World Journal of Gastrointestinal Oncology

Online Submissions: http://www.wjgnet.com/1948-5204office
wjgo@wjgnet.com
doi:10.4251/wjgo.v2.i6.259

World J Gastrointest Oncol 2010 June 15; 2(6): 259-264
ISSN 1948-5204 (online)
© 2010 Baishideng. All rights reserved.

EDITORIAL

From chronic liver disorders to hepatocellular carcinoma: Molecular and genetic pathways

Enzo Ierardi, Rosa Rosania, Mariangela Zotti, Floriana Giorgio, Simonetta Prencipe, Nicola Della Valle, Vincenzo De Francesco, Carmine Panella

Enzo Ierardi, Rosa Rosania, Mariangela Zotti, Floriana Giorgio, Simonetta Prencipe, Nicola Della Valle, Vincenzo De Francesco, Carmine Panella, Section of Gastroenterology, Department of Medical Sciences, University of Foggia, Ospedali Riuniti, Viale L. Pinto 71100-Foggia, Italy

Author contributions: Ierardi E and Panella C designed the study, revised the manuscript and approved the final version; Rosania R, Zotti M, Prencipe S and Giorgio F collected the data; De Francesco V, Della Valle N and Ierardi E analyzed the data; Rosania R, Zotti M, Prencipe S and Giorgio F drafted the manuscript.

Correspondence to: Enzo Ierardi, MD, Professor, Section of Gastroenterology, Department of Medical Sciences, University of Foggia, Ospedali Riuniti, Viale L. Pinto 71100-Foggia, Italy. enzo.ierardi@fastwebnet.it

Telephone: +39-881-733848 Fax: +39-881-733849
Received: August 27, 2009 Revised: November 24, 2009
Accepted: December 1, 2009
Published online: June 15, 2010

Abstract

Hepatocarcinogenesis is a process attributed to progressive genomic changes that alter the hepatocellular phenotype producing cellular intermediates that evolve into hepatocellular carcinoma (HCC). During the preneoplastic phase, the liver is often the site of chronic hepatitis and/or cirrhosis, and these conditions induce liver regeneration with accelerated hepatocyte cycling in an organ that is otherwise proliferatively at rest. Hepatocyte regeneration is accelerated by upregulation of mitogenic pathways involving molecular and genetic mechanisms. Hepatic growth factors, inhibitors and triggers may also play a role. This process leads to the production of monoclonal populations of aberrant and dysplastic hepatocytes that have telomerase re-expression, microsatellite instability, and occasionally structural aberrations in genes and chromosomes. Development of dysplastic hepatocytes in foci and nodules and the emergence of HCC are associated with the accumulation of irreversible structural alterations in genes and chromosomes even if the genomic basis of the malignant phenotype is largely heterogeneous. Therefore, a malignant hepatocyte phenotype may be produced by changes in genes acting through different regulatory pathways, thus producing several molecular variants of HCC. On these bases, a key point for future research will be to determine whether the deletions are specific, due to particular loci in the minimally deleted regions of affected chromosome arms, or whether they are non-specific with loss of large portions of chromosomes or entire chromosome arms leading to passive deletion of loci. The final aim is the possibility of identifying a step where carcinogenetic processes could be terminated.

© 2010 Baishideng. All rights reserved.

Key words: Hepatocarcinoma; Chronic liver disorders; Genetic pathways; Molecular pathways; Hepatic growth factors; Augmenter liver regeneration

Peer reviewer: George G Chen, Professor, Department of Surgery, University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong, China

Ierardi E, Rosania R, Zotti M, Giorgio F, Prencipe S, Della Valle N, De Francesco V, Panella C. From chronic liver disorders to hepatocellular carcinoma: Molecular and genetic pathways. *World J Gastrointest Oncol* 2010; 2(6): 259-264 Available from: URL: http://www.wjgnet.com/1948-5204/full/v2/i6/259.htm DOI: http://dx.doi.org/10.4251/wjgo.v2.i6.259

INTRODUCTION

Hepatocarcinogenesis is a process attributed to progressive genomic changes that alter the hepatocellular phenotype producing cellular intermediates that evolve into hepatocellular carcinoma (HCC). The aim of this review was to trace epidemiological features within actual world distributions and to suggest possible future changes based on

recent advancements. Moreover, risk factors and etiological mechanisms that trigger this complex process have been analyzed and described according to state of art investigations.

