A bile acid transporter as a candidate receptor for hepatitis B and D virus entry

Fei Xiao[1], Jane A. McKeating[2], Thomas Baumert[1, 3,*]

[1]Inserm, U748/1110, Institut de Virologie, Université de Strasbourg, Strasbourg, France, [2]School of Immunity and Infection, NIHR Liver Biomedical Research Unit, University of Birmingham, [3]Pôle Hépato-digestif, Nouvel Hôpital Civil, Strasbourg, France

*Corresponding author:
Thomas F. Baumert, M. D.; Inserm Unité 1110, Université de Strasbourg, 3 Rue Koeberle, F-67000 Strasbourg, France; Phone: (++33) 3 6885 37 03, Fax: (++33) 3 68 85 37 05, e-mail: Thomas.Baumert@unistra.fr

Abbreviations

ASBT, apical sodium-dependent bile acid transporter; DHBV, duck hepatitis B virus; HBV, hepatitis B virus; HDV, hepatitis delta virus; PHH, primary human hepatocytes; PTH, primary *Tupaia* hepatocytes; uPA/SCID, urokinase-plasminogen activator / severe combined immunodeficiency; BMS, mass spectrometry; NTCP, sodium taurocholate cotransporting polypeptide; SLC10, soluble carrier family; TM, transmembrane; SOAT, sodium-dependent organic anion transporter; HS, heparan sulfate; cccDNA, covalently closed circular DNA

Keywords

Antiviral, infection, liver disease, pathogen

Comment on:

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Comment

With 350 million chronically infected individuals worldwide, hepatitis B virus (HBV) is an unsolved global health challenge. Current treatment strategies, based on interferon-alpha or nucleos(t)ide analogues have been shown to control viral infection and reduce liver disease. However, available treatments are far from satisfactory as they largely fail to eradicate HBV or hepatitis delta virus (HDV) [1]. Although the HBV genome replicates in a variety of cell lines, the virus can only infect primary human and *Tupaia* hepatocytes (PHH and PTH) [2, 3] and the bipotent differentiated HepaRG liver progenitor cell line [4]. Despite tremendous progress in the molecular characterization of HBV replication and assembly, the host determinants mediating the first steps of infection remain poorly defined, limiting the development of robust in vitro models supporting the complete HBV life cycle. Although other hepadnaviruses (e. g. duck hepatitis B virus [DHBV]) share some functional and structural properties with HBV and are therefore used as models for HBV-host interactions, functional data suggest that entry pathways of these viruses differ [5]. Indeed, the functional relevance of cellular receptors identified for DHBV (such as carboxypeptidase D) could not be confirmed for HBV (for review see [5]).

The pre-S1 domain of the HBV encoded large surface envelope protein plays a role in particle entry. Indeed, a peptide derived from the pre-S1 protein inhibits HBV infection of human hepatocytes [4, 6, 7] and chimeric uPA/SCID mice [8]. Since HDV utilizes the envelope proteins of HBV it is assumed to enter hepatocytes via a similar mechanism [5]. There is accumulating evidence that HBV attaches to cells via heparan sulfate proteoglycans [9-11]. Several cell surface proteins have been reported to interact with HBV envelope proteins but none of them have been confirmed to be an essential entry factor [5].

A recent study by Wenhui Li's laboratory at the National Institute of

Biological Sciences in Beijing, China, identified a novel HBV and HDV receptor candidate [12]. Based on the previous mapping studies by Schulze et al. [13], Wenhui Li's team established a photo cross-linking assay using a series of synthetic pre-S1 peptides as "bait" to identify interacting proteins expressed in *Tupaia* hepatocytes to screen for putative HBV entry factors. The cross-linked peptide-protein complexes were purified and analyzed by mass spectrometry (MS). Comparing the MS results of the captured proteins with a *Tupaia* protein database obtained by deep-sequencing the *Tupaia* transcriptome, enabled Yan and colleagues to identify sodium taurocholate cotransporting polypeptide (NTCP, also known as SLC10A1) as a hepatocyte surface molecule binding pre-S1. NTCP is a member of the soluble carrier family 10 (SLC10), the major bile acid uptake system in human hepatocytes, that localize to the basolateral hepatocyte membrane. NTCP is a 349-amino acid integral membrane glycoprotein comprising 7 or 9 transmembrane (TM) domains according to topology studies on a related SLC10 family member, apical sodium-dependent bile acid transporter (ASBT) [14-16]. The ability of NTCP to bind HBV pre-S1 was confirmed using NTCP transfected 293T cells. Silencing NTCP expression in PTHs, HepaRG or PHHs partially reduced HBV or HDV infection. NTCP expression in non-permissive HepG2 or Huh7 hepatoma cells rendered these cells susceptible to low level HBV or HDV infection, respectively. Finally, the authors combined phylogenetic analysis with mutagenesis studies to identify a putative role for NTCP amino acids 157-165 in viral infection.

As shown for many other viruses "cellular receptor proteins" can act in several ways to mediate viral entry, including viral attachment, post-binding transport and viral fusion [17]. Hepatic NTCP expression is regulated by a number of familiar pathways, notably the glucocorticoid receptor, the retinoic acid receptor and hepatic nuclear transcription factors HNF1α, HNF4α, and HNF3β [18]. NTCP is a member of a family of 7 related solute carrier family transporters and has been shown to interact with a variety of partner proteins.

In addition to forming a homodimer, NTCP can dimerise with other members of the SLC10A family, notably SLC10A4 and SLC10A6 (sodium-dependent organic anion transporter, SOAT). NTCP interaction with these partner proteins regulate protein trafficking in vitro and hints at possible mechanisms for viral transport [19]. Resolving these outstanding issues will clarify the role of NTCP in HBV internalization. Furthermore, it will of interest to explore NTCP or its regulatory factors as antiviral targets.

The observations that silencing NTCP did not ablate HBV infection of PHHs and that HepG2-NTCP cells support low level HBV infection suggest that NTCP may not be the sole host factor defining liver permissivity to HBV, highlighting the need for additional studies in this area.

In summary, the work of Yan et al. provides an important advance in our understanding of HBV entry and suggests new avenues for the genesis of cell culture and animal model systems that support HBV and HDV infection, enabling the development of new antivirals and immunotherapies.

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Conflict of interest

The authors do not report any conflict of interest.

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Figure legend

Fig. 1. Putative model of sodium taurocholate co-transporting polypeptide (NTCP) as a co-factor for hepatitis B and D virus entry. HBV or HDV first attaches to heparan sulfate (HS) [9-11]. The virus may then interact with NTCP through the pre-S1 domain of the large envelope protein as shown by Yan et al. [12]. NTCP is a glycoprotein localizing to the basal membrane of hepatocytes. The key function of NTCP is the Na^+-dependent uptake of bile acids allowing to maintain the enterohepatic circulation of bile acids [14]. Residues 157 to 165 (aa 157-165) of NTCP have been suggested to be critical for pre-S1 binding [12]. Putative other unknown HBV/HDV receptors during viral entry are indicated. The subsequent steps of HBV/HDV entry are largely unknown. Clathrin [20] and caveolin [21]-mediated pathways have been suggested but remain to be confirmed. After the import of the HBV genome into host cell nucleus, viral relaxed circular DNA is converted into covalently closed circular DNA (cccDNA), from which genomic and subgenomic RNAs are transcribed.