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REVIEW

Similarities and Differences in Hepatitis B and C Virus Induced Hepatocarcinogenesis

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Hepatocellular carcinoma (HCC), the major manifestation of primary liver cancer, is one of the most frequent and malignant diseases worldwide. Among other environmental factors, hepatitis viruses, as the hepatitis B (HBV) and hepatitis C (HCV) viruses, are to be listed in the etiology of HCC. Both of these viruses cause a wide spectrum of clinical manifestations, ranging from healthy carrier state to acute and chronic hepatitis, cirrhosis and HCC. HBV and HCV are different viruses in structure: HBV contains a DNA genome which replicates through an RNA intermediate and requires an active viral reverse transcriptase (RT) polymerase enzyme, while HCV is an RNA virus which has no RT activity and replicates on the cellular membrane by RNA replication. In this review we discuss how these two biologically diverse viruses use common pathways to induce hepatocarcinogenesis despite their significant structural and viral cycle differences. A summary is also given of several observable common and different features. Direct integration of HBV viral sequences into the host genome increases the genomic instability, which does not occur in HCV infection. However, viral proteins may directly play a significant role in the induction of carcinogenesis by both viruses. (Pathology Oncology Research Vol 10, No 1, 5–11)

Keywords: hepatitis B virus, hepatitis C virus, hepatocellular carcinoma, hepatocarcinogenesis

Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide, representing approximately 4% of all malignancies[4,27] (*Figure 1*). An estimated 564,000 new cases and almost as many deaths were registered in 2000,[17,30] which means that HCC has a very unfavorable prognosis. An increase in 1-year but not 5-year survival rates was seen in patients with HCC.[12,13] The death rate due to HCC has been increasing over the last two decades.[2] Recent studies have shown that one of the main causes of this increase is associated with the increased infection with hepatitis C virus (HCV), at least in Japan.[2]

Much is known about the hepatocarcinogenesis and etiopathogenesis of HCC,[4,7,18,42] however, the exact mechanism is still not exactly known. The main causes of HCC are the hepatitis B virus (HBV), HCV, aflatoxin B_1, alcohol, hemochromatosis, with lower magnitude of risk, alpha 1-antitrypsin deficiency, tyrosinemia, glycogen storage disease etc,[4] however, it is estimated that HBV and HCV account for 70-85% of HCC cases worldwide.[4]

An effective vaccine has been available for prevention of new infection with HBV, however, no vaccine exists against HCV infection. Several publications point to the significance of the „worldwide epidemic" of HCV infection expected in this decade,[9] which means that we will be facing increasing incidence of HCC as well.

Understanding the mechanism of HCV and HBV induced hepatocarcinogenesis might help to provide a better therapy, even gene therapy for patients suffering from these infections and diseases.

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Abbreviations: STAT 3: signal transducer and activator of transcription 3. IGF: insulin-like growth factor. PKC: protein kinase C. NFB: nuclear factor kappa

Mechanism of HBV induced HCC

Epidemiology of HBV infection

HBV is a partially double stranded hepatotropic DNA virus, which belongs to the Hepadnaviridae family (*Table 1*). HBV infection causes acute or chronic liver diseases. Recent estimates account about 400 million people chronically infected with HBV (*Table 1*), with 75-80% of the

