

Hepatitis D and hepatocellular carcinoma

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Abstract

Hepatitis D virus (HDV) is a defective circular shape single stranded HDV RNA virus with two types of viral proteins, small and large hepatitis D antigens, surrounded by hepatitis B surface antigen. Superinfection with HDV in chronic hepatitis B is associated with a more threatening form of liver disease leading to rapid

progression to cirrhosis. In spite of some controversy in the epidemiological studies, HDV infection does increase the risk of hepatocellular carcinoma (HCC) compared to hepatitis B virus (HBV) mono-infection. Hepatic decompensation, rather than development of HCC, is the first usual clinical endpoint during the course of HDV infection. Oxidative stress as a result of severe necroinflammation may progress to HCC. The large hepatitis D antigen is a regulator of various cellular functions and an activator of signal transducer and activator of transcription (STAT)3 and the nuclear factor kappa B pathway. Another proposed epigenetic mechanism by which HCC may form is the aberrant silencing of tumor suppressor genes by DNA Methyltransferases. HDV antigens have also been associated with increased histone H3 acetylation of the clusterin promoter. This enhances the expression of clusterin in infected cells, increasing cell survival potential. Any contribution of HBV DNA integration with chromosomes of infected hepatocytes is not clear at this stage. The targeted inhibition of STAT3 and cyclophilin, and augmentation of peroxisome proliferator-activated receptor γ have a potential therapeutic role in HCC.

Key words: Hepatitis D; Hepatocellular carcinoma; Necroinflammation; Epigenetic processes; Cirrhosis; Oxidative stress

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Core tip: Role of hepatitis D virus (HDV) in the oncogenesis of hepatocellular carcinoma (HCC) has not been thoroughly investigated. Many epidemiological studies favour the increased risk of HCC with HDV superinfection. Oxidative stress as a result of severe necroinflammation may trigger the development of HCC. Epigenetic mechanisms like DNA methylation and histone modification may also be operating.

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INTRODUCTION

Hepatitis D virus (HDV) is a small virus, often compared to viroids because of its unique characteristics. It is a defective virus with a circular shape single stranded HDV RNA and two types of viral proteins, small (sHDAG or p24) and large hepatitis D antigens (lHDAG or p27), surrounded by hepatitis B virus (HBV) surface antigen (HBsAg)^[1]. The virus does not code any enzyme to replicate its genome and takes the help from hepatocyte RNA polymerase II for synthesizing its RNAs with positive and negative polarities. Both the smaller sHDAG, which is required for HDV genomic replication, and the larger lHDAG, which represses replication, colocalize with delta RNA throughout the nucleoplasm^[2].

HDV is highly pathogenic. Whereas coinfection evolves to chronicity in only 2% of the cases, superinfection results in chronic infection in over 90% of the cases^[3]. Superinfection with HDV in chronic hepatitis B is associated with a more threatening form of liver disease exacerbating the pre-existing liver damage leading to more rapid progression to cirrhosis in 70% to 80% of the cases^[4]. It may lead to cirrhosis within 2 years in 10%-15% of patients^[5]. HBV DNA levels are low in both hepatitis B e antigen (HBeAg)-negative and HBeAg-positive patients, suggesting suppressive effects of HDV on HBV irrespective of the phase of HBV infection. The clinical long-term outcome of HBeAg-positive patients is not different to HBeAg-negative patients infected with the HDV^[6].

HEPATOCELLULAR CARCINOMA IN HDV INFECTION

Hepatocellular carcinoma (HCC) is the second most common cause of cancer-related death in men worldwide^[7]. Persistent HDV replication and hepatic inflammation end up with cirrhosis and HCC formation^[8]. Active replication of both HBV and HDV may be associated with a more progressive disease pattern leading to early cirrhosis and HCC^[5]. Wu *et al.*^[9] described three phases of HDV superinfection: acute phase, active HDV replication and suppression of HBV with high alanine transaminase (ALT) levels; chronic phase, decreasing HDV and reactivating HBV with moderate ALT levels; and late phase, development of cirrhosis and hepatocellular carcinoma caused by replication of either virus or remission resulting from the marked reduction of both viruses. Therefore, HBV replication, in spite of being inhibited by HDV, appears to play a major role sustaining HDV pathogenicity.

