Review

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Entry of hepatitis B and hepatitis D virus into hepatocytes: Basic insights and clinical implications

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Summary

For almost three decades following the discovery of the human Hepatitis B Virus (HBV) the early events of virus infection (attachment to hepatocytes, specific binding to a receptor on hepatocytes) remained enigmatic. The gradual improvement of tissue culture systems for HBV has enabled the identification of viral determinants for viral infectivity and facilitated the discovery of the human sodium taurocholate co-transporting polypeptide (hNTCP) as a liver specific receptor of HBV and its satellite, the human Hepatitis Delta Virus (HDV). These findings are currently leading basic and clinical research activities in new directions. (1) Stable hNTCP-expressing cell lines have become a valuable platform to study the full HBV replication cycle from its native template, the cccDNA. (2) The suitability of NTCP complemented cell culture systems for high throughput screening approaches will facilitate identification of novel host factors involved in HBV replication (including those that determine the peculiar host specificity of HBV infection) and will enable identification and development of novel drug candidates for improved therapeutics. (3) Since NTCP is a major host-specific restriction factor for HBV and HDV, hNTCP-expressing animals provide the basis for future susceptible *in vivo* models. (4) The concept obtained with the entry inhibitor Myrcludex B demonstrates that NTCP is a suitable target for clinical interference with viral entry. This will foster further clinical approaches aiming at curative combination therapies.

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NTCP acts as a functional receptor of HBV and HDV

The nature of the cellular receptor mediating HBV infection remained elusive long time after the discovery the virus [1–3]. Although some candidate molecules were found to bind to the envelope proteins of HBV, none of them ever demonstrated a virus receptor activity upon expression in non-susceptible cells, which is accepted as a strong criteria regarding whether a candidate is truly a functional virus receptor. Studies of the infection biology of HBV and its satellite virus hepatitis D virus (HDV), an RNA virus using HBV envelope proteins for its packaging and cellular entry, was hampered by the lack of a convenient cell culture system. Primary cultures of human hepatocytes (PHH) [4,5], primary tupaia hepatocytes (PTH) [6–8], and a hepatoma cell line, HepaRG, after differentiation with DMSO [9], were the only cells susceptible to HBV and HDV. PHH and PTH are scarce and their susceptibility to the virus varies depending on the source as well as isolation and culture procedures. HepaRG cells require a 2-week differentiation period before cells become susceptible. Nonetheless, elegant work using these cell culture systems over the past decades has revealed some critical aspects of HBV entry [10]: (1) Infection of HBV exhibits extraordinary species and organ specificity, indicating existence of a highly specific interaction between the virus and the hepatocytes; (2) two of HBV three envelope proteins, namely the small (S) and large (L) envelope protein, but not the middle (M) protein are responsible for HBV entry [11,12]; (3) the antigenic loop (AGL) of S protein [13] and the preS1 domain of L-protein are essential for HBV infection [14]; (4) the N-terminal region of preS1 domain requiring an uninterrupted sequence of 75 amino acids (genotype D) [15] with a myristoylation modification

at the N-terminus [16,17] critically contributes to infectivity, among which amino acid residues 9–16 are of vital importance [18–20]. The corresponding lipopeptide of this domain blocks viral infection *in vitro* and *in vivo* [19–22].

The preS1 lipopeptide as a natural ligand is useful to identify the specific receptor on hepatocytes. However, it was not an easy task to find its binding partner on hepatocytes and several non-functional molecules have been described [10]. Successful discovery of the *bona fide* receptor sodium taurocholate co-transporting polypeptide (NTCP) was achieved using an innovative preS1 peptide ligand combined with proteome analysis of PTH and highly efficient tracing and purification processes [23]. Biochemical studies confirmed the interaction between preS1 and the liver specific bile acids transporter. Remarkably, transfection of HepG2 cells with NTCP expression DNA vector confers susceptibility to this otherwise non-susceptible cell [23]. This finding was quickly confirmed by several important studies, including a study demonstrating that human NTCP can confer susceptibility to HDV pseudotyped with the envelope proteins of bat hepadnavirus [24], woolly monkey HBV (WMHBV) also exploits NTCP for viral entry in tupaia hepatocytes [25] and HBV/HDV exploit NTCP for species-specific entry into hepatocytes [26]. Discovery of the receptor has also engendered new perspectives on HBV and its associated diseases [27].