EPIDEMIOLOGICAL FEATURES

HCC is one of the most frequently occuring neoplasms worldwide. It represents the third most common cause of cancer-related deaths throughout the world with an estimated annual 600 000 deaths[1]. Its incidence, defined as new cases/year, was assessed as 564 000 in 2001[2] and is currently assessed as approximately 550 000. In 70%-90% of cases, HCC develops on a background of chronic liver inflammation or cirrhosis. Regional differences in the incidence of HCC are significant. High incidence areas are sub-Saharan Africa and southeast Asia, whereas low incidence zones are northern/western Europe and North America[3-6]. However, there is a trend towards increasing rates of HCC in developed countries. In the United States, the age-adjusted incidence has doubled over the past two decades[7]. Male predominance has been demonstrated showing a male/female ratio of 2-4:1[8].

RISK FACTORS AND ETIOLOGY

In the areas with a high incidence of HCC, most cases are related to hepatitis B (HBV) and C viruses (HCV)[9]. In developed countries, a high intake of alcohol and non alcoholic steato-hepatitis are additional factors contributing to HCC[6]. Other known causes are aflatoxin and metabolic diseases such as hemochromatosis, α 1-antitrypsin deficiency and hereditary tyrosinaemia. As previously described, a very common etiological factor of HCC is chronic HBV infection. HBV is a DNA virus and it has a characteristic life cycle that includes its integration into the host genome, which can induce chromosomal instability resulting in rearrangements or deletions, such as insertional mutations at specific sites[10]. The process can activate endogenous genes, such as cyclin A and sarcoplasmic endoplasmic reticulum 1 (SERCA 1), which stimulate cell proliferation provoking uncontrolled liver regeneration[11,12]. Integration of HBV DNA may also deregulate the expression of oncogenes or tumour suppressor genes that are able to control cell death/survival[13]. Moreover, HBV can induce hepatocarcinogenesis through the expression of viral proteins that have oncogenic properties. X protein plays a relevant role by activating different promoters and triggering the activation of transcription factors such as activator protein 1 and nuclear factor κ B[14-16]. HBV x protein (HBVx) can affect and modify the expression of a variety of genes that are involved in the control of the cell cycle. In fact, HBx represses p53-mediated transcriptional activation and inactivates p53 mediated apoptosis, which are well-known mechanisms of genomic integrity control. The effects of HBx on p53 may be a possible mechanism for malignant transformation of HBV infected cells[17].

Unlike HBV, HCV is an RNA virus that does not integrate its genome, but acts through a protein interaction. Core protein, non structural protein 3 (NS3) and protein 5 A (NS5A) are the HCV proteins correlated with hepatocarcinogenesis. The HCV core protein seems to play a role in hepatocarcinogenesis through interference with the modulation of cellular proliferation/apoptosis and immunological control[18-20]. Additionally, HCV core protein has a transcriptional regulation function on different cellular genes involved in the regulation of cell growth, including the proto-oncogene c-myc[21]. HCV core protein may also induce carcinogenesis through other mechanisms such as changes in tumour necrosis factor α release, which is known to interfere with proliferation/apoptosis balance[22,23].

Another HCC carcinogen is aflatoxin, a mycotoxin from Aspergillus flavus and Aspergillus parasiticus. Aflatoxin (AFB) can damage hepatocyte DNA after its conversion into exo-8,9-epoxide by cytochrome p 450. After this conversion, AFB-8,9-epoxide reacts with guanine nucleotides to form a connection, which induces a genetic mutation. Aflatoxin, moreover, can alter p53 control of cell proliferation[24-26].

Additional HCC inducing factors are metabolic diseases such as hemochromatosis, which is an excess of iron accumulation in the liver, resulting in fibrosis and cirrhosis. Iron acts on oxidative stress, generating reactive oxygen intermediates that disturb the redox equilibrium of cells leading to lipid peroxidation of unsaturated fatty acids in the membranes of cells and organelles. Moreover, free iron induces immunological abnormalities that may decrease immune surveillance for malignant transformation[27].

Another proposed process is mutational effects on androgen receptors (AR), which act as transcription factors. AR mutations involving the hormone binding domain could increase AR function and promote carcinogenesis. Elevated AR levels in atypical cells could have a tumour promoting effect by stimulating cell growth[28].

HEPATOCARCINOGENESIS TIMING AND MODALITIES

HCC develops in the setting of chronic hepatitis or cirrhosis, in which many hepatocytes die, inflammatory cells invade the liver and connective tissue is deposited[29]. Dysregulation of the balance between cell proliferation and cell death, due to the decreased expression of some pro-apoptotic genes and/or to the overactivation of anti-apoptotic pathways, represents a well supported pro-tumorigenic principle in this context[30]. Pathologically, human HCC develops in a multistep fashion in the following sequence: from low-grade dysplastic nodules (LGDN) to high-grade dysplastic nodules, to early HCC, then well-differentiated HCC and moderately differentiated HCC[31,32].

