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REVIEW

Molecular and Clinical Features of Hepatocellular Carcinoma in Patients with HBV-HDV Infection

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Abstract: Hepatitis D virus (HDV) infection affects more than 10 million people worldwide, with an estimated prevalence of nearly 4.5% among HBsAg-positive individuals. Epidemiological studies have shown a significant increase in the prevalence of hepatocellular carcinoma (HCC) in patients with chronic HDV infection compared to those with chronic hepatitis B virus (HBV) mono-infection. Despite the clinical findings, data on molecular oncogenic mechanisms are limited and fragmentary. Moreover, the role of HDV in promoting the development of HCC has so far been controversial, because it is difficult to weigh the respective contributions of the two viruses. In this review, we focused on the direct oncogenic action of HDV, its role in modifying the tumor microenvironment, and the genetic signature of HDV-related HCC, comparing these features with HBV-related HCC.

Keywords: hepatitis B virus, hepatitis delta virus, chronic hepatitis B, CHB, chronic delta hepatitis, CDH, dual infection, HCC

Introduction

Hepatocellular carcinoma (HCC) is the most frequent histological subtype of primary liver cancer, accounting for nearly 75% of all liver malignancies.[1] With 800,000 new cases annually, a cumulative incidence of 16/100,000 cases and 745,000 deaths per year, HCC ranks as the sixth most frequent cancer in the world, and represents one of the leading causes of cancer-related mortality in men. Moreover, the incidence is expected to increase after 2025, with more than 1,000,000 cases yearly.[2] Asia and Africa have the highest incidence rates worldwide;[1] however, as recent epidemiological data show, the mortality rate from HCC is rising in North America and Europe, where HCC is reported to be the cause of death in 54–70% of individuals with compensated cirrhosis from various etiologies.[3]

In most cases, HCC is preceded by advanced fibrosis/cirrhosis, resulting from chronic liver inflammation.[4] Globally, the underlying aetiology of liver disease is related to chronic viral hepatitis sustained by hepatitis B virus (HBV) or hepatitis C virus (HCV) infections in about 80% of patients.[5] On the other hand, the relationship with hepatitis delta virus (HDV) remains unclear.[6] Further independent risk factors for HCC are alcohol abuse, diabetes mellitus, overweight and obesity, metabolic dysfunction associated fatty liver disease (MAFLD), aflatoxin B1 exposure, hemochromatosis and other more rare conditions.[7] Given its rising prevalence and the steady decline in viral hepatitis-related HCC due to immunization and efficient antiviral therapies, MAFLD is expected to overtake viral infections as the primary cause of HCC in the next ten years.[7,8] However, HCC still remains a fearsome consequence of HBV infection, especially when associated with delta virus (HDV) infection.

In this paper, we will discuss the pathophysiological background and characteristics of HCC arising in this specific clinical setting.

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HBV: Microbiological Characteristics and Natural History

The hepatitis B virion consists of an inner nucleocapsid core that contains the viral DNA and an outside lipoprotein envelope. Viral genome encodes seven proteins: envelope antigens (Small-protein or S or HBsAg, Middle-protein or M, Large-protein or L), a non-structural antigen (HBeAg), the core antigen (HBcAg), HBV polymerase and X protein (HBx). After endocytosis in the hepatocyte, HBV-DNA is incorporated into the genome of the host but it is also transformed into a double-stranded circular DNA structure (the "covalently closed circular DNA"), which serves both as a transcriptional template for viral RNA and as a reservoir for the reactivation of viral replication.

When the host fails to resolve acute HBV infection it becomes chronic, and up to 40% of untreated patients progresses to cirrhosis (Figure 1).[9–11] Roughly one-third of patients with untreated HBV-related liver cirrhosis develops HCC.[11] Although nearly 80% of HCC develop in a cirrhotic liver,[12] and a small percentage in the absence of cirrhosis.[13] Overall, at least one-third of HCC-related death is caused by chronic HBV infection.[14]

