Oncogetic Viruses and Hepatocellular Carcinoma

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INTRODUCTION

Worldwide, approximately 80% of hepatocellular carcinoma (HCC) is caused by hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, especially in the setting of established cirrhosis or advanced fibrosis. There are more than half a million new cases of HCC globally and almost the same number of deaths caused by this disease annually1 because of the very high case-fatality rate.

The risk of developing HCC among carriers of HBV infection ranges from 10- to 100-fold greater compared with the rates in uninfected people, depending on the markers and populations that are evaluated.2 In HCV infection, the relative risk for developing HCC in patients with serologically confirmed HCV infection is estimated to be 17-fold.3 The age-adjusted incidence of HCC is increasing in many countries,

KEY POINTS

- Cirrhosis in patients with chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infection is not a prerequisite step for hepatic tumorigenesis.
- The role of HCV and HBV in promoting hepatocellular carcinoma (HCC) development by either direct or indirect effects is still speculative, yet there is compelling evidence that both mechanisms exist.
- Vaccination plays a central role in the prevention of HBV-related HCC.
- Current antiviral therapies for HBV and HCV, if successful, can reduce but not completely eliminate the risk of HCC.
- The introduction of the new HCV direct-acting antiviral agents has not been in practice long enough to permit an estimate of their likelihood of reducing HCC incidence.
including the United States, and has been widely attributed to the spread of HCV infection in industrialized countries. The geographic distribution of HCC coincides with the distribution of HBV and HCV infections in those areas. In the United States, Europe, Egypt, and Japan, more than 60% of HCC is associated with HCV and about 20% is related to HBV, whereas nonalcoholic fatty liver disease and other causes contribute to the remainder. In Africa and Asia, where HBV is endemic, 60% of HCC is associated with HBV, 20% is related to HCV, and the remainder is distributed among other risk factors (for example aflatoxin). Men are more susceptible to HCC than women; older age, family history of HCC, and advanced disease are also associated with its development. Other risk factors for HCC, apart from viral hepatitis B and C, include alcohol consumption and nonalcoholic fatty liver disease. Although recent clinical observations and translational research have enhanced our understanding of the molecular mechanisms driving the initiation and progression of HCC, much remains unknown. The role of HCV and HBV in promoting HCC development either directly or indirectly is still speculative. The indirect pathways include the development of HCC on a background of chronic inflammation and the associated regenerative wound-healing response that is linked to the development of fibrosis and cirrhosis. The more direct pathways refer to alteration in cellular homeostasis caused by integration of the virus (notably HBV DNA) into the host’s genome or modifications in cell signaling by specific HBV or HCV viral-encoded proteins. The evidence, as described later, is compelling that molecular derangements that are hepatocarcinogenic exist in viral infection; but the cause-and-effect relationships have yet to be confirmed.

In Taiwan, HBV vaccination has decreased the incidence of new infections and HCC; however, there is no vaccine for HCV. Suppression of HBV replication and a sustained viral response (SVR) in the treatment of HCV are associated with a reduction in HCC incidence among treated populations. There is an ongoing controversy regarding the role of antiviral therapy in reducing HCC incidence in cirrhotic patients with HBV. Also a small subset of patients with HCV with advanced fibrosis or cirrhosis who achieve SVR remain at a heightened risk for HCC development. None of the new HCV direct-acting antiviral agents have been in use long enough to evaluate their effect in reducing HCC incidence.

PATHOGENESIS

Current data indicate that HCC tumors are highly complex and heterogeneous resulting from the aberrant function of multiple molecular pathways. The role of HCV or HBV in promoting HCC development by either direct or indirect activity and their relative importance to the pathogenesis of HCC have not been clearly defined.

Hepatitis B Virus

Although HBV integration into the host’s genome is not essential for viral replication, there is substantial evidence showing that such HBV DNA genomic integration occurs. Since the development of new whole-genome sequencing methods, several studies have been done to evaluate the relative extent and the functional impact of such integrations on the development of HCC. Whole-genome sequencing analysis of HCC in patients with HBV revealed that, although HBV sequences were present in both the tumor and their adjacent nontumorous liver tissue, HBV signals were more frequent in the tumor than in the nontumorous tissue. Furthermore, although HBV integration in the nontumorous tissue occurs in many sites, in the tumor, most of the insertions are at major integration sites. These findings suggest that, although in the nontumorous tissue hepatocytes are heterogeneous, HCC tumors are more likely
to result from a clonal expansion. Furthermore, most breaking points of HBV integration in the HCC samples were near coding genes.