Hepatic decompensation, rather than development of liver cancer, is the first clinical endpoint that develops during the course of HDV infection^[10]. A clinical study has suggested that HCC in HDV infection may be a

secondary effect of severe necroinflammation leading to cirrhosis. In this study, decreased liver size was noticed more in cases of HDV HCC compared to an HBV mono-infection group where the liver size was normal or increased. HDV patients had lower platelets and larger varices on endoscopy as an indirect evidence of more severe portal hypertension. HCC presented at an earlier TNM stage compared with HBV mono-infection^[11].

EPIDEMIOLOGICAL STUDIES

Some controversy exists in the epidemiological studies on the role of HDV infection in increasing the risk of HCC. Early studies did not find an increased incidence of HCC in HDV co-infected individuals. But recent studies show an increased incidence of the tumor. The risk of HCC should be reconsidered according to the changing natural history of chronic HDV disease. Though the incidence of HDV infection has decreased in many Western countries, it is still very much prevalent in many parts of the world specially the Asia Pacific Region^[12].

The European Concerted Action on Viral Hepatitis (Eurohep) study done on hepatitis B patients and published in 1995 failed to show any significance of HDV (anti-HDV) markers at presentation on prognosis. However, a later study done by the same group on 200 HDV patients with a median follow up of 6.6 years showed that the adjusted estimated five year risk for HCC was 13% for anti-HDV positive and 2%-4% in anti-HDV negative/HBsAg positive cirrhotics. HDV infection increases the risk for HCC threefold and for mortality two fold in patients with hepatitis B cirrhosis^[13,14]. Analysis of retrospective data from South London showed that the risk of hepatocellular carcinoma was similar in anti-HDV positive and negative patients^[15].

Two studies from Turkey show prevalence of anti-delta antibodies in 18.8% to 23.0% of HBsAg positive HCC^[16,17]. In an older Jordanian study the prevalence of anti-HDV in a small group of HBsAg positive HCC patients was 67% (10/15). However, they were significantly older than patients without hepatitis D viral infection^[18]. In another similar study from Greece done on 87 HBsAg positive HCC patients, 9 were positive for serum anti-delta (10%) whereas among the HBsAg positive controls none was positive for this antibody ($P = 0.067$)^[19]. In a Romanian study, 166 consecutive patients with compensated HDV-related cirrhosis diagnosed since 1994 were followed up. HDV-related cirrhosis in Romania is an aggressive disease with a median time to decompensation less than 2 years and a median survival less than 5 years. Jaundice, the main clinical consequences of portal hypertension and HCC were the most frequent causes of decompensation. HCC developed in 12% cases^[20].

A study from Mongolia considered the sero-epidemiological and social-historical background of the country, and compared HCV related and HDV related HCC prevalence^[21]. In Mongolia co-infection with

HBV and HDV had a stronger association with HCC development at a younger age while patients with HCV mono-infection were older. Their results demonstrated that the viruses had different epidemic dynamics in Mongolia; HCV was characterized by earlier epidemic expansion, whereas HDV spread with approximately 50 years lag. Keeping this in mind, there was a comparable contribution of the HCV-monoinfection and HBV + HDV co-infection in the current HCC rate.

In a study from the Kure district in Japan, where HDV infection of persons infected with HBV in 1990s was about 6%, such superinfection increases the risk of cirrhosis and HCC. The proportion of HCC per 1000 person years was 7.84 among cases with anti-HDV and 2.73 among those without anti-HDV. The overall relative risk of HCC was 2.87, 95%CI: 1.03-6.23^[22]. A study from Taiwan failed to show any acceleration in the development of HCC in patients with HDV superinfection. Nevertheless, the numbers of patients in HDV group were small compared to HBV monoinfection group (42 vs 255)^[23].