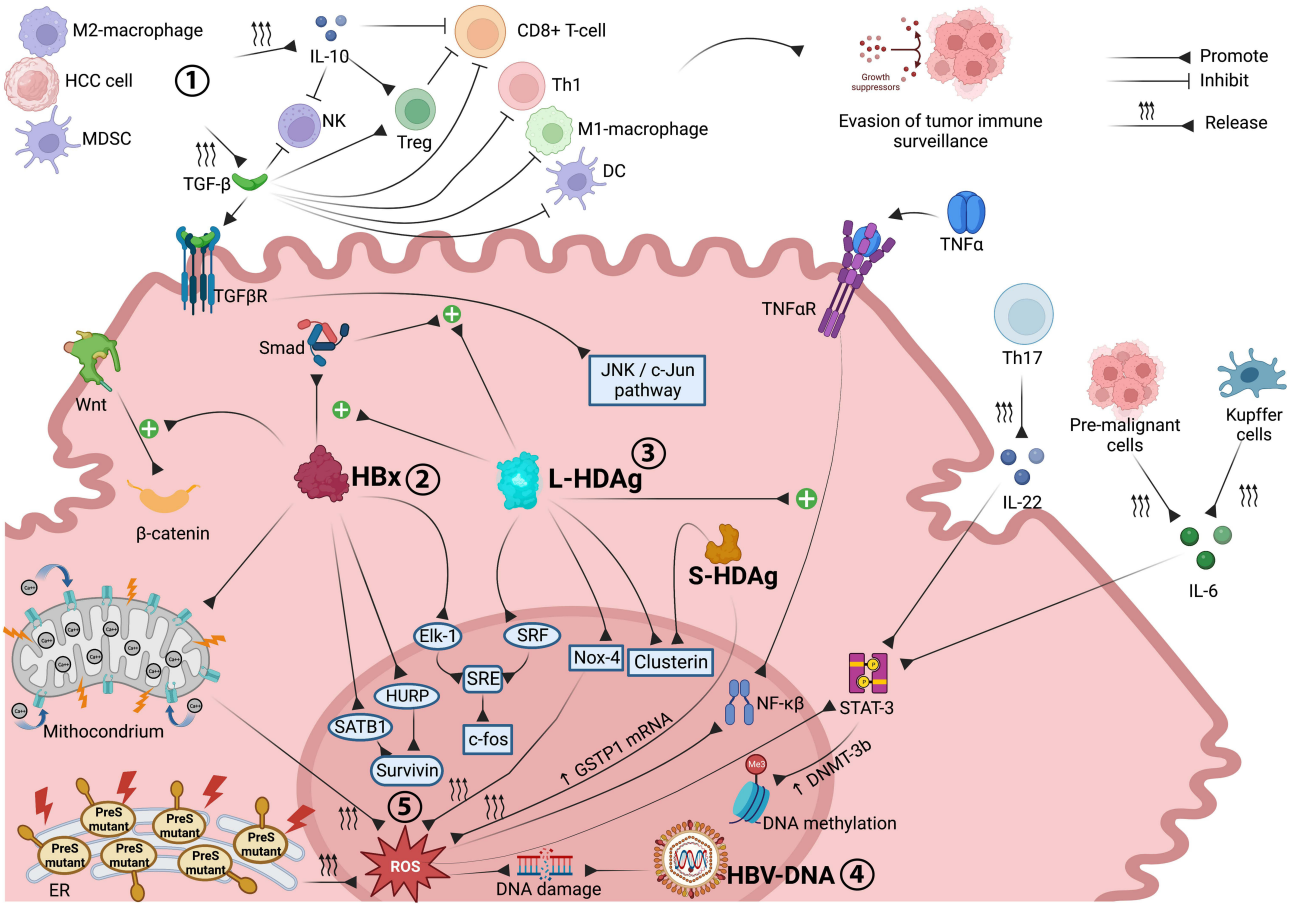

Figure 1 Molecular interactions and oncogenic mechanisms occurring in the HCC microenvironment associated with chronic HBV-HDV infection. Both immune cell impairment and stimulation of pro-oncogenic intracellular signaling pathways are involved in CHB- and CDH-driven hepatocarcinogenesis. Through the release of TGF-β and IL-10, M2 macrophages, MDSCs and tumor cells inhibit the antitumor functions of immune effector cells, promoting tumor immune escape. HBx upregulates Wnt/β-catenin signaling and promotes survivin protein synthesis, supporting tumor cell proliferation and preventing apoptosis. Synergistically, L-HDAg and HBx trigger the JNK/c-Jun and SRE/c-fos cascades, both of which are involved in cancer cell growth, proliferation and survival. The integration of HBV-DNA into the host genome and the overproduction of ROS, triggered by HBx, L-HDAg and S-HDAg, promote DNA damage and genomic instability. ROS also promote cancer cell survival, angiogenesis and invasiveness through upregulation of the NF-κB pathway, which is also stimulated by L-HDAg in a TNFα-dependent manner, and STAT-3 signaling, which is further activated by Th17 (via IL-22), pre-malignant cells and Kupffer cells (via IL-6). Image created with Biorender.com.

