing liver cells to Fas-triggered apoptosis, the latter of which is a major mechanism whereby the immune system eliminates virus-infected cells during a bout of CLD [73]. Other HbxAg-upregulated genes that have not been identified may confer a cellular phenotype that is resistant to cytokine-mediated apoptosis [61]. The latter would protect virus-infected cells with accumulating mutations against immune elimination. HBxAg also upregulates the expression of two additional genes that have properties of oncoproteins [74,75]. Hence, both

upregulated expression of growth promoting genes and downregulated expression of negative growth regulatory genes are operative before tumor development.

The observation that HBxAg stimulates the expression of TGF β 1 [76], the latter of which promotes fibrogenesis and HCC development in transgenic mice [17], provides an additional mechanism whereby HBxAg may contribute to the early stages of tumor formation. Upregulated expression of TGF β 1 by tumor cells may inhibit the growth of surrounding nontumor cells, thereby favoring tumor growth, or by inhibiting antitumor immune responses [77]. The finding that α 3-integrin expression is upregulated in tumor compared with nontumor cells, and that TGF β 1 stimulates α 3-integrin [78], suggests a mechanism whereby TGF β 1 positively stimulates tumor growth. Hence, TGF β 1 appears to play important roles in HCC development and progression.

Microarray analysis

An increasing number of studies have used microarray analysis to distinguish differentially expressed genes in tumor compared with nontumor liver. This approach has shown constitutive upregulation of the MAPK pathway, as well as many genes associated with an activated cell cycle [79–81], in HCC. Interestingly, HBxAg also stimulates MAPK signaling and activates the cell cycle [70,82], suggesting that some of the changes observed by microarray analysis may be virus mediated. These observations were associated with the high incidence of chromosomal instability observed in these tumors. This analysis also showed an upregulated expression of several hepatocyte nuclear factors (eg, HNF-1, -3 β , 4 α , and 4 γ) as well as components involved in protein translation (eg, ribosomal and poly[A] binding proteins), suggesting that both transcriptional and translational changes distinguish tumor from nontumor. In this context, the upregulated expression of the ribosomal protein, S15a (Zhaorui Lian, MD, PhD, et al, unpublished data, 2002), and downregulated expression of the translation initiation factor, *sui1*, by HBxAg [83], suggest that the virus also contributes to transformation post-transcriptionally. Independent work has also shown upregulated expression of a locus that maps to chromosome 6p21–22, which encodes (among other things) a ubiquitin-like protein (SMT3B) that may accelerate the catabolism of selected proteins [72]. The latter suggests post-translational differences in gene expression at the level of protein stability. When downregulated genes were examined, the majority encoded hepatocyte specific gene products (eg, complement components, albumin, and amyloid) and detoxification enzymes (eg, cytochrome P-450), suggesting a less differentiated phenotype [79,81]. Downregulated genes also included several involved in retinoid metabolism (*LY6E* and *RBPI*), whose expression is associated with retinoid-induced differentiation. The independent finding that several downregulated genes in HCC were effectors of liver-enriched

transcription factors, which are important in liver development and differentiation, also suggests that differentiation is altered during hepatocarcinogenesis [80]. These molecular changes are consistent with histopathological changes whereby early, differentiated HCC slowly gives rise to the moderately differentiated or undifferentiated tumors that characterize late tumors. HBV-infected tumor cells also had decreased levels of proteins (such as glutathione peroxidase) that detoxify chemical carcinogens, suggesting that tumor cells have an increased sensitivity to the mutagenic effects of chemical carcinogens. These observations are consistent with the possibility that HBV compromises DNA repair, as discussed above. Although other similar microarray and differential display-PCR studies have been reported [84], the actual roles of up- or downregulated genes in HCC in the development and progression or tumor remain to be studied in most cases. In addition, these studies do not readily distinguish which steps are early versus late, or virus dependent versus independent in the pathogenic pathway, although strides are being made in this direction in a recent study comparing the gene expression patterns in primary tumors compared with intrahepatic metastatic tumors in individual patients [85].

β-catenin signaling

Another important early event involves the mutation of β-catenin, resulting in the constitutive upregulated expression of β-catenin target genes, which include *c-myc*, *c-jun*, cyclin D1, fibronectin, the connective tissue growth factor WISP, and matrix metalloproteinases [86]. In normal liver, β-catenin is a submembranous protein associated with E-cadherin, and participates in cell-cell adhesion. Its phosphorylation results in ubiquitination and proteasome-mediated degradation. Mutation of selected serine residues in β-catenin makes it resistant to phosphorylation and degradation, resulting in the cytoplasmic and nuclear accumulation of β-catenin, which is characteristic of constitutive β-catenin signaling. This is often accompanied by reduced or undetectable expression of E-cadherin [87]. The finding of mutated β-catenin in early stages of human HCC [88–90] and in adenomas of *c-myc* transgenic mice [86], but not in metastatic HCC [91], suggests that mutation results in alterations in normal cell-cell interactions that occur during tumor formation. In addition, the stimulated expression of extracellular matrix (ECM) protein genes by mutant β-catenin suggests that it may play a significant role in the development of fibrosis and cirrhosis, which precede the development of HCC. Further, transgenic mice expressing an oncogenic (mutated) form of β-catenin rapidly develop hepatomegaly [92], demonstrating that constitutively activated β-catenin strongly stimulates hepatocellular growth in vivo. Interestingly, the inhibition of the proteasome by HBxAg [93–95] may block the normal degradation of wild type β-catenin, resulting in its stabilization and accumulation at the early stages of tumor development, although there are presently no data to support this mechanism.