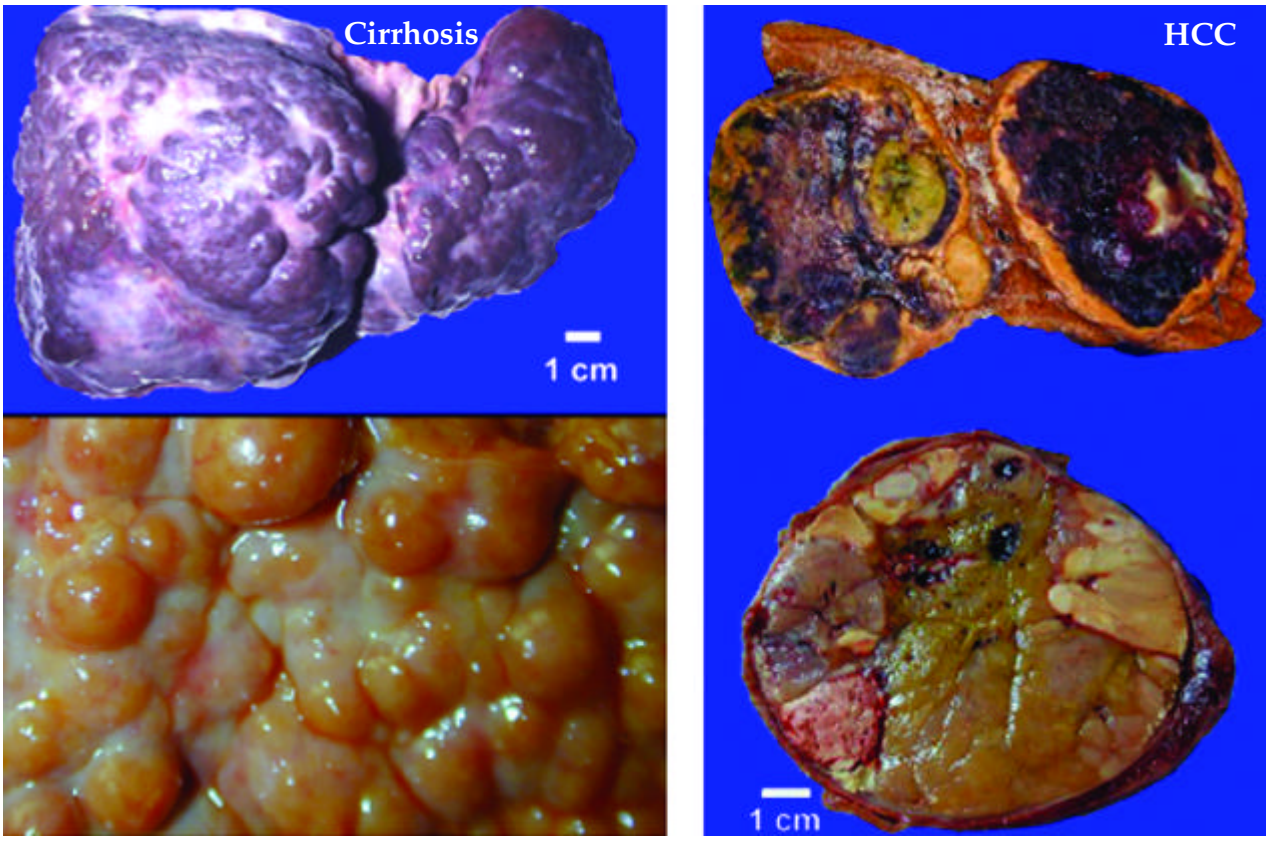

Figure 1. *Macroscopic appearance of cirrhotic liver and HCC.*

cases occurring in Africa, Asia and the Western Pacific.[24] It has been shown that the risk of developing HCC is increased by 100 fold in chronic HBV surface antigen (HBsAg) carriers.[5]

HBV infects all age groups, however, a high percentage (70-80%) of chronic HBV infection occurs in the perinatal period, during infancy or early childhood, while in adults the chronicity is approximately 10%.

Patients in whom HBsAg persists in the serum for more than 6 months are referred to as „chronic HBsAg carriers",[24] the commonly used term „carrier", however, refers to persistently infected individuals with normal serum aminotransferase levels („healthy carriers").

The main problem in patients chronically infected with HBV is the development of progressing chronic liver disease: chronic hepatitis (CH), cirrhosis and HCC (*Figure 2*).

Life cycle of HBV

HBV is a small (3.2 kB) DNA virus with four open reading frames in which several genes overlap; as core, surface, X and polymerase. It is interesting that while HBV is a DNA virus, it replicates through an RNA intermediate and requires an active viral reverse transcriptase (RT) polymerase enzyme (*Figure 3*). The mature virions (Dane particles) attach to the cell surface, however, the membrane receptor is unknown. The viral genome is transferred into the nucleus, where a covalently closed circular form of DNA (cccDNA) is formed, which serves as a template for viral transcription (*Figure 3a*).