Abbreviations: CDH, chronic delta hepatitis; CHB, chronic hepatitis B; DC, dendritic cells; DNMT-3b, DNA methyltransferase-3b; Elk-1, E26 transformation-specific like-1 protein; ER, endoplasmic reticulum; GSTP1, glutathione S-transferase P1; HBV, hepatitis B virus; HBx, X protein; HCC, hepatocellular carcinoma; HDV, hepatitis delta virus; HURP, hepatoma upregulated protein; IL-6, interleukin-6; IL-10, interleukin-10; IL-22, interleukin-22; JNK, c-Jun N-terminal kinase; L-HDAg, large hepatitis D antigen; MDSC, myeloid-derived suppressor cells; NK, natural killer cells; Nox-4, NADPH Oxidase 4; NF-κB, nuclear factor κB; ROS, reactive oxygen species; SATB1, special AT-rich sequence-binding protein-1; S-HDAg, small hepatitis D antigen; Smad, small mother against decapentaplegic proteins; SRE, serum response element; SRF, serum response factor; STAT3, signal transducer and activator of transcription 3; TGF-β, transforming growth factor-β; TGFβR, transforming growth factor-β receptor; Th1, T-helper 1 cells; Th17, T-helper 17 cells; TNFα, tumor necrosis factor α; TNFαR, tumor necrosis factor α receptor; Treg, regulatory T cells; Wnt, wingness-related integration site.

Most often, acute HBV infection is asymptomatic or paucisymptomatic. Once chronic infection occurs, it typically progresses in an asymptomatic manner, so that the diagnosis is often established by chance during laboratory tests.[15] The immune reaction to HBV foreign antigens, rather than the virus itself, promotes inflammation, necrosis and eventually fibrosis of liver parenchyma. However, the immunological activity against HBV is not continuous, so that this process proceeds intermittently; indeed, cirrhosis and HCC development result from repeated periods of immunological activity over several years. Hence, chronic HBV infection has been schematically divided into five, not necessarily sequential, phases, according to the presence of the following biomarkers: HBeAg, HBV-DNA levels, alanine aminotransferase value, and liver necroinflammation and fibrosis. The combination of these indicators reflects the degree of hepatic immunological activity, thus guiding the choice to begin treatment.[16]

Epidemiological and Microbiological Characteristics of HDV

HDV infection affects about 12 million people worldwide, with an estimated prevalence of 4.5% among HBsAg-positive individuals. Prevalence rates are higher in Africa and America (slightly under 6%), although the highest one is registered in Mongolia (36.9%). Also, HCV-positive or human immunodeficiency virus (HIV)-positive subjects, persons who inject drugs and haemodialysis recipients show higher prevalence rates compared to the general population. HDV genotype 1 prevails globally (89.9%), while other genotypes are more regionally specific, such as genotypes 2 and 4 in Asia, genotype 3 in Latin America, genotypes 5, 6 and 8 in Africa (Table 1).[12]

HDV is made up of an inner nucleocapsid carrying a single-stranded circular RNA combined with roughly 200 molecules of hepatitis D antigen (HDAg), and an outer lipid envelope embedded with the HBsAg derived from HBV.[13] In fact, HDV is considered a faulty virus, requiring the simultaneous presence of HBV to be a human pathogen; specifically, it needs HBsAg to assemble virions and penetrate into hepatocytes.[14] After entering the cell via a HBsAg-mediated receptor binding, HDV-RNA is translocated into the nucleus and uses host RNA polymerase for replication. Following viral genome replication, synthesis of HDAg (the only protein encoded by HDV genome, in 2 isoforms: large-HDAg or L-HDAg and small-HDAg or S-HDAg) and post-translational HDAg modifications, HDAg must combine with HBsAg to generate an infectious virion that can exit the hepatocyte.[15]

Pathogenesis and Clinical Evolution of HBV-HDV Dual Infection

Unlike the other main hepatotropic viruses, the pathogenesis of HDV-related liver damage is still unclear. Analogous to HBV infection, HDV infection seems to cause liver damage predominantly via a host-mediated immune response, as suggested by both the presence of HDAg-specific CD4+ T-cells in peripheral blood,[16] and the pronounced necroinflammatory activity at liver histology[17,18] of affected patients. Furthermore, in subjects with HDV/HIV co-infection, the reduction in CD4+ T-lymphocyte counts due to HIV activity has been associated with higher HDV-RNA levels, indicating that adaptive immunity may play a relevant role in the containment of HDV replication.[19] The existence of

Table 1 Estimated Prevalence of HDV Infection in HBsAg-Positive General Population and HDV Genotypes Distribution by Country

Country	HDV Prevalence (%)	HDV Prevailing Genotype
African Region	5.97	1, 5, 6, 7, 8
Regions of Americas	5.91	1, 3
Western Pacific Region	4.09	1, 2, 4
Eastern Mediterranean Region	3.54	1
European Region	3.00	1, 5
South East Asian Region	3.20	1, 2
Global	4.49	1