**Hepatitis B Virus DNA Viral Integration into the Host’s Genome**

There are 3 main consequences of HBV DNA viral integration into the host’s genome:

**Alterations in the transcriptional levels of adjacent genes**

Results of recent studies have provided a long, growing list of HBV integration events and have uncovered 3 cancer-associated genes that were found at frequent integration sites in HBV-positive tumors: TERT,\(^{20,23–26}\) MLL4,\(^{21,23}\) and CCNE1.\(^{24}\) Regardless of whether HBV integration was at the promoter, intron, or exon sites, all 3 genes showed upregulated expression in the tumor relative to nontumorous tissue.

**Genome instability**

To evaluate genome instability in HBV-infected HCC tissue, Sung and colleagues\(^{24}\) measured the somatic copy number variations (CNVs) adjacent to HBV integration sites. They found that CNVs were significantly increased at HBV break point locations where chromosomal instability was likely induced, suggesting that HBV integration might alter chromosomal stability and cause changes in the CNVs.\(^{24}\) One of the consequences of HBV integration that leads to genome instability and alteration in molecular signaling is a heterozygous deletion of a cluster of caspase and caspase-recruiting domain family genes. These genes, which are important for the execution of apoptosis caused by viral integration, colocalized precisely within the junction of a large DNA copy number loss. Jiang and colleagues\(^{23}\) following RNA-sequencing (RNA-seq) analysis, revealed downregulation in the expression levels of these genes.

**Viral-human fusion transcripts**

This phenomenon is common in HBV-associated HCC tumors. According to RNA-seq analysis, the viral arm of such chimeric transcripts is mapped preferentially to a region between 1500 and 2000 base pairs on the viral genome located toward the end of the HBx gene.\(^{23}\) Lau and colleagues\(^{27}\) performed transcriptome sequencing of 6 HBV-positive HCC cell lines. They identified a viral-human chimeric fusion transcript that functions like a long noncoding RNA to promote HCC. Multiple instances of chimeric transcripts resulting from HBV integration were detected, many of which were from the human intergenic regions that contained a portion of a repetitive element, such as a LINE or SINE. The most intriguing and abundant fusion was the HBx-LINE1 chimera, detected in 23% of the HBV-associated HCC tissues examined. The HBx-LINE1 chimera functioned as a hybrid RNA. In addition, they also demonstrated that HBx-LINE1 can promote tumor growth likely through the activation of Wnt/b-catenin signaling.\(^{27}\)

**HBx**

HBx is a 17-kD protein encoded by the X open reading frame of HBV. HBx can complex with cellular proteins and transcriptionally transactivates virus gene expression and replication.\(^{28}\) Furthermore, HBx protects virus-infected cells from immune-mediated destruction during repeated bouts of hepatitis.\(^{29}\) HBx is thought to play a pivotal role in the hepatocarcinogenesis abilities of HBV by interfering with several cellular functions.

**Epigenetic effects**

HBx was shown in several studies to induce promoter hypermethylation and, as a consequence, to downregulate the expression of tumor suppressor genes. DNA methylation is an epigenetic tool that allows cells to repress the transactivation of
certain genes. Methylation of CpG sites within the promoters of genes can lead to their silencing. The DNMT family of genes executes DNA methylation.\textsuperscript{30} HBx can upregulate CpG methylation by transactivation of DNMT1 and DNMT3A expression.\textsuperscript{31,32} Among others, P16INK4A,\textsuperscript{31,33,34} E-cadherin,\textsuperscript{32,35,36} and IGFBP3\textsuperscript{30} were repressed in HBV-infected hepatocytes by the hypermethylation mechanism induced by upregulation of the DNMT genes. Both SFRP1 and SFRP5, antagonists of the Wnt signaling pathway, were epigenetically silenced by HBx in hepatoma cell lines. Downregulation of SFRP1 and SFRP5 correlated positively with DNMT1 overexpression.\textsuperscript{35} HBx can alter the methylation status of specific genes by the recruitment of DNMT1 and DNMT3A to specific methylation sites.\textsuperscript{38,39} However, other studies demonstrated an opposite effect of HBx, namely, the downregulation of levels of methylation on specific promoters and, as a consequence, upregulated expression of those genes.\textsuperscript{40,41} Recently, the effect of HBx on a novel epigenetic regulatory element, the highly methylated CpG islands (mCGIs), was demonstrated.\textsuperscript{42} Severe hypomethylation of intragenic mCGIs was observed in HBx liver before the full development of HCC. Furthermore, hypomethylation of mCGIs was caused by the downregulation of Dnmt3L and Dnmt3a expression levels because of the binding of HBx to their promoters, along with histone deacetylase 1 (HDAC1). These events led to the downregulation of many developmental regulators that could facilitate tumorigenesis.\textsuperscript{42}