Matrix metalloproteinases

Early nodules of HCC are often small, well differentiated, and are surrounded by a fibrotic capsule that originally formed during the development of cirrhosis. In this context, the success of tumor growth depends upon the invasion of the tumor into the neighboring portal tracts in the liver. This process is accompanied by upregulated expression of matrix metalloproteinase-1 (MMP-1) and -7 (MMP-7) [96,97], which break down ECM, thereby permitting outgrowth of tumor. Elevated MMP-1 expression has been detected in early, differentiated HCC, but is absent from moderately or poorly differentiated tumor cells in late HCC. This suggests that early HCCs grow by destroying ECM, whereas late HCCs grow by virtue of a very high proliferative rate, and that MMP-1 expression correlates with the state of cell differentiation. MMP-7 expression is also upregulated in early, differentiated tumor cells, promoting angiogenesis and the intrahepatic spread of tumor. The finding that β -catenin and the Ets oncoprotein stimulate MMP-7 expression [97], and that HBxAg *trans*-activation maps to Ets binding sites on cellular genes [98], implies that HBxAg may modulate the outgrowth of early HCC by defining the integrity of the ECM during chronic infection.

Early mutations involving loss of heterozygosity

LOH occurs at a number of sites early in hepatocarcinogenesis. For example, several studies have reported that LOH at 1p [99], 4q [100–102], 6q [101,103], and 8p [104] occur early in preneoplastic liver or in small, differentiated tumors [105]. Most of these losses have been mapped to tumor suppressor genes that normally limit hepatocellular growth and survival, although LOH may also include genes involved in DNA repair or in carcinogen metabolism, or that protect against oxidative damage. For example, LOH at chromosome 1p includes many putative tumor-suppressor genes [106], including the p53 relative, p73, which is an imprinted gene that maps to 1p36. The findings that p73 mutations in HCC are infrequent [107], and that p73 expression is upregulated in HCC [108], however, suggest that p73 is not the target of LOH at 1p. Independent evidence has documented LOH at 1p36-p34 not only in HCCs, but also in cirrhotic and dysplastic nodules, indicating the presence of one or more tumor suppressors that are lost before tumor development [109]. One gene (designed RIZ), located within 1p36.13-p36.23, appears to be a tumor suppressor that is frequently deleted in HCC [110], verifying that commonly deleted regions encode negative regulators of hepatocellular growth. LOH at 6q26-27 often involves loss of the mannose 6-phosphate/IGF2 receptor [103], which is also an imprinted gene. This gene has been implicated as a tumor suppressor due to its ability to activate TGF- β 1 signaling (which triggers apoptosis) and to degrade IGF2 (which promotes hepatocellular growth). Mutations in the

M6P/IGF2R have been detected in the majority of dysplastic liver lesions, suggesting that it occurs early in hepatocarcinogenesis [103]; however, mutations in the M6P/IGF2R have been observed rarely [111] or not at all in other studies [112]. LOH at chromosome 4q has been reported in several tumor types, although putative tumor suppressor loci within 4q have not been identified [105,106]. Allelic imbalance in 4q has been observed in cirrhotic nodules [102], confirming the early nature of these changes to tumor development. LOH at 8p has been observed in several tumor types [106], and in HCC, a putative tumor suppressor gene has been identified at 8p21.3-22 [113]. The latter encodes a protein with 80% homology to rat p122RhoGAP, which maintains the organization of the actin stress fibers within microfilaments that are important for cell-cell contact. Its loss may contribute to the disorganization of the cytoskeleton, which is characteristic of tumor cells. Independent observations have also pointed to the existence of multiple putative tumor suppressor genes within chromosome 8p [105,114,115]. The recent report of allelic imbalance in 8p in cirrhotic nodules [102] underscores that these genetic changes occur before tumor development. Hence, it is likely that multiple steps cooperate in the appearance of tumor, and that integrated HBV DNA as well as HBxAg may contribute to the development of some of these steps.

The consequences of LOH may be seen as it affects the degree of ploidy among hepatocytes, which increases with the extent of hepatocellular turnover [116]. Increased polyploidy in cirrhotic livers may translate into impaired regeneration, increased apoptosis, and liver failure, whereas the appearance of aneuploidy, resulting from LOH, provides advantages in growth and survival that contribute importantly to tumor formation. Hence, LOH is a risk factor for HCC, in that it permits hepatocytes to escape normal apoptosis and senescence, which are characteristics commonly associated with the transformed phenotype.

Summary

Although the overview above provides a partial molecular picture of the early stages of stepwise hepatocarcinogenesis, it should be emphasized that tumor and nontumor liver contain multiple changes, and that there is variability in their profile among different patients even within single studies. Variability in the number and types of genetic changes has also been observed geographically, and may be dependent upon the etiology of the tumor (viral, chemical or both) [117]. Interestingly, HBxAg inactivates tumor suppressors (such as p53 [by direct binding] and Rb [by stimulating its phosphorylation]) early in carcinogenesis that are mutated later during tumor progression. HBxAg also constitutively activates signal transduction pathways, such as those involving *c-jun* and ras, and activates oncogenes, such as *c-myc*, that are otherwise activated by β -catenin mutations. These findings suggest common molecular targets in hepatocarcinogenesis, despite

different mechanisms of activation or inactivation. These observations need to be exploited in future drug discovery and in the development of new therapeutics.