In a Spanish study, One hundred and fifty-eight patients with chronic HDV were followed for a median period of 158 mo. 18% had hepatic decompensation, 3% developed hepatocellular carcinoma^[24]. Romeo *et al.*^[25] tracked the course of HDV infection in 299 patients over a mean period of 233 mo; 46 developed HCC. Persistent HDV replication led to cirrhosis and HCC at annual rates of 4% and 2.8%, respectively, and was the only predictor of liver-related mortality.

A recent study calculated the standardized incidence ratios (SIRs) for hepatitis D patients. The risk of hepatocellular carcinoma was greatly increased in patients with HBV and HDV (SIR = 137.17, 95%CI: 62.19 to 261.51) when compared with the general population. The risk of HCC among patients with HDV was increased (SIR = 6.11, 95%CI: 2.77 to 11.65) when patients with chronic HBV monoinfection were used as the reference population^[26]. High levels of HDV viremia in non-cirrhotic patients were associated with a considerable likelihood of progression to cirrhosis and the development of HCC; multivariate analysis: OR = 1.42, 95%CI: 1.04-1.95; $P=0.03$. Once cirrhosis has developed, the role of HDV replication as a predictor of a negative outcome lessens^[27]. Table 1 summarizes the epidemiological studies on the role of HDV infection in increasing the risk of HCC.

HDV AND HBV GENOTYPES

Hepatitis D is an immune-mediated disease. Though it is more aggressive than HBV monoinfection, the rate of disease progression may vary, as with other immune mediated diseases. Active replication of both HBV and HDV may be associated with a more progressive disease pattern. HDV and HBV genotypes may play a role in various disease outcomes. Genotype II HDV infection is relatively less frequently associated with fulminant hepatitis at the acute stage and cirrhosis or

HCC at the chronic stage as compared to genotype I^[41,42]. The outcome of patients with genotype IV (IIb) HDV infection is more like of genotype II HDV infection. HBV of the genotype C is also a significant factor associated with adverse outcomes (cirrhosis, HCC or mortality) in patients with chronic hepatitis D in addition to genotype I HDV and age^[42,43].

ONCOGENESIS

The mechanism by which HDV causes HCC remains to be elucidated, but recent advances seem to suggest a number of pathways that result in pathogenesis. HCC development itself is a complex process involving cumulative gain and loss of function mutations affecting tumor suppressor and oncogenic products^[44].

HDV seems to exert epigenetic control over HBV transcription and replication. A possible explanation may be that p24 and p27 both repress HBV enhancers, pIIE1 and pIIE2 inhibit replication, thus accounting for the low serum levels of HBV DNA in co-infected patients^[45]. P27 also inhibits interferon- α signaling by interfering with janus kinase, tyrosine kinase 2, signal transducer and activator of transcription (STAT)1 and STAT2, impairing the transcription of 2', 5' oligoadenylate synthase and protein kinase R but upregulating myxovirus resistance A gene transcription, which causes HBV replication inhibition^[46,47]. In fact HDV has been shown to repress HCV replication as well and chronic HCV infection has been reported to be cleared in the presence of HBV and HDV superinfection^[1]. This implies that HCC is caused by HDV alone in a conviction, but it may not be so, as the active proliferation of both HBV and HDV leads to more aggressive disease and HCC^[5].

It is believed that the pathogenic effects of HDV arise from replication-associated cytopathogenicity rather than a direct effect, since there is little injury observed in liver tissues expressing HDV alone^[48]. An investigation by Taylor confirmed that the expression of the antigen alone had no cytopathic effect, however high levels of the antigen and viral RNA caused cell cycle arrest in the G1 phase within two days and cell death in six^[49]. This experiment models the acute phase of infection wherein a high replicative rate is responsible for tissue injury. However, in chronic infection, wherein adequate levels of the large antigen are built up to suppress HDV RNA synthesis, the problem shifts to the development of HCC.