Differentiation between LGDN and early HCC is the most important issue in a clinical setting. Initial hepatocellular alterations include foci of phenotypically altered and, subsequently, dysplastic hepatocytes[33]. Dysplastic nodules

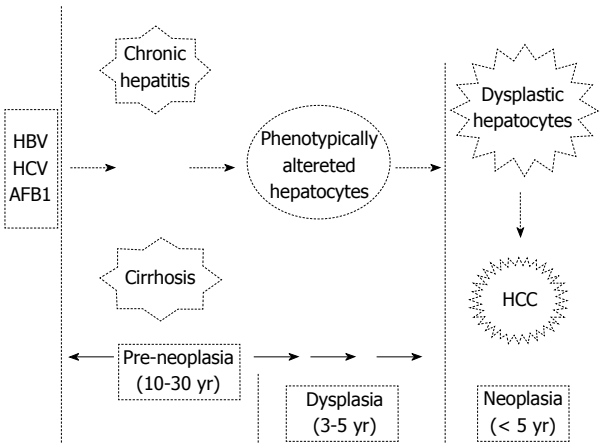

Figure 1 The "timing" of hepatocarcinogenesis from chronic liver disorders[29]. HBV: Hepatitis B viruses; HCV: Hepatitis C viruses; HCC: hepatocellular carcinoma.

are precancerous lesions, which are divided into high and low grades based on histological/cytological changes and the presence/absence of portal tracts[31]. There is no destruction of the underlying hepatic structure in early HCC and tumour invasion into the ntratumoral portal tracts has been suggested as a helpful distinctive sign of LGDN[34]. The "timing" of HCC development from chronic liver disorders is summarized in Figure 1.

MOLECULAR PATHWAYS

The development and progression of HCC is accompanied by complex changes in the pattern of gene expression. In the pre-neoplastic phase, elevated manifestations of transforming growth factor-α (TGF-α) and insulin-like growth factor-2 (IGF-2) are responsible for accelerated hepatocyte proliferation. An upregulation of TGF-α and IGF-2 results from the combined actions of cytokines produced by chronic inflammatory cells, viral transactivation and regenerative responses of the liver. This altered pattern reflects the expression of several genes strongly upregulated during the preneoplastic stage. Consequently, a monoclonal hepatocyte population develops with progressive telomere shortening and re-expression of the telomerase enzyme complex[35,36]. The activation of cellular oncogenes and the inactivation of tumour suppressor genes result in a deregulation of signalling pathways in HCC. The wingless gene family (Wnt), β-catenin, ras, p14ARF/p53, P16INK4A/Rb, TGF-β and PTEN/Akt may show a changed pattern in hepatocytic monoclonal elements[8].

Growth factors

Liver regeneration is controlled by several growth factors that may be involved in the triggering, progression and termination of hepatocyte replication, especially because hepatocytes are usually in a quiescent state (about 1/1000 hepatocytes are outside the G0 phase of the cell cycle)[37,38]. Hepatocyte growth factor (HGF) is a potent mitogen of mature hepatocytes, is produced by mesenchymal cells (Kupffer's, sinusoidal endothelial and Ito cells) and acts on a wide variety of epithelial cells. It acts as a mitogen (stimulation of cell growth), motogen (stimulation of cell motility), and morphogen (induction of multicellular architectures). Moreover, HGF has anti-apoptotic, angiogenic, and immunoregulatory activities. HGF is considered to be a key molecule for the construction of normal tissue structure during embryogenesis, organogenesis, and organ regeneration after damage. HGF may act as a hepatotrophic factor for liver regeneration through two mechanisms: paracrine and endocrine, through mediation in epithelial-mesenchymal interactions[39-41].

Another hepatotrophic factor is the augmenter of liver regeneration (ALR), originally called hepatic regenerative stimulation substance. ALR specifically stimulates hepatic cell proliferation only in the presence of liver damage. It is able to specifically stimulate hepatocyte proliferation and support liver regeneration in an organ specific, but species non-specific manner, by inducing a significant increase in mitochondrial gene expression associated with enhanced cytochrome content and oxidative phosphorylation capacity[41-45]. It is likely that ALR might mediate its activity by regional regulation of natural killer cells, which exert a specific cytotoxicity against regenerating hepatocytes[44]. The ALR gene could be the mammalian version of the ERV-1 gene because it codes for the synthesis/stability of nuclear and mitochondrial transcripts[46].