Heterogeneity in the mechanisms of tumor development, evidenced by the differences in the up- and downregulated genes reported in microarray analyses, as well as in the genetic loci that undergo mutation or LOH in different reports, has now been well documented. This suggests that there are multiple pathways to HCC, and that there is redundancy in the pathways that regulate cell growth and survival. These findings also reflect that, although hepatocarcinogenesis is multistep, the molecular changes that underpin histopathological changes in tumor development are likely to be different or only partially overlapping in individual tumors. Overall, the consequences of these changes suggest that the pathogenesis of HCC is accompanied by a progressive loss of differentiation, loss of normal cell adhesion, loss of the ECM, and constitutive activation of selected signal transduction pathways that promote cell growth and survival. Although mechanisms are important, attention also has to be paid to the target genes whose altered expression actually mediate the neoplastic phenotype.

Other key avenues of work need to be explored. For example, it will be important to try to identify germline mutations in HBV-infected patients that are passed on to their children, resulting in the development of HCC in childhood [20]. Clinical materials will also be important for the validation of new markers with diagnostic or prognostic potential. In this context, there is an urgent need to establish simple and low-cost tests based upon molecular changes that are hallmarks of HCC development. Identification of patients with early HCC will also significantly increase survival through its impact upon treatment. The discovery and validation of HCC markers may permit accurate staging of lesions, determine the proximity of such lesions to malignancy, and determine whether lesions with a particular genetic profile are still capable of remodeling through appropriate therapeutic intervention [118]. The efficient reintroduction of the relevant tumor suppressors, or the inhibition of oncogene expression by siRNA, provide just some of the additional opportunities that will ultimately be useful in patient treatment. Together, these approaches will go far in reducing the very high morbidity and mortality associated with HCC.

References

- [1] Feitelson MA. Hepatitis B virus and cancer. In: Notkins AL, Oldstone MBA, editors. *Concepts in viral pathogenesis II*. New York: Springer-Verlag; 1986. p. 269–75.
- [2] Nalpas B, Feitelson MA, Brechot C, Rubin E. Alcohol, hepatotropic viruses and hepatocellular carcinoma. *Alcohol* 1995;19:1089–95.
- [3] LaBrecque DR. Neoplasia and the liver. In: Kaplowitz N, editor. *Liver and biliary diseases*. Baltimore (MD): Williams & Wilkins; 1992. p. 347–88.

- [4] Chen CJ, Yu MW, Liaw YF. Epidemiological characteristics and risk factors of hepatocellular carcinoma. *J Gastroenterol Hepatol* 1997;12:S294–308.
- [5] Beasley RP, Hwang LY. Epidemiology of hepatocellular carcinoma. In: Vyas GN, Dienstag JL, Hoofnagle JH, editors. *Viral hepatitis and liver disease*. New York: Grune & Stratton, Inc.; 1984. p. 209–24.
- [6] Kim SO, Park JG, Lee IK. Increased expression of the insulin-like growth factor I (IGF-I) receptor gene in hepatocellular carcinoma cell lines: implications of IGF-I receptor gene activation by hepatitis B virus X gene product. *Cancer Res* 1996;56:3831–6.
- [7] Murakami Y, Hayashi K, Hirohashi S, Sekiya T. Aberrations of the tumor suppressor p53 and retinoblastoma genes in human hepatocellular carcinomas. *Cancer Res* 1991;51:5520–5.
- [8] Nishida N, Fukuda Y, Komeda T, Kita R, Sando T, Furukawa M, et al. Amplification and overexpression of the cyclin D1 gene in aggressive human hepatocellular carcinoma. *Cancer Res* 1994;54:3107–10.
- [9] Peng SY, Lai PL, Hsu MC. Amplification of the c-myc gene in human hepatocellular carcinoma: biologic significance. *J Formos Med Assoc* 1993;92:866–70.
- [10] Boige V, Laurent-Puig P, Fouchet P, Flejou JF, Monges G, Bedossa P, et al. Concerted nonsyntenic allelic losses in hyperploid hepatocellular carcinoma as determined by a high-resolution allelotyping. *Cancer Res* 1997;57:1986–90.
- [11] Marchio A, Meddeb M, Pineau P, Danglot G, Tiollais P, Bernheim A. Recurrent chromosomal abnormalities in hepatocellular carcinoma detected by comparative genomic hybridization. *Genes Chromosomes Cancer* 1997;18:59–65.
- [12] Lencioni R, Caramella D, Bartolozzi C, Di Coscio G. Long-term follow-up study of adenomatous hyperplasia in liver cirrhosis. *Ital J Gastroenterol* 1994;26:163–8.
- [13] Santoni-Rugiu E, Nagy P, Jensen MR, Factor VM, Thorgeirsson SS. Evolution of neoplastic development in the liver of transgenic mice co-expressing c-myc and transforming growth factor- α . *Am J Pathol* 1996;149:407–28.
- [14] Sargent LM, Sanderson SD, Thorgeirsson SS. Ploidy and karyotype alterations associated with early events in the development of hepatocarcinogenesis in transgenic mice harboring c-myc and transforming growth factor α transgenes. *Cancer Res* 1996;56:2137–42.
- [15] Nakau M, Miyoshi H, Seldin MF, Imamura M, Oshima M, Taketo MM. Hepatocellular carcinoma caused by loss of heterozygosity in *Lkb1* gene knockout mice. *Cancer Res* 2002;62:4549–53.
- [16] Ueda H, Ullrich SJ, Gangemi JD, Kappel CA, Ngo L, Feitelson MA, et al. Functional inactivation but not structural mutation of p53 causes liver cancer. *Nat Genet* 1995;9:41–7.
- [17] Factor VM, Kao CY, Santoni-Rugiu E, Weitach JT, Jensen MR, Thorgeirsson SS. Constitutive expression of mature transforming growth factor beta1 in the liver accelerates hepatocarcinogenesis in transgenic mice. *Cancer Res* 1997;57:2089–95.
- [18] Murakami H, Sanderson ND, Nagy P, Marino PA, Merlino G, Thorgeirsson SS. Transgenic mouse model for synergistic effects of nuclear oncogenes and growth factors in tumorigenesis: interaction of c-myc and transforming growth factor alpha in hepatic oncogenesis. *Cancer Res* 1993;53:1719–23.
- [19] Collier J, Sherman M. Screening for hepatocellular carcinoma. *Hepatology* 1998;27:273–8.
- [20] Chang MH, Chen DS, Hsu HC, Hsu HY, Lee CY. Maternal transmission of hepatitis B virus in childhood hepatocellular carcinoma. *Cancer* 1989;64:2377–80.
- [21] Simbulan-Rosenthal CM, Haddad BR, Rosenthal DS, Weaver Z, Coleman A, Luo R, et al. Chromosomal aberrations in PARP(–/–) mice: genome stabilization in immortalized cells by reintroduction of poly(ADP-ribose) polymerase cDNA. *Proc Natl Acad Sci U S A* 1999;96:13191–6.
- [22] Tong WM, Cortes U, Hande MP, Ohgaki H, Cavalli LR, Lansdorp PM, et al. Synergistic role of Ku80 and poly(ADP-ribose) polymerase in suppressing chromosomal aberrations and liver cancer formation. *Cancer Res* 2002;62:6990–6.