Subgenomic and pregenomic RNA molecules are transcribed from this template, serving as the template for reverse transcription and the mRNA for the viral proteins (core, polymerase, surface, X), which are formed in the endoplasmic reticulum (ER) (Figure 3b, c). Viral assembly occurs in the ER, too (*Figure 3d*). An interesting step in the replication cycle is the encapsidation of the pregenomic RNA, which is transcribed into a negative-strand HBV DNA, serving later as the template for positive-strand genomic DNA (*Figure 3c*).

HBV DNA integration

From the viewpoint of carcinogenesis, the integration of HBV DNA into the cell genome and the production of the X protein (HBxAg) seem to be of significance. Integration of the provirus into the host genome is important in the replication cycle of the retroviruses, it is not, however, a „necessary" part of the viral cycle in HBV replication.[16,23] The integration is random, usually multiple (3-4), does not

Table 1. **Characteristics of HBV and HCV infection**

	HBV		*HCV*
Viral family	Hepadnaviridae	Hepaci	Flavi
Nucleic acid	dsDNA	ssRNA	
Host genome integration	Yes (random) multiple		No
Reverse transcription	Yes		No
Size	42 nm		55-65 nm
Global prevalence of infected individuals	~ 400 million[10]	3-5 %	~ 170 million[9]
Geographic variation	< 1%–20%		< 1%–>10%
Chronic infection	~ 10%*		~ 85 %
Number of death cases	~ 1 million/yr[24]	?	
Transmission	Parenteral sexual, perinatal etc		Parenteral ?

*adult cases (perinatal, childhood: ~ 80%
[9]Cohen J, 1999; [10]Conjeevaram HS and Lok AS, 2003; [24]Nair S and Perrillo RP, 2003

preserve the viral genome sequence and is variable. The integrated viral DNA might therefore act as a mutagenic agent, causing secondary chromosomal rearrangement (duplications, translocations, deletions) and increasing genomic instability. The deletions might involve loss of tumor suppressor genes, or the amplification, overexpression of growth factor genes which influence cell proliferation and cell cycle control.

HBV x gene/protein

The term „X gene" is applied because its role during acute/chronic viral infection is not known, despite its essentiality for the viral cycle. The protein product (HBx) functions as a transcriptional transactivator of different host genes involved in cellular growth control.[3,6,14,20,23,34]

HBx transactivates cellular genes involved in cell proliferation control (c-jun, c-fos, c-myc). This transactivation activity appears to involve stimulation of the protein kinase C (PKC) and nuclear factor kappa B (NFB) pathways.[14] The hepatitis B virus X protein deregulates cell cycle control, interferes with cellular DNA repair and apoptosis. It is important that HBx may interact with p53 and RB (for review see Andrisani and Barnabas[3]).

Mechanism of HCV induced hepatocarcinogenesis

Epidemiology of HCV infection

It is estimated that about 170 million people are chronically infected with HCV worldwide.[9] The rate is around 1% in North America and Western Europe, while it is up to 10-20% in some African and Asian countries. The number of HCV infected people is lower than in case of HBV infection, the chronicity, however, is much higher in every age group, reaching up to 85%. The pathological alterations caused by HCV are similar to the HBV-related disease; acute and chronic hepatitis, cirrhosis and HCC (*Figure 4*).

Life cycle of HCV

HCV has been classified as the Hepacivirus genus of the Flaviviridae family.[45] The single stranded RNA genome (of approximately 9600 nucleotide length) encodes a single polyprotein precursor (approximately 3000 amino acids), which is cleaved into several smaller structural (core, envelope 1, 2) and non-structural (NS_1, NS_3, NS_4, NS_5)