NTCP is coded by the *SLC10A1* gene. It is a bile acid transporter, responsible for hepatic uptake of the majority of conjugated bile acids from blood, and plays a critical role in bile acids homeostasis and enterohepatic circulation [28]. The expression of NTCP and its subcellular distribution is subjected to precise regulation at multiple levels. NTCP contains up to nine transmembrane segments embedded in the cell membrane; its structure remains to be elucidated. Interestingly, although the NTCP gene is conserved in mammals, disrupting NTCP in mice in short term (ca. 2 months) did not result in significant abnormalities in the animals but led to elevated serum bile acids, in particular conjugated ones [29]. A single point mutation of R252H of human NTCP interferes with its surface expression on hepatocytes. Recently, a five-year old girl bearing this mutation was identified and she exhibited mild hypotonia, growth retardation, delayed motor milestones but no severe health problem [30]. Therefore, although the long-term consequence of disrupting NTCP needs more studies, the physiological role of NTCP can probably be compensated by other, yet not defined mechanism(s), and redundant pathways may operate in the absence of NTCP. Whether there is another receptor(s) for HBV entry is not known at present, however several lines of evidence support that NTCP is well suited as a key receptor for HBV (Fig. 1). It is predominantly expressed in hepatocytes [31], as also shown by the hepatotropism of its preS-ligand *in vivo* [32]. This is consistent with the high liver specificity of HBV. Furthermore NTCP resides on the sinusoidal side of hepatocytes [33–35], which is in line with the blood transmission of the virus. There is only very low or no expression of NTCP in hepatocarcinoma cell lines like HepG2 or HuH7 and the expression of NTCP rapidly decreases after isolation of primary hepatocytes from animals [23,36–38]. These two features at least partially explain why normal liver cancer cells are resistant to HBV infection and why the susceptibility of primary hepatocytes persists for only a few days after isolation.

Key point

The human sodium taurocholate co-transporting polypeptide (hNTCP) is a bona fide receptor for HBV and HDV.

Interestingly, a human population study on 1899 HBV patients from southern China (Guangdong) showed that a single mutation of NTCP, S267F ((c.800G>A, rs2296651) on *SLC10A1*), is associated with resistance to chronic hepatitis B [39]. The S267F is a mutation found mainly in Asian populations, with a minor frequency of ca. 10 % [40–42]. In cell cultures the mutation abolished taurocholate (TC) transport and over expression of S267F NTCP in HepG2 cells failed to support genotype D HBV and HDV infection [41]. The marked protection of this mutation against HBV infection at population level strongly argues that NTCP is a major viral receptor in human. A more recent genetic association study based on 3801 chronic hepatitis B (CHB) patients from Taiwan confirmed that the same mutation confers resistance to HBV infection and is independently associated with decreased risk of cirrhosis and HCC [42]. It is currently unclear how the mutation would reduce the risk of cirrhosis and HCC.

Key point

hNTCP expressing hepatoma cell lines are important tools to study HBV and HDV replication.

Interestingly, there are five HBV patients with homozygous S267F NTCP mutation in the Guangdong cohort [39] the Taiwan study also found five of such HBV patients [42]. How these patients were infected is an interesting and important question. There are several possibilities: first, cell surface molecules other than NTCP could mediate the viral infection; second, the virus was adapted to S267F-NTCP; third, the patients were genetically mosaic meaning some of their hepatocytes might still have wildtype NTCP. Clearly more work is needed to clarify these possibilities and related studies would help to deepen our understandings of HBV infection.

NTCP determines viral infection specificity at entry level

HBV only infects human, chimpanzees and northern treeshrew (*Tupaia belangeri*). It cannot infect non-human primates, mouse, rat and other