- [23] Werner M, Nolte M, Georgii A, Klempnauer J. Chromosome 1 abnormalities in hepatocellular carcinoma. *Cancer Genet Cytogenet* 1993;66:130.
- [24] Nagai H, Pineau P, Tiollais P, Buendia MA, Dejean A. Comprehensive allotyping of human hepatocellular carcinoma. *Oncogene* 1997;14:2927–33.
- [25] Chernet BW, McBride OW, Chen D, Alkhatib H, Bhatia KG, Hensley P, et al. cDNA sequence, protein structure, and chromosomal location of human gene for poly(ADP-ribose)polymerase. *Proc Natl Acad Sci U S A* 1987;84:8370–4.
- [26] Blunt T, Taccioli GE, Priestley A, Hafezparast M, McMillan T, Liu J, et al. A YAC contig encompassing the XRCC5 (Ku80) DNA repair gene and complementation defective cells by YAC protoplast fusion. *Genomics* 1995;30:320–8.
- [27] Giardiello FM, Welsh SB, Hamilton SR, Offerhaus GJA, Gittelsohn AM, Booker SV, et al. Increased risk of cancer in the Peutz-Jeghers syndrome. *N Engl J Med* 1987; 316:1511–4.
- [28] Tiainen M, Ylikorkkala A, Makela TP. Growth suppression by *Lkb1* is mediated by a G1 cell cycle arrest. *Proc Natl Acad Sci U S A* 1999;96:9248–51.
- [29] Ylikorkkala A, Rossi DJ, Korsisaari N, Luukko K, Alitalo K, Henkemeyer M, et al. Vascular abnormalities and deregulation of *VEGF* in *Lkb1*-deficient mice. *Science* 2001; 293:1323–6.
- [30] Feitelson MA, Duan XL. Hepatitis B virus x antigen in the pathogenesis of chronic infections and the development of hepatocellular carcinoma. *Am J Pathol* 1997;150: 1141–57.
- [31] Feitelson MA. Hepatitis B virus in hepatocarcinogenesis. *J Cell Physiol* 1999;181:188–202.
- [32] Lee T, Elledge SJ, Butel JS. Hepatitis B virus X protein interacts with a probably cellular DNA repair protein. *J Virol* 1995;69:1107–14.
- [33] Becker SA, Lee TH, Butel JS, Slagle BL. Hepatitis B virus X protein interferes with cellular DNA repair. *J Virol* 1998;72:266–72.
- [34] Hwang BJ, Liao JC, Chu G. Isolation of a cDNA encoding a UV-damaged DNA binding factor defective in xeroderma pigmentosum group E cells. *Mutat Res* 1996;362:105–17.
- [35] Kazantsev A, Mu D, Nichols AF, Zhao X, Linn S, Sancar A. Functional complementation of xeroderma pigmentosum complementation group E by replication protein A in an *in vitro* system. *Proc Natl Acad Sci U S A* 1996;93:5014–8.
- [36] Feitelson MA, Zhu M, Duan LX, London WT. Hepatitis B X antigen and p53 are associated *in vitro* and in liver tissues from patients with primary hepatocellular carcinoma. *Oncogene* 1993;8:1109–17.
- [37] Wang XW, Forrester K, Yeh H, Feitelson MA, Gu JR, Harris CC. Hepatitis B virus X protein inhibits p53 sequence-specific DNA binding, transcriptional activity, and association with transcription factor ERCC3. *Proc Natl Acad Sci U S A* 1994;91: 2230–4.
- [38] Bootsma D, Hoeijmakers JHJ. Engagement with transcription. *Nature* 1993;363:114–5.
- [39] Schaeffer L, Roy R, Humbert S, Moncollin V, Vermeulen W, Hoeijmakers JHJ, et al. DNA repair helicase: A component of BTF2 (TFIIH) basic transcription factor. *Science* 1993;260:58–63.
- [40] Madden CR, Fingegold MJ, Slagle BL. Expression of hepatitis B x protein does not alter the accumulation of spontaneous mutations in transgenic mice. *J Virol* 2000;74:5266–72.
- [41] Feitelson MA. Hepatocellular injury in hepatitis B and C infections. In: Feitelson MA, Zern M, editors. *Clinics in laboratory medicine*, vol. 16, no. 2. Philadelphia: WB Saunders Co.; 1996. p. 307–24.
- [42] Dunsford HA, Sell S, Chisari FV. Hepatocarcinogenesis due to chronic liver cell injury in HBV transgenic mice. *Cancer Res* 1990;50:3400–7.
- [43] Hagen TM, Suang S, Curnutte J, Fowler P, Martinez V, Wehr CM, et al. Extensive oxidative DNA damage in hepatocytes of transgenic mice with chronic active hepatitis destined to develop hepatocellular carcinoma. *Proc Natl Acad Sci U S A* 1994;91: 12808–12.