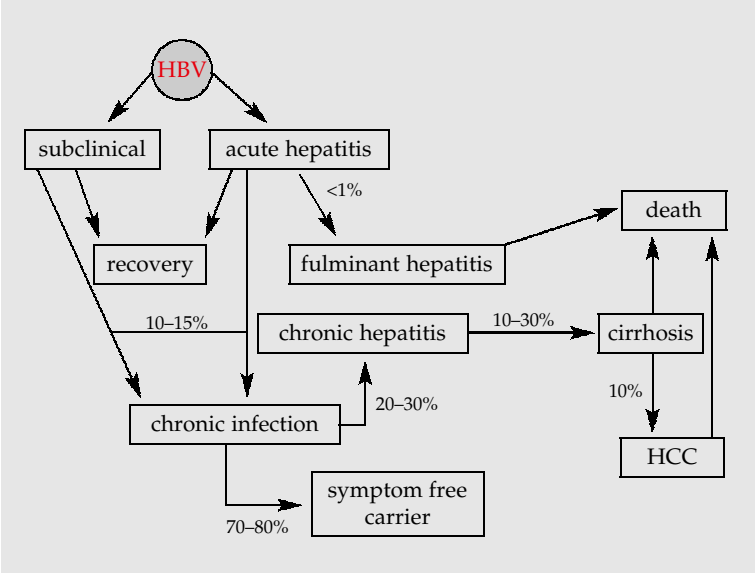

Figure 2. *Possible outcome of hepatitis B virus infection.*

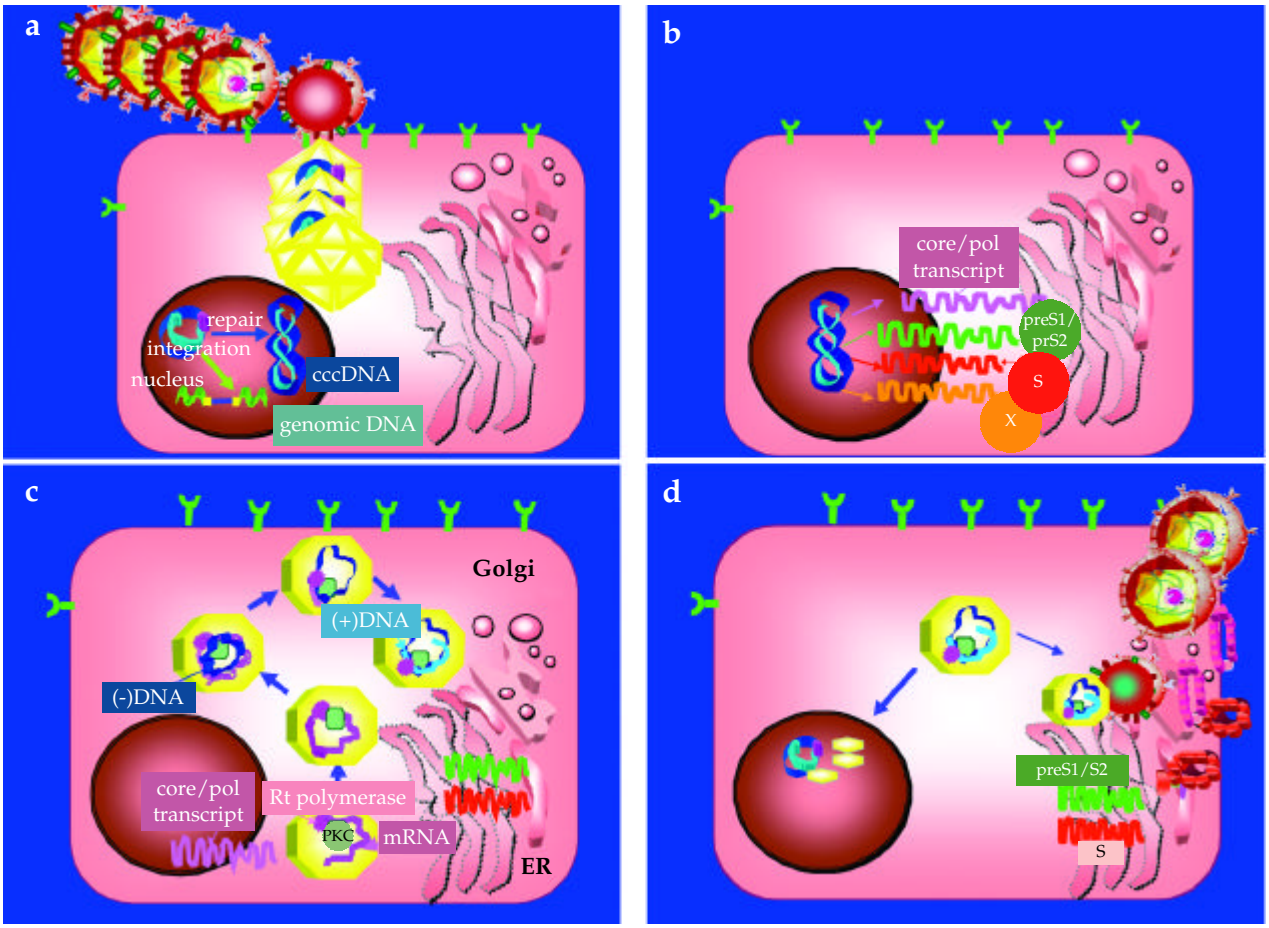

Figure 3. ***(a)*** *Attachment of virions to the unknown receptors. Viral entry into hepatocytes. Uncoating and intracellular transport of viral genome into the nucleus; conversion of the relaxed circular form of HBV DNA into a double-stranded covalently closed circular DNA.* ***(b)*** *cccDNA further serves as a template for viral transcription. The mRNA transcripts are translated to the viral proteins: core, polymerase, surface (pre S1/pre S2, S) and X protein.* ***(c)*** *Packaging of the pregenome RNA into core; reverse transcription of pregenomic RNA into (-) strand DNA by viral RT polymerase enzyme. This RNA serves as a template for (+) strand DNA, which forms a partially double stranded genomic DNA.* ***(d)*** *The core could be transported to the nucleus or the nucleocapsid undergoes maturation and interacts with the HBsAg surface protein in the ER. The viral assembly occurs in the Golgi, the infectious (Dane-particle) and non-infectious virus particles are released from the hepatocyte.*

proteins (Szabó et al[40]). The virus has no RT activity and does not integrate into the cell genome. The low fidelity of the RNA-dependent RNA polymerase is partly responsible for genetic heterogeneity.[36]