HDV cytopathic activity is still debated, as some studies support direct virus-mediated hepatocellular damage,[20] while others do not.[21]

Because HDV is a defective virus that requires the concomitant presence of HBV to establish infection, which can occur at the same time as HBV infection (co-infection) or in the context of chronic HBV infection (superinfection). Compared with acute HBV monoinfection, HBV-HDV coinfection is associated with a higher mortality rate in the short term, as it can result in life-threatening acute or fulminant hepatitis with acute liver failure.[22] However, 90–95% of HBV-HDV coinfections resolve spontaneously, as a consequence of HBV clearance.[23] In contrast, HDV superinfection often results in exacerbation of hepatitis[24] and evolves into chronic HDV infection in the vast majority of cases.[23] Chronic delta hepatitis (CDH) is considered the most aggressive form of hepatitis, as it is associated with a more rapid progression to cirrhosis[23,25] and a higher rate of liver decompensation leading to death[23,26] than chronic HBV infection alone.

Since HBV infection is mandatory to allow HDV to initiate the infection and subsequently accomplish an oncogenic role, the role of HDV in promoting the development of HCC is still controversial, because it is difficult to specifically weigh the respective contributions of the two viruses. In fact, as of 1994, HDV is still included in International Agency for Research on Cancer (IARC) Group 3 (not sufficient evidence of carcinogenicity), while HBV and HCV are classified in Group 1 (high evidence of carcinogenicity).[27] Although some studies have found no statistically significant difference in HCC incidence rates between patients with dual HBV-HDV infection and those mono-infected with HBV,[28,29] many others have found a higher cancer risk in the former population than in the latter.[26,30–32] Among HBV-HDV-infected individuals, the development of HCC has been associated with higher levels of HDV-RNA in peripheral blood,[33] which could be a further indication of the oncogenic effect of HDV. In addition, two very recent meta-analyses comparing dual-infected and single-infected patients confirmed the oncogenic potential of HDV, pointing to a higher incidence rate of HCC in the former group.[34,35] Furthermore, subgroup analyses performed by Chang et al[34] found that the risk of HCC was higher in HBV-HDV dual infection, regardless of ethnic group or concomitant infection with HIV or HCV, with no statistically significant difference according to the severity of liver fibrosis.

Impact of HBV and HDV on Hepatocarcinogenesis

Liver Tumor Microenvironment in Chronic Hepatitis B

HBV conventionally does not exhibit cytopathic activity, and liver tissue damage results from the immune response against surface and core viral antigens. Over time, this leads to a state of chronic inflammation and promotes immune depression, which in turn promotes hepatocarcinogenesis. Specifically, immunological alterations in the tumor microenvironment involve dysregulation of specific key signaling pathways, cytokine production, and functional impairment of T cells and innate immune cells (Figure 2).

Inflammation promotes the activation and interaction between nuclear factor (NF)-κB and signal transducer and activator of transcription 3 (STAT3), which act preventing pre-malignant and tumor cells from apoptosis and promoting tumor immune escape, angiogenesis and invasiveness.[36,37] STAT3 activation is mainly mediated by interleukin (IL)-6,[38] which is initially secreted in an autocrine manner by pre-malignant cells.[39] Once HCC is developed, Kupffer cells become critical in the tumor growth process, through IL-6 release.[40] The STAT3 signaling cascade can also be triggered by IL-22 produced by T helper (Th) 17 cells,[41] the role of which in HCC progression has recently been underlined.[42] As for the NF-κB pathway, some evidence has revealed that it is also involved in anti-oncogenic processes;[35] hence, a strategy to inhibit NF-κB signalling may be a double-edged sword.

Recent evidence has linked a higher concentration of tumor-associated macrophages in HCC microenvironment, with a poorer prognosis.[43] Specifically, M2-phenotype tumor-associated macrophages (a pro-oncogenic subtype of macrophages) release transforming growth factor-β (TGF-β), which triggers CD8+ T-cells to expressmarkers of "exhaustion",[44] such as programmed cell death-1 (PD-1), T-cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), lymphocyte-activation gene-3 (LAG-3) and T cell immunoreceptor with Ig and ITIM domains (TIGIT).[45] Exhausted CD8+ T-cells exhibit defective cytotoxic activity and impaired ability to release antiviral cytokines,[46] thus promoting the development of HCC. TGF-β negatively affects the host immune response through several other mechanisms; in particular, it inhibits the