The cell regulates gene expression by acetylation of histones in the nucleosome. These reactions are typically catalyzed by enzymes with histone acetyltransferase (HAT) or HDAC activity. p300/CBP is a transcriptional coactivator complex of proteins that harbors intrinsic HAT activity.\textsuperscript{43,44} HBx can bind directly to the CREB-binding domain of CREB-responsive promoters of endogenous cellular genes, such as interleukin (IL) 8 and PCNA; increase the recruitment of p300 to these promoters; and, as a result, upregulate those gene expressions.\textsuperscript{45} Conversely, HBx was shown to inhibit the expression of other genes (CDH1 and IGFBP3) by recruitment of HDAC complexes to their promoters.\textsuperscript{46,47}

**MicroRNA**

MicroRNAs (miRNAs) can be important mediators of HBV infection leading to HCC development and progression. HBx regulates miRNAs activity and is associated with both the downregulation and upregulation of different miRNAs expression. Wang and colleagues\textsuperscript{48} reported that HBx upregulated the expression of 7 miRNAs and downregulated 11 miRNAs. Eight out of the 9 members of the let-7 family were downregulated in the HBx-transfected cells. The most highly expressed let-7 family member, let-7a, negatively regulated cellular proliferation partly through targeting STAT3.\textsuperscript{48} HBx was shown to upregulate the oncoprotein astrocyte elevated gene-1 via downregulation of miR-375 and miR-136 in HBx-transfected cells.\textsuperscript{49} Additionally, Zhang and colleagues\textsuperscript{50} have shown that HBx directly targeted miR-205. HBx inhibits miR-205 expression probably by hypermethylation of its promoter. The forced miR-205 expression remarkably inhibited HBx-enhanced proliferation of hepatoma cells in vitro and in vivo, suggesting that miR-205 is a potential tumor-suppressive gene in HCC.\textsuperscript{50} Conversely, Liu and colleagues\textsuperscript{51} have shown that HBx\textsubscript{Δ}127 (a naturally occurring HBx mutant) was able to significantly increase miR-215 expression relative to wild-type HBx in hepatoma HepG2 and H7402 cells. Upregulation of miR-215 targeted the protein tyrosine phosphatase receptor type T, a tumor suppressor gene, indicating that HBx\textsubscript{Δ}127 strongly enhances proliferation of hepatoma cells.\textsuperscript{51}

Yip and colleagues\textsuperscript{52} infected nontumorigenic human hepatocytes with lentivirus-expressing full-length and carboxyl-terminal truncated HBx (Ct-HBx) for cell growth assay and miRNA profiling. Ct-HBx decreased, whereas full-length HBx increased
the expression of a set of miRNAs with growth-suppressive functions. Ct-HBx inhibited the transcriptional activity of some of these miRNA promoters.52

**Apoptosis**

HBx protein contributes to the development of HCC by its effects on apoptosis. HBx has both proapoptotic and antiapoptotic effects. The p53 gene and other tumor suppressor genes mediate both effects, at least partially.53 These contradictory effects might be explained by the evidence that high levels of HBx protein promote apoptosis, whereas low levels inhibit apoptosis.54 Knoll and colleagues55 showed that, in HCC tumor cell lines differing in their p53 status, HBx was proapoptotic but exhibited opposite effects in nontumor cells. In normal cells, p53 and p73 were retained in the cytoplasm, whereas, in hepatoma cells, HBx led to nuclear translocation of p53 and p73, followed by enhanced transactivation of p53-dependent promoters, supporting the dual function of HBx.55

**DNA repair pathways and genetic instability**

Gene array analysis comparing mRNA expression in fetal hepatic cell lines transfected with the HBx gene found increased expression of a large group of genes that are involved in cellular DNA damage repair and checkpoint signaling, such as DDB1, UGT1A9, UNG, XRCC1, XRCC3, XRCC4, and RAD17.56 HBx required binding to UV-damaged DNA binding protein 1 (DDB1)–ubiquitin ligase, a protein involved in DNA repair and cell cycle regulation, in order to stimulate transcription from the viral episomal DNA genome.57,58 HBx also induced lagging chromosomes during mitosis, leading to formation of aberrant mitotic spindles and multinucleated cells. These effects required the binding of HBx to DDB1 and were unexpectedly attributable to HBx interfering with S-phase progression and not directly with mitotic events. The binding of HBx to DDB1 may induce genetic instability in regenerating hepatocytes and, thereby, contribute to HCC development.59