In contrast to HBV, the putative receptors for binding HCV are known, as CD81,[31] LDL-R,[38] human scavenger receptor B1,[1] and others.[37] An interesting step in the „regular" viral cycle is the entrance of smaller viral core portions (p19, p21) into the nucleus.[38] HCV core proteins can modulate various cellular signal transduction pathways, namely by mediating the transcription activity of NFB and STAT-3 proteins.[47]

HCV is not considered as a directly cytotoxic virus, hepatitis occurs as a result of the reaction of the host immune system against the virus infected cells.

Common pathways in HBV and HCV induced hepatocarcinogenesis

It is generally accepted that neither HBV nor HCV are directly cytopathic viruses.[25] An important effect of both viruses, however, is causing chronic infection, a repeating attack of the host immune system against the viral infection. Continuous cell death, mainly by apoptosis, and reactive proliferation occur through the inflammation-necrosis-regeneration sequence, as the basis of cirrhosis.[44]

The question is, how could the same „final outcome" of HBV and HCV infection be explained, given the above discussed significant structural and viral cycle differences between the two viruses? Several data have shown the extensive heterogeneity of genomic alterations in HCCs of

Table 2. **Common genetic alterations in HCC***

G → T transversion in codon 249 (AFB1) of the p53 gene
p53 mutation (15-50%)
loss of heterozygosity (LOH) on 8p, 17p
β-catenin mutation (20-40%)
$p16^{INK4a}$ promoter methylation (~ 70%)
loss of $p16^{INK4a}$ expression
E-cadherin promoter methylation (~ 70%)
decreased p27 expression (50%)

* based on Edamoto Y et al, 2003[11]