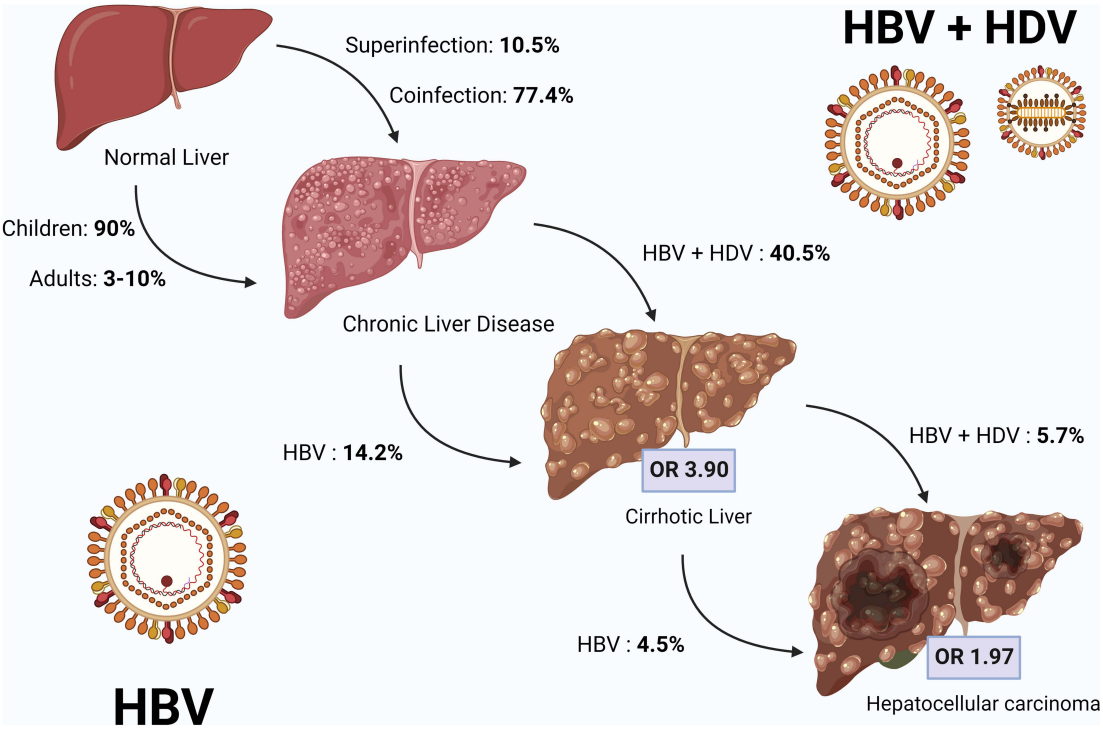

Figure 2 Natural history of HBV and HBV/HDV infected patients. Chronic HBV infection occurs in 90% of infected children and up to 10% of infected adults, while HDV superinfection orcoinfection lead to chronic disease in 10% and 77% of exposed patients, respectively. Compared with HBV mono-infection, HDV/HBV coinfection more frequently leads to liver cirrhosis (OR 3.84), and in a shorter time. The risk of HCC development is increased in both HBV mono-infection and HBV/HDV coinfection (OR 1.66). Image created with Biorender.com.
Abbreviations: HBV, hepatitis B virus; HDV, hepatitis delta virus; OR, odds ratio.

anti-oncogenic M1-macrophages subtype, reduces the antitumor function of Th1 cells by inducing their differentiation to Th2 cells, and suppresses the cytotoxic activity of natural killer (NK) and dendritic cells.[47,48] In addition to M2-macrophages, myeloid-derived suppressor cells and tumor cells also secrete TGF-β and IL-10; IL-10 exerts analogous inhibitory effects on CD8 T-cells and NK cells.[38,49]

Furthermore, TGF-β and IL-10 enable the activation of regulatory T cells (T_{reg}), which represent a subpopulation of T cells (expressing CD4, CD25 and the forkhead box P3 transcriptional regulator [Foxp3]) with tolerogenic and immunosuppressive properties, mostly antagonizing the killing function of CD8+ T-cells. Their role in promoting immune escape in HBV-related HCC is well documented.[50] T_{reg} can be activated by TGF-β and IL-10 both directly (receptor–ligand interaction)[38] and indirectly, as high levels of TGF- β (typically found in chronic hepatitis B) down-regulate the transcription of microRNA-34a, eliciting the synthesis of chemokine C-C Motif Chemokine Ligand 22 (CCL22) and the subsequent T_{reg} recruitment.[51]

Oncogenic HBV Mechanisms

In addition to chronic liver inflammation, virus-related factors significantly contribute to tumorigenesis in chronic hepatitis B (CHB)-related HCC (Figure 2).