**Hepatitis C Virus**

HCV, a positive-strand RNA virus, replicates in the cytoplasm and does not integrate into the host genome. Thus, HCV has a far lower direct oncogenic potential than HBV. It is currently thought that the main mechanism leading to HCC development in HCV infection is the indirect effect of chronic inflammation resulting from immune responses against infected hepatocytes, associated with apoptosis and enhanced hepatocellular proliferation, which leads to fibrosis with eventual progression to cirrhosis and the subsequent development of HCC.4 Data from several studies have implicated the direct role of HCV-specific mechanisms by both HCV structural and nonstructural proteins that are also involved in hepatocellular carcinogenesis.4 Transgenic mice expressing the HCV polyprotein develop liver cancer in the absence of inflammation or immune recognition of the transgene, thus, supporting a direct role for HCV proteins in carcinogenesis.60 Other evidence suggests that HCV protein expression may have broader cocarcinogenic effects. No single viral protein has been shown to consistently cause liver cancer when expressed at a low abundance comparable with that present in most patients with HCV-related liver disease.

**Core protein**

In 1998, Moriya and colleagues60 published the results of experiments with 2 independent transgenic mouse lines, each with a complementary DNA fragment containing the complete core gene of HCV genotype 1b. The expression of HCV core protein in both lines resulted in progressive morphologic and biochemical changes that ultimately resulted in the development of HCC. Chronic hepatitis, with continuous cell
death and regeneration, was not an absolute prerequisite for the development of HCC, indicating that the HCV core protein probably has a direct role in the development of HCC. HCV core protein induced spontaneous, persistent, age-dependent, and heterogeneous activation of PPARα in transgenic mice, which may contribute to multicentric hepatocarcinogenesis. Furthermore, in order to demonstrate the molecular mechanism of HCV core protein induction of hepatocyte growth regulation, microarray analysis of RNA purified from primary human hepatocytes and HCV core gene transfected hepatocytes suggested that the expression of HCV core protein resulted in an increase in expression of IL-6, gp130, leptin receptor, and STAT3. Upregulation of these genes in turn may regulate c-Myc and cyclin D1, downstream the STAT3 signaling pathway. Core protein also activates the Wnt/β-catenin cascade, which is known to play a role in the development of HCC. In HCCs samples, 40% to 70% were shown to harbor nuclear accumulation of the β-catenin protein, the hallmark of the Wnt/β-catenin pathway activation. One of the genes that was overexpressed in cells transfected with the core protein is the Wnt1 gene. Recently, Huang and colleagues have shown that cell lines transfected with HCV core protein significantly suppressed miR-152 expression. Reduction in miR-152 expression upregulated Wnt1 transcript expression leading to proliferation of liver cancer cells. The core protein also activates the Wnt signaling pathway by silencing SFRP1, a regulator of this pathway. HCV core protein markedly increased the expression level and binding of DNMT1 and HDAC1 to the SFRP1 promoter region, resulting in epigenetic silencing of SFRP1 expression. Silencing of SFRP1 may lead to the activation of the Wnt signaling pathway and, thus, contribute to HCC aggressiveness. HCV core protein can also activate the transforming growth factor-β (TGF-β) signaling pathway. Stellate cells growing in coculture with hepatoma cells expressing HCV core were activated by TGF-β, indicating a dual impact of HCV core on liver fibrosis and liver carcinogenesis, acting both in an autocrine manner by modulating the TGF-β responses in hepatocytes and by affecting stellate cell activation in a paracrine mechanism via TGF-β activation. Additionally, HCV core protein can upregulate and stabilize the levels of hypoxia-inducible factor 1α in Huh7.5.1 cells and transactivate the expression of vascular endothelial growth factor, one of the important angiogenic factors associated with the maintenance of liver carcinogenesis.

NS3
Results of experiments, both with tissue culture and nude mice models, have shown that NS3 has a direct role in the induction of cellular transformation. Furthermore, results indicated that the serine protease activity of NS3 is crucial for this transformation. The importance of the serine protease domain was emphasized by the authors’ findings that significant amino acid changes were defined at the catalytic domain of the NS3 serine protease gene isolated from HCC tissue. These changes were not detected in nontumorous tissues or in serum. In addition, NS3 protein can specifically repress the promoter activity of p21 in a dose-dependent manner probably via a protein–protein interaction with p53. Furthermore, the NS3 N-terminal peptide significantly upregulated the phosphorylation of p44/42MAPK but did not affect the expression of the total MAPK protein resulting in proliferation and transformation of hepatocytes. The same group later demonstrated that the NS3 protein can promote cell growth and contribute to hepatocarcinogenesis by the activation of ERK/AP-1 and NF-κB/cyclin D1 cascades. Cheng and colleagues showed that NS3 interacts with Smad3 and represses TGF-β/Smad3-mediated transactivation and growth inhibition and, thus, antagonizes the host defenses during hepatocarcinogenesis. However, recently it was shown that NS3 in the presence of NS4A interacted with
SMURF2, a negative regulator of TGF-β signaling. As a result, cells expressing the combination of NS3 and NS4A stimulate TGF-β induction and increase the expression of SMAD-dependent genes compared with control cells, pointing to the tumor suppressor function of SMURF2 as an interesting target to prevent HCC progression.