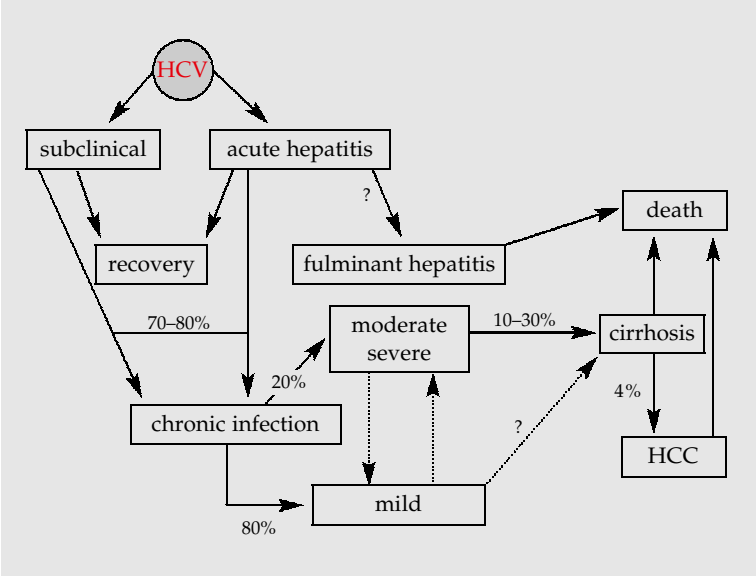

Figure 4. *Possible outcome of hepatitis C virus infection.*

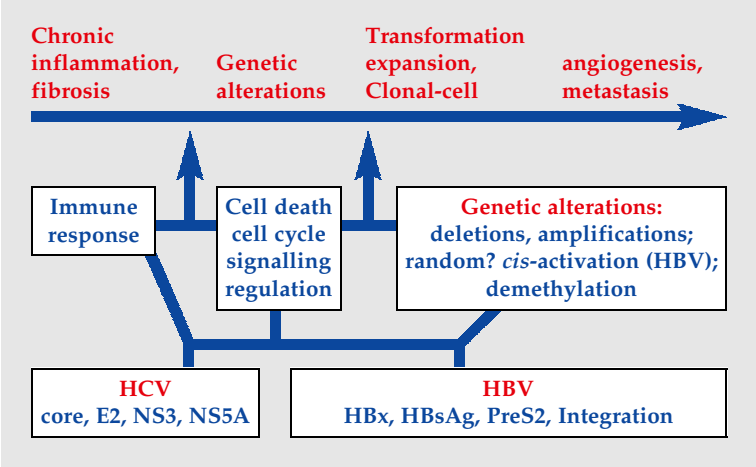

Figure 5. *Mechanisms involved in HBV- and HCV-related chronic liver disease and HCC.*

different etiology[8,15,19-22,26,28,29,32,33,35,41-44,46,48,49] (*Table 1*). The most common genetic alterations in HCC have been revealed,[11] which can be grouped into 3 main routes (*Table 3*). Iizuka et al[21] compared the tumors and surrounding cirrhotic livers in HBV-HCC and HCV-HCC cases. HCC can be classified on the basis of gene expression profiles using high density oligonucleotide microarrays.[21] It has been shown that the groups are determined by the infectious agents and the presence of cirrhosis. A higher number of genes (89) were expressed differently between HBV-HCCs associated with and those not associated with cirrhosis.[21] Low number of genes (8) were expressed differently between HCV-HCCs associated with and without cirrhosis. In accordance with previous data it has been shown that HBV can transform hepatocytes even in the absence of chronic inflammation and cirrhosis, while the role and significance of the inflammation is more important in the development of HCV-associated HCCs. Recently it has been demonstrated that many transcription-related and signaling-related genes were upregulated in HBV-HCCs without cirrhosis. The IGF signal pathway seems to be playing a central role in HBV-HCCs, especially when developing from a noncirrhotic liver.

Table 3. **Common altered pathways in HCC***

– p53 pathway (p53 mutations, p14ARF promoter methylation)
– Wnt pathway (mutation of β-catenin)
– RB1 pathway (p16INK4a methylation, loss of RB1 expression cyclin D1 amplification)

*based on Edamoto Y et al, 2003[11]

Summarizing the role of pathways playing a role in HBV and HCV induced HCC, several common and differing features can be observed (*Figure 5*). Chronic inflammation, cell death and proliferation, as a result of the oxidative stress, and up- and down-regulation of several growth factors and cytokines, play a central role. Viral integration is an essential part of cell transformation by HBV, which does not occur in HCV infection. However, viral proteins, especially HBx and to a certain extent (at least it is now believed) the core component of HCV, may directly participate in the hepatocarcinogenesis.

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