The integration of viral DNA is one of the most well-known oncogenic factors, since its integration in the host genome is thought to provoke deletions, translocations, chromosomal aberrations, fusion transcript synthesis and generalized genomic instability.[52] Moreover, the presence of viral genetic material in the nucleus of hepatocytes elicits the activation of DNA repair mechanisms, increasing the DNA recombination rate.[53] Accordingly, HBV-DNA incorporation occurs more commonly in cancer cells than in healthy hepatocytes.[54] In addition, chronic inflammation leads to hepatocyte death and subsequent compensatory proliferation, which increases the viral DNA insertion rate, and exacerbates the potential direct oncogenic effects of HBV-DNA.[55]

HBx is another viral factor that strongly promotes HCC tumorigenesis, as suggested by its significantly higher expression in HBV-related HCC as compared to first stages of HBV infection.[56] HBx acts by altering several pathways; for example, it prevents cancer cells from apoptosis by increasing the expression of the hepatoma upregulated protein (HURP) and special AT-rich binding -1 (SATB1) gene, resulting in the overproduction of the anti-apoptotic protein survivin.[57] Moreover, HBx upregulates the Wingness-related integration site (Wnt)/β-catenin signaling pathway, favouring the adhesion of epithelial cells.[58] HBx can also interfere with transcriptional regulation and DNA-binding of p53, which acts as regulator of apoptosis, cell cycle arrest and DNA repair.[59] Also, it can hinder the activity of mitochondria by altering their membrane potential[60] and increasing their calcium content,[61] both resulting in the overproduction of reactive oxygen species (ROS) and oxidative stress, whose carcinogenic potential is well established.[62] HBx promotes HCC metastatization too, by inducing epithelial-mesenchymal transition.[63] These are only a small subset of the multitude of molecular mechanisms through which HBx can boost hepatocarcinogenesis, as it is also implicated in promoting genetic instability,[64] preventing cellular senescence[65] and inducing epigenetic modifications.[66]

Further potential viral factors implied in tumorigenesis are envelope proteins, such HBsAg and others encoded by specific mutant variants of preS/S genes. PreS mutants can lead to the overproduction of mutated envelope proteins, which accumulate into the endoplasmic reticulum (ER), resulting in ROS release with subsequent DNA damage and genomic instability. Furthermore, ER stress can trigger the activation of signaling pathways leading to hepatocyte proliferation, angiogenesis and eventually HCC development.[67]

HDV Infection Influences the Molecular Profile of HCC

Although HDV infection seems to increase the risk of HCC in individuals already infected by HBV, data concerning the oncogenic molecular mechanisms specifically related to HDV are limited and fragmentary (Figure 2).

A significant contribution comes from Choi et al,[68] who described one of the potential oncogenic properties of L-HDAg. They demonstrated that L-HDAg upregulates TGF-β-mediated transcriptional activity of c-Jun, which modulates cell growth, proliferation and apoptosis.[69] The same signaling cascade is activated by the interaction between HBx and Small mother against decapentaplegic (Smad) proteins, which was found to be enhanced by L-HDAg in a dose-dependent manner;[68] this synergistic molecular mechanism could, at least partially, explain why HBV-HDV dual infection is associated with a higher HCC incidence rate, as compared with HBV mono-infection. The activation of TGF-β and c-Jun pathways is mainly related to the post-transcriptional isoprenylation of a cysteine residue at the C-terminal of L-HDAg;[68] indeed, S-HDAg lacks both the isoprenylated cysteine and the TGF-β-stimulation activity. Goto et al[70] provided another example of molecular synergy between HBV and HDV in promoting HCC onset, demonstrating that HBx can trigger the transcriptional ability of E26 transformation-specific like-1 (Elk-1) protein, and that L-HDAg can activate the transcriptional function of the serum response factor (SRF). Both Elk-1 and SRF can enhance the serum response element (SRE)-dependent pathway, which in turn mediates the *c-fos* proto-oncogene transcription. L-HDAg can also enhance the activation of NF-κB and STAT3 signaling cascades, which are already overstimulated in CHB. Indeed, L-HDAg can elicit the expression of *Nox4* gene, leading to ROS overproduction, which is a trigger for the above-mentioned pathways.[71] NF-κB can also be upregulated by L-HDAg via the induction of TNFα; however, this mechanism is not related to the isoprenylated cysteine residue.[72] STAT3 activation induced by HDV also accounts for the overexpression of DNA methyltransferase-3b, which is involved in potentially oncogenic methylation events occurring during DNA replication.[73] HDAg-mediated histone acetylation is another epigenetic alteration possibly involved in tumorigenesis. L-HDAg and S-HDAg can induce histone H3 hyperacetylation within the clusterin promoter,[74] resulting in increased expression of clusterin, a molecule involved in cell death control.[75]