TGF-α is an autocrine stimulator of hepatocyte proliferation, which increases transiently in replicating hepatocytes both *in vivo* and *in vitro*[47]. Constitutive TGF-α overexpression in young transgenic mice causes liver hypertrophy and enhanced proliferation that progresses to hepatic tumor development[48]. A transient increase of TGF-β 1 in regenerating liver may promote the formation of extracellular matrix components and signal the end of hepatocyte proliferation. Prolonged overexpression of the factor in nonparenchymal cells causes liver fibrosis both in humans and experimental animals[49,50].

Epidermal growth factor (EGF) is still the most frequently used polypeptide hormone to induce hepatic DNA synthesis in cultures. In addition, it affects amino acid transport and protein synthesis in the liver[51,52]. HGF (hepatopoietin A-HPTA) is a heparin-binding growth factor. HPTA stimulates mitogenesis and causes morphological change in hepatocytes, including cellular enlargement. Its mitogenic effect is inhibited by TGF-β[53]. Hepatopoietin B is a complete hepatocytes mitogen but is less active than EGF or HPTA. In addition, it interacts in a synergistic manner with both[49].

Finally, bile salts can stimulate proliferation in culture hepatocytes and increase proliferation in cholangiocytes and colonocytes. In experimental animals, it has been shown that a persistently elevated proliferative activity enhances tumour development not only favoring genomic mutation but also inducing immunosuppression of the locoregional immune system. Moreover, bile salts are able to activate the phosphorylation cascade stimulated by the epidermal growth factor receptor[54].

Table 1 Main growth factors and growth triggers of the liver

Hepatic growth factors	Hepatic growth triggers
HGF	Norepinephrine
ALR	Vasopressin
TGF-α	Angiotensin Ⅱ and Ⅲ
Epidermal growth factor	Estrogens
HGF (Hepatopoietin A-HPTA)	Insulin
Hepatopoietin B	Glucagon

HGF: Hepatocyte growth factor; ALR: Augmenter of liver regeneration; TGF-α: Transforming growth factor-α.

Growth inhibitors

These substances inhibit EGF mitogenesis in culture. Three main factors have been identified: TGF-β, interleukin 1β and hepatocyte proliferation inhibitor. TGF-β also inhibits the growth of many epithelia in culture including bronchial and mammary cells. In hepatocyte culture, it inhibits mitogenesis induced by EGF or HPTA. Interleukin 1 β inhibits hepatocyte proliferation; its effect on hepatocyte DNA synthesis might reflect a "reprogramming" in gene expression[55,56].

Growth triggers

Growth triggers are a group of substances that affect hepatocyte growth in a positive direction but in an indirect manner. They enhance the mitogen effect of growth stimulator and decrease the effect of growth inhibitors thus regulating the balance between these factors. Growth triggers are norepinephrine, vasopressin, angiotensin Ⅱ, angiotensin Ⅲ, estrogens, insulin and glucagon[49]. The main growth factors and triggers are reported in Table 1.

GENETICS

Recently, advances in the understanding of HCC genetics have been reported. This newly available genomic data could provide a rich source for understanding the molecular basis of HCC. In fact, despite many efforts to improve the diagnosis and treatment of HCC, therapeutic options remain limited. Chromosomal aberrations have been frequently reported in HCC, involving regions that contain key players in hepatocarcinogenesis, even if data on correlation with the clinical course of the disease are not available. The most common chromosomal aberrations consist of the amplifications of 1q, 8q, 6p and 17q (p53 region), and the loss of 8p,16q, 4q, 17p and 13q (Rb gene)[57].

p53 mutations affect over 200 codons in a central position. In HCC, p53 mutations vary in different geographic areas, reflecting differences in etiological agents and susceptibility factors. In particular, in sub-Saharan Africa and China, the p53 point mutation at the third position of codon 249, resulting in G:C to T:A transversion, is the most frequent[26,58]. Members of the wingless gene family (Wnt gene family), originally identified in Drosophila melanogaster, are essential to establish body pattern and axis formation during embryonic development, cell/cell interaction and proliferation. The Wnt pathway may play a role as a key regulator in HCC development and differentiation. In fact, normally, the binding of Wnt proteins to specific cell-surface receptors results in an increased amount/accumulation of β-catenin reaching the nucleus[59,60].