- [44] Guyton KZ, Kensler TW. Oxidative mechanisms in carcinogenesis. *Br Med Bull* 1993;49: 523–44.
- [45] Ohsima H, Bartsch H. Chronic infections and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis. *Mutat Res* 1994;305:253–64.
- [46] Meyer M, Caselmann WH, Schluter V, Schreck R, Hofschneider PH, Baeuerle PA. Hepatitis B virus transactivator MHBS¹: activation of NF- κ B, selective inhibition by antioxidants and integral membrane localization. *EMBO J* 1992;11:2991–3001.
- [47] Hino O, Tabata S, Hotta Y. Evidence for increased in vitro recombination with insertion of human hepatitis B virus DNA. *Proc Natl Acad Sci U S A* 1991;88:9248–52.
- [48] Livezey KW, Simon D. Accumulation of genetic alterations in a human hepatoma cell line transfected with hepatitis B virus. *Mutat Res* 1997;377:187–98.
- [49] Laurent-Puig P, Legoix P, Bluteau O, Belghiti J, Franco D, Binot F, et al. Genetic alterations associated with hepatocellular carcinomas define distinct pathways of hepatocarcinogenesis. *Gastroenterology* 2001;120:1763–73.
- [50] Wang J, Chenivresse X, Henglein B, Brechot C. Hepatitis B virus integration in a cyclin A gene in a hepatocellular carcinoma. *Nature* 1990;343:555–7.
- [51] Benbrook D, Lernhardt E, Pfahl M. A new retinoic acid receptor identified from a hepatocellular carcinoma. *Nature* 1988;333:669–72.
- [52] Gozuacik D, Mrakami Y, Saigo K, Chami M, Mugnier C, Lagorce D, et al. Identification of human cancer-related genes by naturally occurring Hepatitis B Virus DNA tagging. *Oncogene* 2001;20:6233–40.
- [53] Dejean A, de The H. Hepatitis B virus as an insertional mutagen in a human hepatocellular carcinoma. *Mol Biol Med* 1990;7:213–22.
- [54] Dejean A, Sonigo P, Wain-Hobson S, Tiollais P. Specific hepatitis B virus integration in hepatocellular carcinoma DNA through a viral 11-base-pair direct repeat. *Proc Natl Acad Sci U S A* 1984;81:5350–4.
- [55] Diamantis ID, McGandy CE, Chen TJ, Liaw YF, Gudat F, Bianchi L. Hepatitis B X-gene expression in hepatocellular carcinoma. *J Hepatol* 1992;15:400–3.
- [56] Paterlini P, Poussin K, Kew M, Franco D, Brechot C. Selective accumulation of the X transcript of hepatitis B virus in patients negative for hepatitis B surface antigen with hepatocellular carcinoma. *Hepatology* 1995;21:313–21.
- [57] Haruna Y, Hayashi N, Katayama K, Yuki N, Kasahara A, Sasaki Y, et al. Expression of X protein and hepatitis B virus replication in chronic hepatitis. *Hepatology* 1991;13:417–21.
- [58] Wang W, London WT, Feitelson MA. Hepatitis B x antigen in hepatitis B virus carrier patients with liver cancer. *Cancer Res* 1991;51:4971–7.
- [59] Wang W, London WT, Lega L, Feitelson MA. Hepatitis B x antigen in liver from carrier patients with chronic hepatitis and cirrhosis. *Hepatology* 1991;14:29–37.
- [60] Poussin K, Kienes H, Sirma H, Urban S, Beaugrand M, Franco D, et al. Expression of mutated hepatitis B virus X genes in human hepatocellular carcinomas. *Int J Cancer* 1999; 80:497–505.
- [61] Elmore L, Hancock AR, Chang SF, Wang XW, Chang S, Callahan CP, et al. Hepatitis B virus X protein and p53 tumor suppressor interactions in the modulation of apoptosis. *Proc Natl Acad Sci U S A* 1997;94:14707–12.
- [62] Kim H, Lee H, Yun Y. X-gene product of hepatitis B virus induces apoptosis in liver cells. *J Biol Chem* 1998;273:381–5.
- [63] Sirma H, Giannini C, Poussin K, Paterlini P, Kremsdorf D, Brechot C. Genetic and functional analysis of the effects of hepatitis B viral transactivator HBx on cell growth and apoptosis: implications for viral replication and hepatocarcinogenesis. In: Fleig WE, editor. Normal and malignant liver cell growth: FALK workshop. Norwell (MA): Kluwer Academic Publishers; 1999. p. 171–86.
- [64] Tu H, Bonura C, Giannini C, Mouly H, Soussan P, Kew M, et al. Biological impact of natural COOH-terminal deletions of hepatitis B virus X protein in hepatocellular carcinoma tissues. *Cancer Res* 2001;61:7803–10.