A further oncogenic mechanism, promoted by S-HDAg, involves the glutathione S-transferase P1 (GSTP1) gene. S-HDAg can bind GSTP1 mRNA, thus decreasing the GSTP1 protein expression, leading to ROS accumulation and subsequent rise in cellular apoptosis and DNA damage in proliferative cells.[76]

More recently, Yu et al[77] performed a sophisticated study based on microarray dataset analysis, comparing cancerous and para-cancerous specimens from individuals with CHB or CDH-related HCC. Seven genes closely involved in mitotic cell cycle and DNA replication (CDC6, CDC45, CDCA5, CDCA8, CENPH, MCM4, MCM7) were found to be differently expressed (mostly upregulated) only in the CDH-driven HCC subgroup; therefore, the alteration of pathways in which these genes are involved seems selectively mediated by HDV. Overall, these findings show that the molecular

profile of CDH-related HCC is characterized by an overexpression of genes involved in cell cycle and DNA replication/repair, highlighting genomic instability as a key mechanism of hepatocarcinogenesis.

Epidemiology of HCC in CBH and CDH

In the past, chronic HDV infection was thought to be at minor HCC risk, due to its rapidly progressive liver disease pattern, while nowadays evidence shows that CDH leads to early cirrhosis and a higher risk of HCC development. Even if cirrhosis decompensation, rather than incidence of HCC, is the main clinical endpoint that occurs during HDV infection, some clinical studies suggest that HCC and cirrhosis could be secondary effects of the same necro-inflammatory process.[78]

However, evidence-based clinical data point to a significant increase in the global risk of HCC development in patients with chronic HDV infection compared to those with chronic HBV mono-infection. Among CDH patients, HCC cumulative incidence was, respectively, 2.3% at 1 year, 5.4% at 3 years, and 7.5% at 5-year in non-cirrhotic patients, rising to 5.4% at 1 year, 15.9% at 3 years and 23.1% at 5 years in cirrhotic patients.[79] Major risk factors are liver cirrhosis (hazard ratio [HR] 9.98), higher HDV-RNA serum level (HR 5.73), age >50 years (HR 3.64), male gender (HR 2.69), higher body mass index (BMI) (HR 1.11), and lower platelet count.[30,79,80]

In a systematic review and meta-analysis from Alfaiate et al, the correlation between CDH and HCC was proven to be clinically relevant in cohort studies (pooled odds ratio [OR] 1.67; 95% confidence interval [CI] 1.28–2.1, p-value [p] <0.001), and even stronger in prospective studies (pooled OR 2.77; 95% CI 1.79–4.28, $p < 0.001$).[35] According to a recent study, the risk of HCC is increased in patients with CDH when compared to those with CHB (standardized incidence ratio [SRI] = 137.17, 95% CI: 62.19 to 261.51) or with HBV mono-infected patients (SRI = 6.11, 95% CI: 2.77 to 11.65).[78] Another study also found an increased HCC risk in acute (relative risk [RR] 6.1) or chronic (RR 3.9) HDV infection when compared toHBV mono-infection.[81] Other studies identified a 3-fold increase in the risk of CDH-related HCC, and a 2-fold increase in overall mortality compared to HBV mono-infected patients.[82] Moreover, a further increase in the risk of HCC development has been reported in human immunodeficiency virus (HIV)/HBV/HDV coinfection.[83]

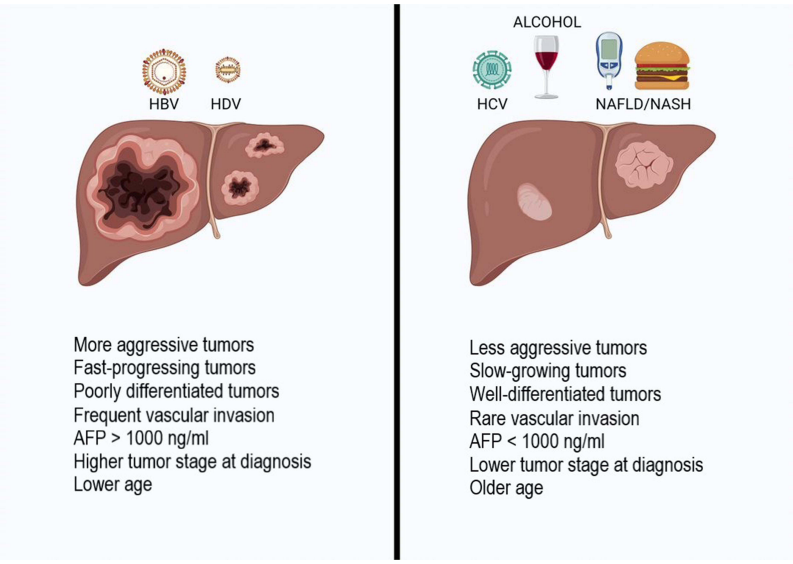

Figure 3 HCC clinical features at diagnosis in HBV/HDV patients and other aetiologies of liver disease. HBV/HDV-related HCC shows a more aggressive behavior compared with HCC related to other risk factors (such as alcohol, NAFLD, HCV, autoimmune diseases, biliary diseases), with a faster growth rate, larger nodule size, higher AFP serum levels, and a more advanced tumor stage. Image created with Biorender.com.
Abbreviations: AFP, alpha-fetoprotein; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HDV, hepatitis delta virus.