NS3 protease mimics TGF-β2 and directly exerts its activity via binding to and activating TβRI, thereby, enhancing liver fibrosis.

NS5A
NS5A is an HCV nonstructural protein that was shown to have transformation abilities in tissue culture and nude mice. NS5A binds directly to p53 and inhibits its transcriptional activity and, thereby, downregulates endogenous p21/waf1 expression. As a result, p53-induced apoptosis was abrogated by NS5A, thereby, contributing to the hepatocarcinogenesis of HCV. Moreover, NS5A downregulates the expression of GADD45α in a p53-dependent manner and subsequently triggers cellular proliferation. GADD45 proteins serve as tumor suppressors. Defects in the GADD45 signaling pathway can be related to the initiation and progression of malignancies. NS5A can also affect the Wnt/β-catenin cascade by stabilization of β-catenin and stimulation of β-catenin–dependent transcription by activation of PI3K. NS5A also binds directly to β-catenin and stimulates its activity. NS5A stabilization of β-catenin stimulated the expression of c-Myc that led to increased production of reactive oxygen species, mitochondrial perturbation, enhanced DNA damage, and aberrant cell-cycle arrest—all suggesting that HCV has a direct effect in liver tumorigenesis.

NS5B
NS5B the viral RNA-dependent RNA polymerase nonstructural protein forms a cytoplasmic complex with the retinoblastoma tumor suppressor protein (Rb) and, thereby, strongly negatively regulates HCV infection in cultured cells. NS5B recruits E6-associated protein to the process. The recruitment leads to polyubiquitination of Rb and Rb degradation through the proteasome. The end result is activation of E2F-responsive promoters, which would be expected to stimulate entry into the S phase of the cell cycle. The disruption of Rb/E2F regulatory pathways in cells infected with HCV is likely to promote hepatocellular proliferation and chromosomal instability, factors important for the development of liver cancer. These findings, which are unique among RNA viruses and may share attributes in common with many DNA tumor viruses, suggest a novel theoretic framework for the origins of liver cancer.

MicroRNAs and hepatitis C virus–associated cancer
HCV replication critically depends on miR-122. There is some evidence that miR-122 may have tumor suppressor properties. Expression of miR-122 has been shown to be low or undetectable in the human hepatoma cell lines, Hep3B and HepG2, in which its overexpression inhibited tumor formation in nude mice. Although several studies have profiled miRNA expression in HCC, it remains unclear whether miR-122 abundance is altered in HCV-associated HCC and what the exact role is of miRNAs in the development of HCC.

RISK OF HEPATOCELLULAR CARCINOMA IN A NONCIRRHOTIC LIVER
Hepatitis B Virus
Cirrhosis in patients with chronic HBV infection is only present in about 70% to 80% of HBV-related HCC cases and, thus, is not a prerequisite for tumorigenesis, especially in Asian and African patients. Patients with HBV can develop HCC with minimal liver damage. The REVEAL–HBV study demonstrated that 1932 inactive noncirrhotic HBV carriers, followed for a mean period of 13.1 years, were at a substantial risk of
developing HCC. The multivariate adjusted hazard ratio for carriers of inactive HBV, compared with controls, was 4.6 (95% confidence interval 2.5–8.3) for HCC. The REVEAL-HBV study also evaluated the risk of HCC across a biological gradient of serum HBV DNA levels. The incidence of HCC was significantly associated with serum HBV DNA levels in a dose-response manner from less than 300 (undetectable) to greater than 1,000,000 copies per milliliter. The serum level of HBV DNA was a prominent predictor of HCC risk, independent of the presence of cirrhosis (P<.001). Liu and colleagues reported that the accumulation of mutations in the basal core promoter and a high viral load (10^4–5 copies per milliliter) were independent predictors of HCC development in the absence of cirrhosis.

Occult Hepatitis B Virus

In occult HBV infection, HBV virus can continue to replicate at low levels causing persistent inflammation and injury, which may contribute to the development of HCC, implying that the occult viral strains maintained the transcriptional activity and the pro-oncogenic property of the overt (termed clear by the investigators) HBV infection (ie, showing HBsAg positivity). However, knowledge of the role of occult HBV in the development of HCC is still very limited and often confounded by current therapy and testing methodology.