- [65] Huo TI, Wang XW, Forgues M, Wu CG, Spillare EA, Giannini C, et al. Hepatitis B virus X mutants derived from human hepatocellular carcinoma retain the ability to abrogate p53-induced apoptosis. *Oncogene* 2001;20:3620–8.
- [66] Feitelson MA, Reis H, Pan J, Lian Z, Fang J, Liu J, et al. Abrogation of negative growth regulatory pathways by hepatitis B virus encoded X antigen in the development of hepatocellular carcinoma. In: Fleig WE, editor. Normal and malignant liver cell growth: FALK workshop. Norwell (MA): Kluwer Academic Publishers; 1999. p. 156–70.
- [67] Sun BS, Zhu X, Clayton MM, Pan J, Feitelson MA. Identification and preliminary characterization of a protein involved in cellular senescence which binds to hepatitis B virus X antigen. *Hepatology* 1998;27:228–39.
- [68] D'Arville CN, Nouri-Aria KT, Johnson P, Williams R. Regulation of insulin-like growth factor II gene expression by hepatitis B virus in hepatocellular carcinoma. *Hepatology* 1991;13:310–5.
- [69] Aihara T, Noguchi S, Miyoshi Y, Nakano H, Sasaki Y, Nakamura Y, et al. Allelic imbalance of insulin-like growth factor II gene expression in cancerous and precancerous lesions of the liver. *Hepatology* 1998;28:86–9.
- [70] Benn J, Schneider RJ. HBV X protein activates ras-GTP complex formation and establishes a ras, raf, MAP kinase signaling cascade. *Proc Natl Acad Sci U S A* 1994;91:10350–4.
- [71] Lucito R, Schneider RJ. Hepatitis B virus X protein activates transcription factor NF-kappa B without a requirement for protein kinase C. *J Virol* 1992;66:983–91.
- [72] Shirota Y, Keneko S, Honda M, Kawai HF, Kobayashi K. Identification of differentially expressed genes in hepatocellular carcinoma with cDNA microarrays. *Hepatology* 2001;33:832–40.
- [73] Lian Z, Liu J, Pan J, Tufan NLS, Zhu M, Arbuthnot P, et al. A cellular gene up-regulated by hepatitis B virus encoded X antigen promotes hepatocellular growth and survival. *Hepatology* 2001;34:146–57.
- [74] Tufan NLS, Lian Z, Liu J, Pan J, Arbuthnot P, Kew M, et al. Hepatitis B x antigen stimulates expression of a novel cellular gene, URG4, that promotes hepatocellular growth and survival. *Neoplasia* 2002;4:355–68.
- [75] Lian Z, Liu J, Li L, Li X, Tufan NLS, Clayton MM, et al. Up-regulated expression of a unique gene by hepatitis B x antigen promotes hepatocellular growth and tumorigenesis. *Neoplasia* 2003;5:229–44.
- [76] Yoo YD, Ueda H, Park K, Flanders KC, Lee YI, Jay G, et al. Regulation of transforming growth factor-beta 1 expression by the hepatitis B virus (HBV) X transactivator. Role in HBV pathogenesis. *J Clin Invest* 1996;97:388–95.
- [77] Rossmann W, Schulte-Hermann R. Biology of transforming growth factor beta in hepatocarcinogenesis. *Microsc Res Tech* 2001;52:430–6.
- [78] Giannelli G, Fransvea E, Marinosci F, Bergamini C, Colucci S, Schiraldi O, et al. Transforming growth factor- β 1 triggers hepatocellular carcinoma invasiveness via α 3 β 1 integrin. *Am J Pathol* 2002;161:183–93.
- [79] Okabe H, Satoh S, Kato T, Kitahara O, Yanagawa R, Yamaoka Y, et al. Genome-wide analysis of gene expression in human hepatocellular carcinomas using cDNA microarray: identification of genes involved in viral carcinogenesis and tumor progression. *Cancer Res* 2001;61:2129–37.
- [80] Xu XR, Huang J, Xu ZG, Qian BZ, Zhu ZD, Yan Q, et al. Insight into hepatocellular carcinogenesis at transcriptome level by comparing gene expression profiles of hepatocellular carcinoma with those of corresponding nontumor liver. *Proc Natl Acad Sci U S A* 2001;98:15089–94.
- [81] Chen X, Cheung ST, So S, Fan ST, Barry C, Higgins J, et al. Gene expression patterns in human liver cancers. *Mol Biol Cell* 2002;13:1929–39.
- [82] Benn J, Schneider RJ. Hepatitis B virus HBx protein deregulates cell cycle checkpoint controls. *Proc Natl Acad Sci U S A* 1995;92:11215–9.