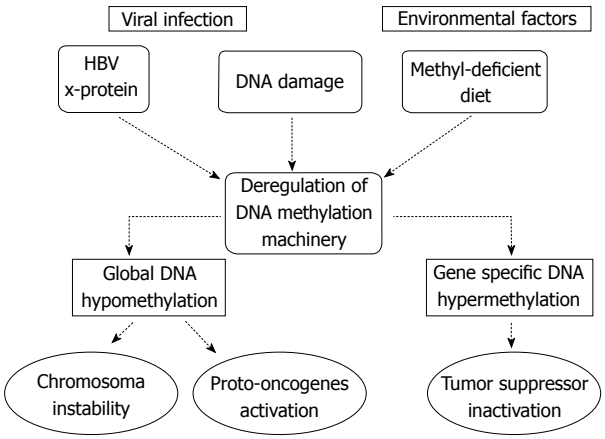

Figure 2 Genetics of HCC: the sequence of DNA methylation[63].

The tumour suppressor retinoblastoma protein (Rb) is critical for the development of several cancer types. In normal cells, genetic signaling Rb prevents cell division and cell cycle progression. In HCC, the Rb gene is inactivated and members of its network have aberrant expression[61]. In cancer, a methylation-imbalance is frequently observed where a genome-wide hypomethylation is accompanied by localized hypermethylation and an increased expression of DNA methyltransferase. Aberrant methylation alters groups of genes and chromosomal segments in livers with chronic hepatitis and cirrhosis.

Expression of DNA methyltransferases (DNMTs) is increased in chronic hepatitis and cirrhosis and both DNMT1 and DNMT3a are strongly upregulated in HCC[62,63]. The sequence of DNA methylation is reported in Figure 2.

Hepatocyte microsatellite instability occurs in chronic hepatitis, cirrhosis and HCC. Simultaneous examination of multiple loci detects allelic deletions in 30%-50% of chronic hepatitis or cirrhosis cases, in 70%-80% of dysplastic nodules and in almost all HCC cases. Aberrant loci detected in preneoplastic cells differ from those of adjacent HCC, suggesting that despite many cells harboring early genomic aberrations in chronic liver disorders, they do not evolve necessarily into the malignant phenotype[64].

CONCLUSION

In conclusion, hepatocarcinogenesis is associated with a large accumulation of chromosomal, genetic and epigenetic alterations. Some of these alterations occur in different stages of hepatocarcinogenesis and result in deregulation of important molecular cellular pathways. An additional role may be played by growth stimulators, inhibitors and triggers. On these bases, future challenges will be to identify molecular variants of HCC and their correlation with

clinical courses. Currently, there is no commonly accepted effective molecular/genetic therapy. Nevertheless, some HCC, such as those characterized by catenin mutations, have a limited repertory of genomic alterations and could be amenable to molecular therapeutic intervention. The genetic changes identified in phenotypically altered and dysplastic hepatocytes before HCC development constitute an interesting field of investigation since an intervention at this stage could be more feasible. Indeed, laser-capture microscopy enables these individual lesions to be selectively recovered as cellular clones for genomic analysis and recent reports of preneoplastic lesions suggest that aberrations of particular genes and chromosomes may presage the emergence of HCC. On these bases, a pool of non-diseased livers could be an appropriate control group and livers harbouring chronic hepatitis and cirrhosis or dysplastic lesions could supply reference tissues for detecting stepwise changes during the progression of hepatocarcinogenesis. A key point will be to determine whether the deletions are specific or nonspecific, whether there are particular loci in the minimally deleted regions of affected chromosome arms or whether loss of large portions of chromosomes or entire chromosome arms leads to passive (nonspecific) deletion of loci. The final aim could be identifying a step where the carcinogenetic process could be terminated.

REFERENCES

1 **Schütte K**, Bornschein J, Malfertheiner P. Hepatocellular carcinoma--epidemiological trends and risk factors. *Dig Dis* 2009; **27**: 80-92

2 **Parkin DM**, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. *Int J Cancer* 2001; **94**: 153-156

3 **Motola-Kuba D**, Zamora-Valdés D, Uribe M, Méndez-Sánchez N. Hepatocellular carcinoma. An overview. *Ann Hepatol* 2006; **5**: 16-24

4 **McGlynn KA**, London WT. Epidemiology and natural history of hepatocellular carcinoma. *Best Pract Res Clin Gastroenterol* 2005; **19**: 3-23

5 **Srivatanakul P**, Sriplung H, Deerasamee S. Epidemiology of liver cancer: an overview. *Asian Pac J Cancer Prev* 2004; **5**: 118-125