HBV infection can trigger hepatic carcinogenesis independent of the development of cirrhosis. Different molecular mechanisms may be involved underlying the development of HCC with and without cirrhosis. For instance, as previously mentioned, the HBV viral genome can directly integrate into the host human genome and act as an oncogenic factor, a process that is independent of the chronic inflammation that commonly characterizes cirrhosis. By using comparative genomic hybridization, it has been observed that copy number gains in 8q and 20q and the loss of 4q were more frequent in HBV-associated HCCs with no underlying cirrhosis than in cirrhotic HCCs. Telomere length has emerged as a promising risk predictor of various cancers, including HCC. Longer relative telomere length in circulating cell-free serum DNA was significantly associated with an increased risk of noncirrhotic HBV-related HCC. HBx protein increased both the expression of telomere reverse transcriptase and telomerase activity, the enzyme responsible for the maintenance of telomere length, thus, prolonging the lifespan of hepatocytes and contributing to malignant transformation.

Hepatitis C Virus

To date there are very few data on HCC arising in HCV infected but noncirrhotic livers. Of HCV-related HCC, 6% to 17% were reported in noncirrhotic livers. In the prospective HALT-C trial of 1005 chronically infected patients in the United States, 17% of HCCs were found in the absence of advanced fibrosis. The mechanism of HCV-associated HCC arising in noncirrhotic livers is not clear. There is general agreement that ongoing necroinflammation resulting from HCV infection seems to contribute significantly to the risk of developing HCC, independent of hepatic fibrosis. Elevated levels of transaminases, a marker for liver inflammation, correlate with HCC risk among patients with HCV independently of fibrosis stage. As stated earlier, several HCV gene products (core, NS3, NS4B, and NS5A) possess a transformation potential to alter several potentially oncogenic pathways suggesting that HCV also has a direct hepatocarcinogenic potential. The development of HCC in persons with noncirrhotic fibrosis raises the question of whether such patients should undergo HCC surveillance, as is recommended for persons with established cirrhosis. Currently, screening in noncirrhotic fibrosis is optional.
PREVENTION OF HEPATOCELLULAR CARCINOMA IN PATIENTS WITH CHRONIC VIRAL HEPATITIS B AND C

Hepatitis B Virus Vaccine

It is now well established that vaccination against hepatitis B is very effective in preventing HBV infection. Control and significant reduction in incidence of new HBV infections as well as a reduced incidence of HCC have been repeatedly reported in countries in East Asia and Africa. Data from Taiwan have shown convincingly that rates of childhood HCC have decreased significantly since the implementation of universal infant vaccination in Taiwan in 1984. The prevention of HCC by HBV vaccination extends from childhood to early adulthood; failure to prevent HCC resulted mostly from incomplete HBV vaccination or unsuccessful control of HBV infection in highly infectious mothers. In a recently published follow-up study, the 30-year outcomes of the HBV Taiwanese immunization program were evaluated and HCC incidence decreased by more than 80% for individuals aged 5 to 29 years. Likewise, a reduction in HCC has been demonstrated among Thai and native Alaskan children who received hepatitis B vaccination at birth. Hepatitis B vaccination is now a part of the National Infant Immunization Schedule in 162 countries and constitutes the first example of cancer prevented by vaccination, although confirming the mortality reductions from HBV-associated HCC may require several decades.

Hepatitis B Virus Antiviral Therapy: Interferon and Nucleoside/Nucleotide Analogues

It has been difficult to prove that HBV treatment reduces the incidence of HCC. Prevention of HCC in patients with HBV with a maintained virological response to therapy is not, as yet, convincingly demonstrated because it takes many years for long-term outcomes, such as HCC development, to present. The exclusion of patients from treatment while continuing to observe them clinically is obviously unethical and untenable. All therapeutic trials designed to assess antiviral efficacy of anti-HBV regimens adopted surrogate end points and, therefore, were underpowered to capture hard end points of hepatitis B infection, including HCC. The two accepted treatment modalities are interferon-α (IFN-α) given subcutaneously for a limited time period and nucleoside/nucleotide analogues given orally on a long-term basis. These treatments are effective in suppressing viral activity and improving disease markers in short-term studies. There are no studies directly comparing IFN-α and nucleoside/nucleotide analogues.