Oxidative stress

Oxidative stress as a result of severe necroinflammation in HDV infection may progress to HCC. Large hepatitis D antigens or p27 was shown by Williams *et al.*^[50] to be a regulator of various cellular functions and an activator of STAT3 and the nuclear factor kappa B (NF- κ B) pathway (Figure 1). Studies on HCV and HBV have linked the activation of NF- κ B and STAT3, *via* the overproduction of reactive oxygen species (ROS), to the pathology of the virus^[51-58]. These proteins have been implicated in

Table 1 The epidemiological studies on the role of hepatitis D virus infection in increasing the risk of hepatocellular carcinoma

¹ Romeo <i>et al</i> ^[27]	193 patients with HDV co-infection were investigated for a median of 9.5 yr. HDV RNA levels appeared significantly associated with HCC
¹ Romeo <i>et al</i> ^[25]	299 HDV infected patients investigated over 28 yr. Persistent HDV leads to cirrhosis and HCC at annual rates of 4% and 2.8%
¹ Oyunsuren <i>et al</i> ^[28]	292 chronic hepatitis patients were investigated retrospectively. HDV co-infection has a stronger association with HCC development at a younger age than HCV mono-infection
¹ Fattovich <i>et al</i> ^[14] (EUROHEP study group)	A retrospective cohort study of 200 Western European patients was carried out with a follow-up median period of 6.6 yr. HDV infection increases the risk of HCC three-fold
¹ Cenac <i>et al</i> ^[29]	89 Sahelian African patients were tested alongside 47 controls. 55% of HDV patients had HCC compared to the 17% who had HBV mono-infection with HCC
¹ Oliveri <i>et al</i> ^[30]	Patients with HDV co-infection developed HCC at a significantly younger age than those affected by HBV alone, by about 10 yr
¹ Tamura <i>et al</i> ^[22]	1127 patients were followed for at least 3 yr. The prevalence was 4.05 per thousand person years in HDV co-infection patients compared to 2.73 in patients with HBV alone
¹ Verme <i>et al</i> ^[31]	62 patients were investigated. The findings suggest that HDV co-infection causes HCC at an earlier age
¹ Smedile <i>et al</i> ^[32]	85 patients were investigated. The outcome in patients with HDV co-infection was significantly worse than others
¹ Trichopoulos <i>et al</i> ^[19]	116 patients were investigated. There is a higher prevalence of HCC amongst HDV co-infected patients
¹ Toukan <i>et al</i> ^[18]	The highest prevalence of HCC was found in those patients co-infected with HDV
¹ Ji <i>et al</i> ^[26]	650 out of 9160 HBV patients had HDV. The median follow up was 11 yr. The risk of HCC was increased. HDV was a strong risk factor
² Huang <i>et al</i> ^[33]	114 HCC patients were investigated prior to surgery. A higher prevalence of hepatic inflammation was observed in HCV patients and also, possibly, in HDV patients
² Abbas <i>et al</i> ^[11]	92 HDV positive and 92 negative patients with HCC were compared. HDV causes HCC in a different manner to HBV
³ Heidrich <i>et al</i> ^[6]	71 out of 534 patients had HBV and HDV co-infection. The median follow-up period was 4.25 yr. The long-term outcome for HBeAg positive and negative was the same
³ Huo <i>et al</i> ^[23]	42 HDV co-infected patients were compared to 255 HBV patients, all with HCC, over a period of 8 yr. HDV co-infection does not accelerate HCC development, and the outcomes are the same as HBV mono-infection
³ Fattovich <i>et al</i> ^[13] (EUROHEP study group)	349 Western European patients were investigated for 5 yr. HDV co-infection had no prognostic value for the development of HCC
³ Realdi <i>et al</i> ^[34] (EUROHEP)	366 caucasian patients were investigated for 6 yr. HDV infection did not influence the prognosis
³ Kage <i>et al</i> ^[35]	58 patients were investigated. HDV is unlikely to have a role in the development of HCC
³ Tzonou <i>et al</i> ^[36]	185 cases with HCC and 432 hospital controls were investigated. HDV was not a significant cause of HCC
³ Tassopoulos <i>et al</i> ^[37]	47 patients with HCC were investigated. None of the 47 had any evidence of HDV infection
³ Chen <i>et al</i> ^[38]	60 patients were investigated. However, the study indicated that HDV co-infection does not lead to a rise in HCC development amongst Chinese living in Taiwan
³ Govindarajan <i>et al</i> ^[39]	Sera from 39 patients with HBV associated with HCC were studied for the presence of HDV. Only one patient tested positive
³ Negro <i>et al</i> ^[40]	Liver tissues of 19 patients with chronic HDV were investigated and compared to tissues from 16 patients with chronic HBV, and 3 normal patients. Hepatocyte proliferation in HDV was similar to HBV, but higher than normal