6 **Clark JM**. The epidemiology of nonalcoholic fatty liver disease in adults. *J Clin Gastroenterol* 2006; **40** Suppl 1: S5-S10

7 **El-Serag HB**. Hepatocellular carcinoma: recent trends in the United States. *Gastroenterology* 2004; **127**: S27-S34

8 **El-Serag HB**, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007; **132**: 2557-2576

9 **Teo EK**, Fock KM. Hepatocellular carcinoma: an Asian perspective. *Dig Dis* 2001; **19**: 263-268

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

11 **Wang J**, Chenivesse X, Henglein B, Bréchot C. Hepatitis B virus integration in a cyclin A gene in a hepatocellular carcinoma. *Nature* 1990; **343**: 555-557

12 **Chami M**, Gozuacik D, Saigo K, Capiod T, Falson P, Lecoeur H, Urashima T, Beckmann J, Gougeon ML, Claret M, le Maire M, Bréchot C, Paterlini-Bréchot P. Hepatitis B virus-related insertional mutagenesis implicates SERCA1 gene in the control of apoptosis. *Oncogene* 2000; **19**: 2877-2886

13 **Minami M**, Daimon Y, Mori K, Takashima H, Nakajima T, Itoh Y, Okanoue T. Hepatitis B virus-related insertional mutagenesis in chronic hepatitis B patients as an early drastic genetic change leading to hepatocarcinogenesis. *Oncogene* 2005; **24**: 4340-4348

14 **Feitelson MA**, Duan LX. Hepatitis B virus X antigen in the pathogenesis of chronic infections and the development of hepatocellular carcinoma. *Am J Pathol* 1997; **150**: 1141-1157

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

16 **Henkler F**, Lopes AR, Jones M, Koshy R. Erk-independent partial activation of AP-1 sites by the hepatitis B virus HBx protein. *J Gen Virol* 1998; **79** (Pt 11): 2737-2742

17 **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 USA* 1994; 91: 2230-2234

18 **Ghebranious N**, Sell S. The mouse equivalent of the human p53ser249 mutation p53ser246 enhances aflatoxin hepatocarcinogenesis in hepatitis B surface antigen transgenic and p53 heterozygous null mice. *Hepatology* 1998; **27**: 967-973

19 **Kwun HJ**, Jung EY, Ahn JY, Lee MN, Jang KL. p53-dependent transcriptional repression of p21(waf1) by hepatitis C virus NS3. *J Gen Virol* 2001; **82**: 2235-2241

20 **Ghosh AK**, Steele R, Meyer K, Ray R, Ray RB. Hepatitis C virus NS5A protein modulates cell cycle regulatory genes and promotes cell growth. *J Gen Virol* 1999; **80** (Pt 5): 1179-1183

21 **Ray RB**, Lagging LM, Meyer K, Steele R, Ray R. Transcriptional regulation of cellular and viral promoters by the hepatitis C virus core protein. *Virus Res* 1995; **37**: 209-220

22 **Marusawa H**, Hijikata M, Chiba T, Shimotohno K. Hepatitis C virus core protein inhibits Fas- and tumor necrosis factor alpha-mediated apoptosis via NF-kappaB activation. *J Virol* 1999; **73**: 4713-4720

23 **Ierardi E**, Di Leo A, Barone M, Marangi S, Burattini O, Panarese A, Margiotta M, Francavilla R, Panella C, Francavilla A, Cuomo R. Tumor necrosis factor alpha and apoptosis in Helicobacter pylori related progressive gastric damage: a possible mechanism of immune system involvement in epithelial turnover regulation. *Immunopharmacol Immunotoxicol* 2003; **25**: 203-211

24 **Smela ME**, Currier SS, Bailey EA, Essigmann JM. The chemistry and biology of aflatoxin B(1): from mutational spectrometry to carcinogenesis. *Carcinogenesis* 2001; **22**: 535-545

25 **Shen HM**, Ong CN. Mutations of the p53 tumor suppressor gene and ras oncogenes in aflatoxin hepatocarcinogenesis. *Mutat Res* 1996; **366**: 23-44

26 **Bressac B**, Kew M, Wands J, Ozturk M. Selective G to T mutations of p53 gene in hepatocellular carcinoma from southern Africa. *Nature* 1991; **350**: 429-431

27 **Kew MC**. Hepatic iron overload and hepatocellular carcinoma. *Cancer Lett* 2009; **286**: 38-43

