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

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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.

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

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 occurring 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 areas are northern/western Europe and North America\(^3\)-\(^4\). 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\(^5\). Male predominance has been demonstrated showing a male/female ratio of 2.4:1\(^6\).

**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)\(^7\). In developed countries, a high intake of alcohol and non-alcoholic steato-hepatitis are additional factors contributing to HCC\(^8\). 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 insertionional mutations at specific sites\(^9\). 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\(^10\). Integration of HBV DNA may also deregulate the expression of oncogenes or tumour suppressor genes that are able to control cell death/survival\(^11\). 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\(^12\)-\(^14\). 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\(^15\).

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\(^16\)-\(^24\). 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\(^25\). 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\(^25\)-\(^29\).

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\(^29\)-\(^31\).

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\(^32\).

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\(^33\).

**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\(^34\). 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\(^35\). 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\(^36\)-\(^38\). 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\(^39\).

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\(^40\). Dysplastic nodules
are precancerous lesions, which are divided into high and low grades based on histological/cytological changes and the presence/absence of portal tracts. There is no destruction of the underlying hepatic structure in early HCC and tumour invasion into the nontumoral portal tracts has been suggested as a helpful distinctive sign of LGDN. 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-β 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 in altered hepatocytes. The wingless gene family (Wnt), β-catenin, ras, p14ARF/p53, P16INK4A/Rb, TGF-β and PTEN/Akt may show a changed pattern in the hepatocytes, including cellular enlargement. 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/Rh, TGF-β and PTEN/Akt may show a changed pattern in hepatocytes, including cellular enlargement. 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. It is likely that ALR might mediate its activity by regional regulation of natural killer cells, which exert a specific cytotoxicity against regenerating hepatocytes. 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.

TGF-α is an autocrine stimulator of hepatocyte proliferation, which increases transiently in replicating hepatocytes both in vivo and in vitro. Constitutive TGF-α overexpression in young transgenic mice causes liver hypertrophy and enhanced proliferation that progresses to hepatic tumor development. 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.

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. 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. HGF-β is a complete hepatocytes mitogen but is less active than EGF or HPTA. In addition, it interacts in a synergistic manner with both.

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.

**Figure 1** The “timing” of hepatocarcinogenesis from chronic liver disorders. HBV: Hepatitis B viruses; HCV: Hepatitis C viruses; HCC: hepatocellular carcinoma.
Hepatocellular carcinoma

**Table 1  Main growth factors and growth triggers of the liver**

| Hepatic growth factors | Hepatic growth triggers |
|------------------------|------------------------|
| HGF                    | Norepinephrine         |
| ALR                    | Vasopressin            |
| TGF-β                  | 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[35,56].

**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)[59].

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.

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| June 15, 2010 | Volume 2 | Issue 6 |
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