Interferon-α therapy

Results of long-term follow-up studies of IFN-α therapy were inconsistent regarding the reduction of HCC development, probably related to IFN-α’s moderate suppressive ability on HBV replication. Although in some studies there was prevention of HCC by IFN, in others no benefits of IFN therapy were demonstrated. Patients with less severe disease were included in these IFN studies in order to improve patient compliance, which resulted in a selection bias. There were also differences in the duration of follow-up between patients who responded to INF-α versus nonresponders. Moreover, in most of the studies, hepatitis B e antigen (HBeAg) seroconversion was used as the end point of treatment, although most patients continued to have detectable HBV DNA after HBeAg seroconversion. Currently it can be surmised that the beneficial effect of IFN-α in reducing the development of HCC is limited to patients with cirrhosis who are sustained responders, although they compose a relatively small proportion of patients studied.
**Nucleoside/Nucleotide analogues**

In the long-term studies of treatment with lamivudine (and adefovir), there was a consistent reduction in the development of liver cancer in patients who achieved a virological response irrespective of the presence of cirrhosis; but in some cases, nonetheless, HCC still developed. This beneficial effect is blunted by the development of HBV resistance. In a systematic review of studies of NUC treatment of patients with HBV, it was clearly determined that HCC was prevented in patients with chronic hepatitis but not in those with cirrhosis and, in general, in patients who could not achieve complete virological suppression. This finding was also confirmed by a cohort study from Greece in which cirrhotic patients on long-term lamivudine were shown to remain at risk of developing liver cancer. Recently the effect of the more potent anti-HBV drugs, such as entecavir and tenofovir, was evaluated in terms of HCC prevention in responders with cirrhosis. In a large US-based longitudinal observational cohort study in 2671 patients with HBV, HBV antiviral therapy (94% received NUCs: lamivudine, entecavir, tenofovir, telbivudine, or adefovir) was associated with a significantly decreased risk of HCC (follow-up 9 years) than those who did not receive antiviral therapy (adjusted hazard ratio, 0.39; 95% confidence interval, 0.27–0.56; P<.001). The beneficial effects were not associated with the fibrosis stage (using various surrogates to estimate the severity of underlying liver disease). Treated patients with viral loads greater than 20,000 IU/mL had a significantly lower risk of HCC than untreated patients with comparable viral loads. In a recent Japanese study, entecavir significantly reduced the incidence of HCC among chronic HBV-infected patients and did so to a greater extent than lamivudine did. Patients at higher risk for HCC (ie, those with cirrhosis) derived greater benefit from treatment compared with lower-risk subjects. However, this was not the case in a large nationwide Greek study that included 321 patients treated with entecavir for a median of 40 months; the HCC risk remained increased in entecavir-treated HBeAg-negative patients with HBV with cirrhosis. In another multicenter study from Italy, patients with compensated cirrhosis and undetectable serum HBV DNA during 5 years of entecavir monotherapy showed an annual rate of HCC of approximately 2.5%, which is similar to the HCC rates in untreated HBeAg-negative patients in Europe. A prediction model (REACH-B risk calculator) was used to compare the incidence of HCC in 641 patients treated for 6 years with tenofovir in the tenofovir long-term registration trial. In non-cirrhotic patients, the effect of tenofovir became noticeable at approximately 2 years of therapy and became significant (55% reduction) at 6 years. But the benefit was less pronounced in cirrhotic patients.

Persistence of HCC risk in cirrhotic patients responding to NUCs therapy may be the consequence of the extended survival provided by NUCs, or it can be assumed that HBV-related liver carcinogenesis is promoted by cellular events that are established early during chronic infection with HBV and are independent of the onset of cirrhosis.

**Hepatitis C Virus Antiviral Therapy**

In chronic HCV infection, HCC is usually associated with advanced fibrosis or cirrhosis. There is limited evidence for the role of IFN-based therapy in the prophylaxis of HCC in patients with chronic HCV, as most studies were primarily designed to assess the antiviral effect of treatment and not the long-term impact on the natural history of the disease. HCV eradication decreases the risk of HCC in antiviral responders compared with that observed in patients failing therapy. Significantly lower incidences of HCC and mortality were observed in sustained virological responders (both for IFN monotherapy and IFN/ribavirin in combination), but not in nonresponders,
when compared with untreated patients. Preexisting cirrhosis, nonresponse, HCV genotype-1, and age were associated with HCC, as well as steatosis, male sex, diabetes, and alcohol consumption. SVR had a strong independent positive influence on the incidence of HCC in 127 HCV patients with bridging fibrosis and 180 with cirrhosis, treated with pegylated IFN (pegIFN) and ribavirin. In a recent Japanese multicenter study of 1013 patients with HCV (noncirrhosis n = 863 and cirrhosis n = 150), SVR and complete viral suppression during treatment in relapsers were associated with a lower risk of HCC development in cirrhotic patients when compared with nonresponders. In a meta-analysis that combined data from 30 studies, the incidence of HCC was 1.05% per person-year in those with SVRs compared with 3.3% in those without an SVR. In patients with advanced fibrosis (Ishak fibrosis score 4–6), the cumulative occurrence of HCC after 10 years was 21.8% without an SVR and 5.1% with an SVR. Results of several studies suggested that low-dose IFN may delay the development of HCC even in patients with cirrhosis. However, this latter finding remains controversial; there are also conclusive and negative studies.