28 **Barone M**, Margiotta M, Scavo MP, Gentile A, Francioso D, Papagni S, Castellaneta A, Mallamaci R, Di Leo A, Francavilla A. Possible involvement of androgen receptor alterations in hepatocarcinogenesis. *Dig Liver Dis* 2009; **41**: 665-670

29 **Thorgeirsson SS**, Grisham JW. Molecular pathogenesis of human hepatocellular carcinoma. *Nat Genet* 2002; **31**: 339-346

30 **Chen X**, Cheung ST, So S, Fan ST, Barry C, Higgins J, Lai KM, Ji J, Dudoit S, Ng IO, Van De Rijn M, Botstein D, Brown PO. Gene expression patterns in human liver cancers. *Mol Biol Cell* 2002; **13**: 1929-1939

31 **Ferrell LD**, Crawford JM, Dhillon AP, Scheuer PJ, Nakanuma Y. Proposal for standardized criteria for the diagnosis of benign, borderline, and malignant hepatocellular lesions arising in chronic advanced liver disease. *Am J Surg Pathol* 1993; **17**: 1113-1123

32 **Roncalli M**. Hepatocellular nodules in cirrhosis: focus on diagnostic criteria on liver biopsy. A Western experience. *Liver Transpl* 2004; **10**: S9-S15

33 **Buendia MA**. Genetics of hepatocellular carcinoma. *Semin Cancer Biol* 2000; **10**: 185-200

34 **Kojiro M**, Roskams T. Early hepatocellular carcinoma and dysplastic nodules. *Semin Liver Dis* 2005; **25**: 133-142

35 **Takaishi H**, Kitamoto M, Takahashi S, Aikata H, Kawakami Y, Nakanishi T, Nakamura Y, Shimamoto F, Kajiyama G, Ide T. Precancerous hepatic nodules had significant levels of telomerase activity determined by sensitive quantitation using a hybridization protection assay. *Cancer* 2000; **88**: 312-317

36 **Takahashi S**, Kitamoto M, Takaishi H, Aikata H, Kawakami Y, Nakanishi T, Shimamoto F, Tahara E, Tahara H, Ide T, Kajiyama G. Expression of telomerase component genes in hepatocellular carcinomas. *Eur J Cancer* 2000; **36**: 496-502

37 **Barone M**, Francavilla A, Polimeno L, Ierardi E, Romanelli D, Berloco P, Di Leo A, Panella C. Modulation of rat hepatocyte proliferation by bile salts: in vitro and in vivo studies. *Hepatology* 1996; **23**: 1159-1166

38 **Panella C**, Ierardi E, De Marco MF, Barone M, Guglielmi FW, Polimeno L, Francavilla A. Does tauroursodeoxycholic acid (TUDCA) treatment increase hepatocyte proliferation in patients with chronic liver disease? *Ital J Gastroenterol* 1995; **27**: 256-258

39 **Nakamura T**. Hepatocyte growth factor as mitogen, motogen and morphogen, and its roles in organ regeneration. *Princess Takamatsu Symp* 1994; **24**: 195-213

40 **Jiang WG**, Martin TA, Parr C, Davies G, Matsumoto K, Nakamura T. Hepatocyte growth factor, its receptor, and their potential value in cancer therapies. *Crit Rev Oncol Hematol* 2005; **53**: 35-69

41 **Mizuno S**, Nakamura T. Hepatocyte growth factor: a regenerative drug for acute hepatitis and liver cirrhosis. *Regen Med* 2007; **2**: 161-170

42 **Francavilla A**, Hagiya M, Porter KA, Polimeno L, Ihara I, Starzl TE. Augmenter of liver regeneration: its place in the universe of hepatic growth factors. *Hepatology* 1994; **20**: 747-757

43 **Giorda R**, Hagiya M, Seki T, Shimonishi M, Sakai H, Michaelson J, Francavilla A, Starzl TE, Trucco M. Analysis of the structure and expression of the augmenter of liver regeneration (ALR) gene. *Mol Med* 1996; **2**: 97-108

44 **Polimeno L**, Margiotta M, Marangi L, Lisowsky T, Azzarone A, Ierardi E, Frassanito MA, Francavilla R, Francavilla A. Molecular mechanisms of augmenter of liver regeneration as immunoregulator: its effect on interferon-gamma expression in rat liver. *Dig Liver Dis* 2000; **32**: 217-225

45 **Polimeno L**, Capuano F, Marangi LC, Margiotta M, Lisowsky T, Ierardi E, Francavilla R, Francavilla A. The augmenter of liver regeneration induces mitochondrial gene expression in rat liver and enhances oxidative phosphorylation capacity of liver mitochondria. *Dig Liver Dis* 2000; **32**: 510-517