¹Studies favoring role of HDV in HCC; ²Inconclusive; ³Studies against role of HDV in HCC. HDV: Hepatitis D virus; HCC: Hepatocellular carcinoma; HBV: Hepatitis B virus; HBeAg: Hepatitis B e antigen; HCV: Hepatitis C virus.

cell transformation and tumorigenesis, indeed STAT3 over expression is associated with leukemia, prostate cancer and melanoma^[59-62]. The ROS are produced by endoplasmic reticulum (ER) stress, the NADPH oxidase (Nox) family (HCV induces Nox1 and Nox4 in hepatocytes)^[63], the direct action of the HBV and HCV proteins and the ER overload response. Williams *et al*^[50] found that in the presence of antioxidants (PDTC, NAC) or calcium inhibitors (TMB-8, BAPTA-AM, Ruthenium Red), p27-induced activation of STAT3 and NF- κ B was dramatically reduced. They described that p27 caused an increase in ROS production, partly due to the isoprenylation process. P27 has a prenylation site on C211, which binds to farnesyl residues, and a nuclear export signal, which allows transport of the neosynthesized ribonucleoprotein to the ER^[64,65]. HDV proteins also cause some ER stress, as p27 activates ER stress elements present in the promoter of target genes, GRP78 and GRP94, and the antigen also triggers Nox4 activity *via* transforming growth factor (TGF) β 1. TGF β 1 and c-Jun signaling cascades may also induce

epithelial-mesenchymal transition and fibrogenesis^[66,67] and cause cirrhosis. Isoprenylation inhibitors, still in early development, may play a key role in preventing these undesirable outcomes^[11].

In a dose dependent manner, p27 also significantly increases (3.2 fold) NF- κ B activity^[50]. NF- κ B complex activation requires the phosphorylation of the serine 32 and 36 (and possibly Tyr42) residues by an Inhibitor of kappa B kinases, I κ B kinase (IKK) α and IKK β , of I- κ B (which is then proteosomally degraded), hence allowing the nuclear translocation and DNA binding of the active dimmer (p50/65)^[50]. Park *et al*^[68] demonstrated that p27 might also increase NF- κ B activation *via* tumor necrosis factor α (TNF- α) induction. TNF- α is involved in a wide range of inflammation and immunity related actions^[69-71]. The study also found that the large antigen increased TNF receptor associated factor (TRAF2), IKK β and p65 mediated NF- κ B activation. The investigators found TRAF2 (a protein involved in early signal transduction events) to interact with both SHDAg and LHDAg. An interesting parallel can be drawn to HCV,