- [83] Lian Z, Pan J, Liu J, Zhu M, Arbutnot P, Kew MC, et al. The translation initiation factor, SUI1, may be a target of hepatitis B x antigen in hepatocarcinogenesis. *Oncogene* 1999;18:1677–87.
- [84] Kim MY, Park E, Park JH, Park DH, Moon WS, Cho BH, et al. Expression profile of none novel gene differentially expressed in hepatitis B virus-associated hepatocellular carcinomas. *Oncogene* 2001;20:4568–75.
- [85] Cheung ST, Chen X, Guan XY, Wong SY, Tai LS, Ng IOL, et al. Identify metastasis-associated genes in hepatocellular carcinoma through clonality delineation for multinodular tumor. *Cancer Res* 2002;62:4711–21.
- [86] Calvisi DF, Factor VM, Loi R, Thorgeirsson SS. Activation of beta-catenin during hepatocarcinogenesis in transgenic mouse models: relationship to phenotype and tumor grade. *Cancer Res* 2001;61:2085–91.
- [87] Xu L, Hui L, Wang S, Gong J, Jin Y, Wang Y, et al. Expression profiling suggested a regulatory role of liver-enriched transcription factors in human hepatocellular carcinoma. *Cancer Res* 2001;61:3176–81.
- [88] De La Costa A, Romagnolo B, Billuart P, Renard CA, Buendia MA, Soubrane O, et al. Somatic mutations of the beta-catenin gene are frequent in mouse and human hepatocellular carcinomas. *Proc Natl Acad Sci U S A* 1998;95:8847–51.
- [89] Hsu HC, Jeng YM, Mao TL, Chu JS, Lai PL, Peng SY. Beta-catenin mutations are associated with a subset of low-stage hepatocellular carcinoma negative for hepatitis B virus and with favorable prognosis. *Am J Pathol* 2000;157:763–70.
- [90] Miyoshi Y, Iwao K, Nagasawa Y, Aihara T, Sasaki Y, Imaoka S, et al. Activation of the beta-catenin gene in primary hepatocellular carcinomas by somatic alterations involving exon 3. *Cancer Res* 1998;58:2524–7.
- [91] Mao TL, Chu JS, Jeng YM, Lai PL, Hsu HC. Expression of mutant nuclear beta-catenin correlates with non-invasive hepatocellular carcinoma, absence of portal vein spread, and good prognosis. *J Pathol* 2001;193:95–101.
- [92] Cadoret A, Ovejero C, Saadi-Kheddouci S, Souil E, Fabre M, Romagnolo B, et al. Hepatomegaly in transgenic mice expressing an oncogenic form of beta-catenin. *Cancer Res* 2001;61:3245–9.
- [93] Huang J, Kwong J, Sun ECY, Liang TJ. Proteasome complex as a potential cellular target of hepatitis B virus X protein. *J Virol* 1996;70:5582–91.
- [94] Hu Z, Zhang Z, Doo E, Coux O, Goldberg AL, Liang TJ. Hepatitis B virus X protein is both a substrate and a potential inhibitor of the proteasome complex. *J Virol* 1999;73:7231–40.
- [95] Zhang Z, Torii N, Furusaka A, Malayaman N, Hu Z, Liang TJ. Structural and functional characterization of interaction between hepatitis B virus X protein and the proteasome complex. *J Biol Chem* 2000;275:15157–65.
- [96] Okazaki I, Wada N, Nakano M, Saito A, Takasaki K, Doi M, et al. Difference in gene expression for matrix metalloproteinase-1 between early and advanced hepatocellular carcinomas. *Hepatology* 1997;25:580–4.
- [97] Ozaki I, Mizuta T, Zhao G, Yotsumoto H, Hara T, Kajihara S, et al. Involvement of the Ets-1 gene in over-expression of matrilysin in human hepatocellular carcinoma. *Cancer Res* 2000;60:6519–25.
- [98] Park US, Park SK, Lee YI, Park JG, Lee YI. Hepatitis B virus-X protein upregulates the expression of p21waf1/cip1 and prolongs G1→S transition via a p53-independent pathway in human hepatoma cells. *Oncogene* 2000;19:3384–94.
- [99] Kuroki T, Fujiwara Y, Tsuchiya E, Nakamori S, Imaoka S, Kanematsu T, et al. Accumulation of genetic changes during development and progression of hepatocellular carcinoma: loss of heterozygosity of chromosome arm 1p occurs at an early stage of hepatocarcinogenesis. *Genes Chromosomes Cancer* 1995;13:163–7.
- [100] Konishi M, Kikuchi-Yanoshita R, Tanaka K, Sato C, Tsuruta K, Maeda Y, et al. Genetic changes and histopathological grades in human hepatocellular carcinomas. *Jpn J Cancer Res* 1993;84:893–9.