46 **Lisowsky T**, Lee JE, Polimeno L, Francavilla A, Hofhaus G. Mammalian augmenter of liver regeneration protein is a sulfhydryl oxidase. *Dig Liver Dis* 2001; **33**: 173-180

47 **Mead JE**, Fausto N. Transforming growth factor alpha may be a physiological regulator of liver regeneration by means of an autocrine mechanism. *Proc Natl Acad Sci USA* 1989; **86**: 1558-1562

48 **Brenner DA**, Koch KS, Leffert HL. Transforming growth factor-alpha stimulates proto-oncogene c-jun expression and a mitogenic program in primary cultures of adult rat hepatocytes. *DNA* 1989; **8**: 279-285

49 **Michalopoulos GK**. Liver regeneration: molecular mechanisms of growth control. *FASEB J* 1990; **4**: 176-187

50 **Houck KA**, Michalopoulos GK. Altered responses of regenerating hepatocytes to norepinephrine and transforming growth factor type beta. *J Cell Physiol* 1989; **141**: 503-509

51 **Raper SE**, Burwen SJ, Barker ME, Jones AL. Translocation of epidermal growth factor to the hepatocyte nucleus during rat liver regeneration. *Gastroenterology* 1987; **92**: 1243-1250

52 **McGowan JA**, Strain AJ, Bucher NL. DNA synthesis in primary cultures of adult rat hepatocytes in a defined medium: effects of epidermal growth factor, insulin, glucagon, and cyclic-AMP. *J Cell Physiol* 1981; **108**: 353-363

53 **Zarnegar R**, Michalopoulos G. Purification and biological characterization of human hepatopoietin A, a polypeptide growth factor for hepatocytes. *Cancer Res* 1989; **49**: 3314-3320

54 **Barone M**, Maiorano E, Ladisa R, Cuomo R, Pece A, Berloco P, Caruso ML, Valentini AM, Iolascon A, Francavilla A, Di Leo A, Ierardi E. Influence of ursodeoxycholate-enriched diet on liver tumor growth in HBV transgenic mice. *Hepatology* 2003; **37**: 880-886

55 **Russell WE**. Transforming growth factor beta (TGF-beta) inhibits hepatocyte DNA synthesis independently of EGF binding and EGF receptor autophosphorylation. *J Cell Physiol* 1988; **135**: 253-261

56 **Nakamura T**, Arakaki R, Ichihara A. Interleukin-1 beta is a potent growth inhibitor of adult rat hepatocytes in primary culture. *Exp Cell Res* 1988; **179**: 488-497

57 **Moinzadeh P**, Breuhahn K, Stützer H, Schirmacher P. Chromosome alterations in human hepatocellular carcinomas correlate with aetiology and histological grade--results of an explorative CGH meta-analysis. *Br J Cancer* 2005; **92**: 935-941

58 **Oda T**, Tsuda H, Scarpa A, Sakamoto M, Hirohashi S. p53 gene mutation spectrum in hepatocellular carcinoma. *Cancer Res* 1992; **52**: 6358-6364

59 **Luu HH**, Zhang R, Haydon RC, Rayburn E, Kang Q, Si W, Park JK, Wang H, Peng Y, Jiang W, He TC. Wnt/beta-catenin signaling pathway as a novel cancer drug target. *Curr Cancer Drug Targets* 2004; **4**: 653-671

60 **Johnson ML**, Rajamannan N. Diseases of Wnt signaling. *Rev Endocr Metab Disord* 2006; **7**: 41-49

61 **Dannenberg JH**, te Riele HP. The retinoblastoma gene family in cell cycle regulation and suppression of tumorigenesis. *Results Probl Cell Differ* 2006; **42**: 183-225

62 **Teufel A**, Staib F, Kanzler S, Weinmann A, Schulze-Bergkamen H, Galle PR. Genetics of hepatocellular carcinoma. *World J Gastroenterol* 2007; **13**: 2271-2282

63 **Wong CM**, Ng IO. Molecular pathogenesis of hepatocellular carcinoma. *Liver Int* 2008; **28**: 160-174

64 **Maggioni M**, Coggi G, Cassani B, Bianchi P, Romagnoli S, Mandelli A, Borzio M, Colombo P, Roncalli M. Molecular changes in hepatocellular dysplastic nodules on microdissected liver biopsies. *Hepatology* 2000; **32**: 942-946

S- Editor Li LF **L- Editor** Lutze M **E- Editor** Yang C