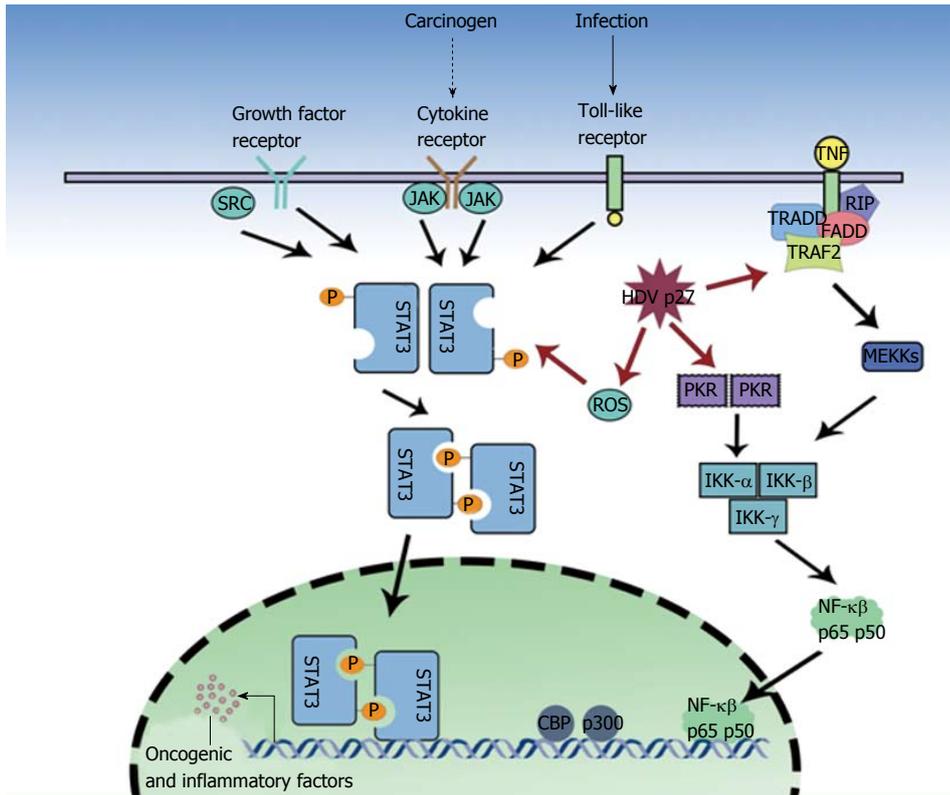


Figure 1 The influence of large hepatitis D antigen in activating oncogenic pathways. JAK: Janus kinase; SRC: Proto-oncogene tyrosine-protein kinase Src; TRADD: Tumor necrosis factor receptor type 1-associated DEATH domain protein; FADD: Fas-associated protein with death domain; TRAF2: TNF receptor associated factor 2; TNF: Tumor necrosis factor; RIP: Receptor-interacting protein; STAT3: Signal transducer and activator of transcription 3; NF-κB: Nuclear factor kappa beta; ROS: Reactive oxygen species; MEKK: Mitogen-activated protein kinase kinase kinase (MEK kinase); PKR: Protein kinase R; IKK: IκB kinase; CBP: CREB-binding protein.

which *via* NS5A and NS5B proteins also modulates TNF-α induced NF-κB activation^[72,73]. Furthermore, the HBX protein directly interacts with I-κB, preventing its association with NF-κB^[74]. However Williams *et al.*^[50] showed that HDV proteins could not directly interact with NF-κB and STAT3 but could act to transcribe various unknown genes by binding to endoplasmic reticulum stress response element (ERSE) motifs in target genes.

The discussion above demonstrates some of the possible mechanisms by which the HDV induces HCC. Furthermore, clinical observations seem to reinforce the view that HCC in HDV infection may be a secondary to the necroinflammation and cirrhosis of the liver^[11]. The investigators noted a decrease in liver size with HDV as opposed to HBV mono-infection and saw that HDV patients had lower platelets and larger varices.

DNA methylation

It has been suggested that another mechanism by which HCC forms is the aberrant silencing of tumor suppressor genes by DNA methyltransferases (DNMT1) and DNMT 3b^[75]. DNMT1 is responsible for the maintenance of methylation patterns whereas DNMT 3a and 3b catalyze new methylation events^[76]. Hence DNMT 3b is potentially oncogenic. Indeed, a study by Mota *et al.*^[77] noted that at least 32 proteins had differential expression in the presence of HDV components, pointing towards

possible epigenetic links. The study did not identify the mechanism of pathogenesis, but noted that HMGB1 (over expression of which is associated with metastasis in various cancer types) was over expressed in Huh7-D12 cells while NASP, TPI and PABP2 (which interact with DNMT 3a and 3b) were found to be down regulated, hence promoting cell proliferation. Proteins involved in cellular metabolism, transport, signal transduction and growth (PCNA and FEN1 Endonuclease) were also found to be affected^[77]. Indeed Negro *et al.*^[40] found that in the cirrhotic tissue of patients with HCC, HDV RNA occasionally co-localized with PCNA (a marker of hepatocyte proliferation).