- [101] Niketeghad F, Decker HJ, Caselmann WH, Lund P, Geissler F, Dienes HP, et al. Frequent genomic imbalances suggest commonly altered tumour genes in human hepatocarcinogenesis. *Br J Cancer* 2001;85:697–704.
- [102] Yeh SH, Chen PJ, Shau WY, Chen YW, Lee PH, Chen JT, et al. Chromosomal allelic imbalance evolving from liver cirrhosis to hepatocellular carcinoma. *Gastroenterology* 2001;121:699–709.
- [103] DeSouze AT, Hankins GR, Washington MK, Fine RL, Orton TC, Jirtle RL. Frequent loss of heterozygosity on 6q at the mannose 6-phosphate/insulin-like growth factor II receptor locus in human hepatocellular tumors. *Oncogene* 1995;10:1725–9.
- [104] Kishimoto Y, Shiota G, Wada K, Kitano M, Nakamoto K, Kamisaki Y, et al. Frequent loss in chromosome 8p loci in liver cirrhosis accompanying hepatocellular carcinoma. *J Cancer Res Clin Oncol* 1996;122:585–9.
- [105] Huang SF, Hsu HC, Fletcher JA. Investigation of chromosomal aberrations in hepatocellular carcinoma by fluorescence in situ hybridization. *Cancer Genet Cytogenet* 1999;111:21–7.
- [106] Knuutila S, Aalto Y, Autio K, Bjorkqvist AM, El-Rifai W, Hemmer S, et al. DNA copy number losses in human neoplasms. *Am J Pathol* 1999;155:683–94.
- [107] Peng CY, Tsai SL, Yeh CT, Hung SP, Chen MF, Chen TC, et al. Genetic alternations of p73 are infrequent but may occur in early stage hepatocellular carcinoma. *Anticancer Res* 2000;20:1487–92.
- [108] Tannapfel A, Wasner M, Krause K, Geissler F, Katalinic A, Hauss J, et al. Expression of p73 and its relation to histopathology and prognosis in hepatocellular carcinoma. *J Natl Cancer Inst* 1999;91:1154–8.
- [109] Sun M, Eshleman JR, Ferrell LD, Jacobs G, Sudilovsky EC, Tuthill R, et al. An early lesion in hepatic carcinogenesis: loss of heterozygosity in human cirrhotic livers and dysplastic nodules at the 1p36-p34 region. *Hepatology* 2001;33:1415–24.
- [110] Fang W, Piao Z, Simon D, Sheu JC, Huang S. Mapping of a minimal deleted region in human hepatocellular carcinoma to 1p36.13-p36.23 and mutational analysis of the RIZ (PRDM2) gene localized to the region. *Genes Chromosomes Cancer* 2000;28:269–75.
- [111] Kawate S, Takenoshita S, Ohwada S, Mogi A, Fukusato T, Makita F, et al. Mutation analysis of transforming growth factor beta type II receptor, Smad2, and Smad4 in hepatocellular carcinoma. *Int J Oncol* 1999;14:127–31.
- [112] Wada I, Kanada H, Nomura K, Kato Y, Machinami R, Kitagawa T. Failure to detect genetic alteration of the mannose-6-phosphate/insulin-like growth factor 2 receptor (M6P/IGF2R) gene in hepatocellular carcinomas in Japan. *Hepatology* 1999;29:1718–21.
- [113] Yuan BZ, Miller MJ, Keck CL, Zimonjic DB, Thorgeirsson SS, Popescu NC. Cloning, characterization, and chromosomal localization of a gene frequently deleted in human liver cancer (DLC-1) homologous to rat RhoGAP. *Cancer Res* 1998;58:2196–9.
- [114] Piao Z, Kim NG, Kim H, Park C. Deletion mapping on the short arm of chromosome 8 in hepatocellular carcinoma. Identification of three distinct regions of allelic deletions on the short arm of chromosome 8 in hepatocellular carcinoma. *Cancer Lett* 1999;138:227–32.
- [115] Pineau P, Nagai H, Prigent S, Wei Y, Gyapay G, Weissenbach J, et al. Identification of three distinct regions of allelic deletions on the short arm of chromosome 8 in hepatocellular carcinoma. *Oncogene* 1999;18:3127–34.
- [116] Gupta S. Hepatic polyploidy and liver growth control. *Semin Cancer Biol* 2000;10:161–71.
- [117] Wong N, Lai P, Pang E, Fung LF, Sheng Z, Wong V, et al. Genomic aberrations in human hepatocellular carcinomas of differing etiologies. *Clin Cancer Res* 2000;6:4000–9.
- [118] Wu TC, Tong MJ, Hwang B, Lee SD, Hu MM. Primary hepatocellular carcinoma and hepatitis B virus infection during childhood. *Hepatology* 1987;7:46–8.