In the HALT-C cohort, HCV-positive patients with bridging fibrosis or cirrhosis who did not respond to PegIFN and ribavirin, maintenance PegIFN for 3.5 years did not reduce the incidence of HCC. A lower incidence of HCC in patients randomized to long-term low-dose PegIFN only emerged after 5 to 7 years of follow-up. Maintenance therapy with PegIFN α-2b in the Evaluation of PegIntron in Control of Hepatitis C Cirrhosis (EPIC) 3 program was deemed unwarranted in cirrhotic patients with chronic HCV, as there was no decrease in the development of HCC with therapy. Altogether, long-term suppressive therapy with IFN to prevent HCC is not currently a realistic strategy especially with the emergence of the new HCV direct-acting antiviral agents, even though none of them have been in use long enough to evaluate their effect in reducing HCC incidence.

**Risk for Hepatocellular Carcinoma Development in Patients Without Cirrhosis After Successful Antiviral Therapy**

Whether there is an increased risk for HCC development among noncirrhotic patients after successful antiviral therapy remain unclear. In a recently published study a total of 642 patients with an SVR after PegIFN/RBV therapy were studied in Taiwan for a median follow-up period of 53.0 months (range: 6–133 months). Of the 556 noncirrhotic patients, only 17 (3.1%) versus 16 (18.6%) of the 86 cirrhotic patients developed HCC (P < .001). Older noncirrhotic patients with high-baseline γ-glutamyl transferase levels had as great a risk for HCC development as cirrhotic patients.

**Advanced fibrosis/patients with cirrhosis achieving sustained viral response and risk for hepatocellular carcinoma**

There is clear evidence that patients with advanced fibrosis or cirrhosis who achieve SVR remain at heightened risk for HCC. In a Japanese study, 1193 patients with HCV-related chronic liver disease and IFN monotherapy or IFN plus ribavirin-induced SVR were followed up for a mean period of 8.3 years. The crude rates of hepatocarcinogenesis at 5, 10, and 15 years were 1.5%, 2.4%, and 4.1%, respectively. Moreover, HCC was diagnosed in 26% of 562 consecutive SVR patients, more than 10 years after completion of IFN therapy. F2 fibrosis (ie, the presence of periportal or portal septa) was detected in 42% of these patients. Patients with the risk factors of advanced age at HCV eradication and heavy alcohol intake were at heightened risk for the development of HCC within 5 years after HCV eradication. Van der Meer and colleagues reported the results of an international, multicenter, long-term follow-up study from 5 large tertiary care hospitals in Europe and Canada.
of 530 patients with chronic HCV infection who started an IFN-based treatment regime between 1990 and 2003, following histologic proof of advanced hepatic fibrosis or cirrhosis. Patients who achieved SVR had a lower incidence of HCC (0.55 vs 1.01 per 100 person-years), liver failure (0.31 vs 3.62 per 100 person-years), liver-related mortality (0.23 vs 3.20 per 100 person-years), and, most importantly, all-cause mortality (1.01 vs 2.93 per 100 person-years) than patients who did not achieve SVR. A recent Swedish study\textsuperscript{142} prospectively evaluated the long-term effect of antiviral therapy on the risk of developing HCC in a cohort of 351 patients with HCV with Child-Turcotte-Pugh class A cirrhosis. The incidence of HCC was significantly reduced in patients who achieved SVR (1.0 per 100 person-years) compared with those who failed treatment (2.3 per 100 person-years) or those who were never treated (4.0 per 100 person-years). The incidence of HCC was similar in the first 3 years and the subsequent 3 years after viral clearance. In both studies,\textsuperscript{17,142} better outcomes were detected in patients who achieved SVR; however, they have also documented that some patients were still at risk of developing HCC, with an incidence of approximately 0.5% to 1.0% per year. Investigators of the HALT-C study\textsuperscript{107} showed that patients with bridging fibrosis had a 0.82% risk per year of developing HCC. These findings suggest that long-term screening for HCC should be done in patients with cirrhosis even after achieving SVR. However, the guidelines from the American Association for the Study of Liver Disease do not recommend screening such patients because the incidence is less than the cost-effectiveness analysis threshold of 1.5% per year.\textsuperscript{143}

REFERENCES