It has been established that DNMT1 and DNMT 3b knockdown causes a global methylation reduction of over 95%, causing the loss of insulin-like growth factor 2 imprinting and the loss of silencing of the vital tumor suppressor p16INK4a^[76]. Hence their roles in human cancers are clear. Benegiamo *et al.*^[75] went on to show the large antigen activates STAT3 *via* phosphorylation of Tyrosine 705 residue. STAT3 in turn regulates DNMT1 and causes the over expression of DNMT3b. Among the 24 genes investigated by the study, the promoter of *E2F1*, a vital regulator of the cell cycle (bound by the Retinoblastoma protein) was found to be hypermethylated. It has been proposed that *E2F1* may also be responsible for Nox4 activation. *E2F1* is

often targeted by other small DNA and RNA viruses as well. The virus was thus found to cause cell cycle disruption and a 2-fold increase in G2/M phase arrest was observed^[75]. It has been suggested by Kannan that following arrest, the cell acquires further mutations that allow it to proceed with the cycle, giving rise to cancerous cells^[78].

Histone modification

HDAgs have also been associated with increased histone H3 acetylation of the clusterin promoter^[79]. This enhances the expression of clusterin in infected cells, increasing cell survival potential. Histone acetyltransferases, CREB-binding protein and p300^[80] are key to this process, as they interact with the antigens while the linker histone H1e binds to the small antigen^[81]. Kang *et al.*^[82] reported that clusterin is over expressed in HCC, with the expression increasing with metastatic HCC^[83]. Indeed, it has already been noted that increased levels of the protein is an important factor in determining the aggressiveness of a breast tumor^[84]. It is believed that at least in human renal cell carcinoma clusterin contributes to a phenotype resistant to Fas-mediated apoptosis^[85]. However, some conflicting results have been noted in the literature regarding the roles of clusterin, which has been involved in cell cycle arrest^[86], cell death^[87] and inhibition of proliferation^[84]. An explanation suggested is that although clusterin may initially cause senescence in problematic cells, over time the molecule may be responsible for survival and with the accumulation of further mutations, may allow tumorigenesis^[88].

Metabolic and autoimmune changes

Another factor to consider is the down-regulation of the Rho GDP dissociation inhibitor and guanine binding proteins^[74]. These proteins are involved in the regulation of the mitogen activated protein kinase (MAPK) pathway, which is frequently implicated in cancer^[89]. A lower availability of Triosephosphate Isomerase and Pyruvate Carboxylase, which lead to an abnormal retention of lipids may also be responsible for microvesicular steatosis during HDV infection^[77].

Furthermore, Wedemeyer *et al.*^[45] suggest that hepatitis D is an immune mediated disease, noting a rise in CD4⁺ T cells in individuals with a HDV infection. Although the role of the host's immune system seems unlikely, various autoantibodies have been detected in infected patients. Prominent amongst them is liver-kidney microsomal antibody type 3, directed against uridine diphosphate glucuronyl transferase^[90]. The disruption of metabolism in this way could contribute to HCC. Indeed Hanahan *et al.*^[91] have already labeled some changes in cellular metabolism as hallmarks of cancer.

HBV DNA integration

It is interesting to note that the HBX product has been found to directly interact with p53 and has been associated with the MAPK pathway and hence causes

HCC^[92]. It was previously thought that HBV DNA integration with chromosomes of infected hepatocytes would be responsible for HCC. However, the process of integration has been noted to be entirely random rather than targeted to specific genes and the length and components of the integrant has found to vary considerably^[93]. Interestingly, when Woodchuck hepatitis virus targets the intronless *N-myc2* gene as a site of integration, it predisposes to HCC^[94]. Together with the activity of the protein product, the increased expression of mechanistic of rapamycin (mTOR) and PI3K/Akt were found to be responsible for cancer development^[95]. Indeed mTOR promotes cell proliferation, apoptosis resistance and vascularization of tumors^[96] by regulating the transcriptional activity of FOXO1-3a and protein translation by pS6 and eIF-4E^[95]. To the authors' knowledge, no study has yet investigated the association of the HDV antigens with mTOR or the downregulation of MiR-101^[97] (which is done by HBX protein and interacts with DNMT3A) and this could be a potential area of research.