Clinical Presentation of HCC in CBH and CDH

Clinical features of HBV-HDV-related HCC are summarized in Figure 3. Usually, CHB-related HCC frequently presents with large tumor size, vascular invasion, aggressive growth pattern and high AFP serum levels at the time of diagnosis when compared to other aetiologies of liver disease.[81,84,85]

Concerning the clinical differences between HBV-related and HDV-related HCC, one study from Abbas et al showed a decreased liver size in patients affected by CDH compared to those affected by CHB ($p < 0.05$), associated with lower platelet count ($p = 0.05$) and larger varices ($P < 0.05$), underlining that HCC occurs in CDH with a higher burden of portal hypertension.[84] Moreover, multifocal tumors ($p < 0.05$), a higher TNM stage ($p < 0.05$) and an alpha-fetoprotein level >1000 IU/mL ($p = 0.06$) were more common in the CHB group than in coinfected patients.[84] These data are explained by a lead time bias, probably due to earlier diagnosis made while liver decompensation is investigated.[78,84]

Prevention of HCC in CBH and CDH

Worldwide hepatitis B vaccination and antiviral maternal prophylaxis in selected patients aim to achieve the goal of primary prevention of HBV infection and HBV-related HCC.[86] Some studies confirmed the lower infection and HCC development rate in populations undergoing vaccination and maternal prophylaxis programs.[87,88]

Secondary prevention of HBV-related HCC can be achieved by treating CHB patients, monitoring for drug resistance, and establishing tailored surveillance programs (based on age, familial history of HCC, HBV viral load, and PAGE B score).[86,89] Some consistent evidence has shown that the risk of HCC occurrence can be reduced, even if not eliminated, by current antiviral treatment in CHB patients. However, HCC still occurs in a percentage of patients obtaining a long-term virological suppression, and, the presence of persistent intrahepatic cccDNA and a high viral relapse rate after discontinuation of nucleoside analogues (NAs) represent still a challenge for these patients.[86] The use of new drugs such as bulevirtide, alone or in combination with pegylated interferon alpha, has been shown to reduce or eliminate HDV viremia in addition to normalising transaminases.[90] Although little is known about the long-term effects, especially on complications such as HCC, it can be expected that bulevirtide will lead to a reduction in the incidence of HCC, as already occurred with the use of antivirals in patients with HBV mono-infection. Schedule and duration of treatment are currently under investigation, and especially in the setting of HBV/HDV infection a "learning- by-doing" approach is currently adopted.

Moreover, tertiary prevention can lower HBV-related HCC recurrence after curative treatment.[91] The protective effect of NAs in reducing HCC recurrence in CHB patients was also confirmed by a meta-analysis, showing a better recurrence free survival (HR: 0.66; $P < 0.0001$).[92]

Conclusion

In conclusion, the most recent data suggest that HDV infection is a major risk factor for HCC occurrence worldwide, in overt contradiction with the old hypothesis proposing that the rapid progression towards decompensation, leading to early liver transplantation or death, minimizes the risk of HCC development. This increment seems to be more clinically significant in high-quality studies with a robust design, in particular prospective studies adjusted for confounders with well-established inclusion criteria.

While HBV molecular pathways are well-known, the comprehension of HDV molecular pathways and its effect on cell microenvironment and epigenetic modifications have strengthened the evidence of its oncogenic role in acutely and chronically infected patients only in the recent years. Indeed, the increasing evidence on the oncogenic role of chronic HDV infection should be a reason to implement screening policies worldwide. In reason of the elevated biological risk of HCC occurrence in CDH patients, these patients should be considered as a very high-risk subgroup, suggesting that a personalized screening schedules should be adopted to detect HCC at its earlier stages.

HBV infection and HBV-related HCC primary prevention through vaccination programs and maternal prophylaxis, as well as CHB treatment before or after the first HCC occurrence (secondary and tertiary HCC prevention) have shown an incredible efficacy in lowering HCC occurrence rates. Concerning HDV, bulevirtide effect on HCC development is still

under investigation. We therefore look forward to seeing the forthcoming data on how bulevirtide may change the natural history of HCC associated with CDH-related HCC.

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Disclosure

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