Peroxisome proliferator-activated receptor and HCC

Peroxisome proliferator-activated receptor (PPAR) has been shown to play a role in the development of HCC^[98]. PPAR α (which normally has a role in lipid metabolism), found in the liver, kidney, heart, and small intestine, has been shown to be involved in the regulation of the cell cycle. In mice, knocking down PPAR α led to HCC suppression^[99]. However, conflicting reports of the role of PPAR α exist. Meanwhile PPAR γ , found in adipose tissue and macrophages, inhibits HCC^[100-102]. These control epithelial-mesenchymal transition and prevent metastasis by increasing E-cadherin through TIMP3^[103]. PPAR γ is also involved in cell cycle arrest^[103] and induces Fas dependent apoptosis, hence combating HCC. PPAR δ (a gene derived from the TCF/ β -catenin pathway) is found universally and has been reported to be involved in highly malignant colon cancer^[104]. It is thus necessary to explore in the future whether PPAR are somehow exploited by HDV in the development of HCC. If so, thiazolidinediones, which act on PPAR γ , could be used to treat HCC. Together with retinoic acid, PPAR agonists and antagonists could become the frontline therapeutic drugs in HCC treatment.

TOWARDS THERAPEUTICS AND A BETTER UNDERSTANDING OF HDV

A better understanding of the molecular events underlying HCC development following HDV infection is vital to not only the approach to the virus but also for the development of new drugs, which can target specific parts of the pathways involved if not the virus itself and prevent development of HCC in patients infected with HDV. For example the targeted inhibition of STAT3 with a decoy 15-mer double-stranded oligonucleotide, which corresponds to the

STAT3 response element in the c-fos promoter region, has been experimentally proven to abrogate head and neck cancer growth^[105] and could eventually be used to prevent or treat HCC as well.

Cyclophilins are a class of proteins localized in various cellular compartments, involved in metabolism and homeostasis and are upregulated during inflammation and cancer. Cyclophilin A (CypA), in the cytoplasm, is involved in the virus life cycle, while extracellular CypA and CypB are pro-inflammatory in nature. Cyclosporins are potential cyclophilin inhibitors and could have therapeutic potential for the treatment of virus induced liver diseases. Indeed cyclosporin A (CsA) has been shown to inhibit HBV and HDV entry *via* sodium taurocholate co-transporting polypeptide. There is a direct interaction between the drug and the NTCP receptor (which is also a bile salt transporter), with overlap at the preS1 domain (which mediates viral entry). CsA also has immunosuppressive effects, exercised *via* cyclophilin dependent inhibition of calcineurin^[106].

Interestingly, HDV can, *in vivo*, infect the cells of hepadnavirus-induced hepatocellular carcinoma in Woodchucks^[107]. Since it had been previously hypothesized that hepadnavirus-induced HCCs are resistant to reinfection, the experiment proves that the cells still have functioning *woodchuck* hepatitis virus receptors and if a resistance does exist, it occurs downstream of the receptor^[108]. This information may facilitate development of novel strategies further dissecting the mechanism of liver carcinogenesis associated with HDV infection

The spread of HDV can be prevented by depriving the defective HDV of HBV necessary to propagate its infection. Countries with effective vaccination programs have shown a dramatic decrease in the incidence of HCC^[109]. As there is no effective treatment for HDV and the only treatment available is interferon, which is of limited efficacy^[110], vaccination against HBV should be stressed. Carriers of HBs should be informed of the risk of superinfection from carriers coinfecting with HDV and educated about preventive practices.

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