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

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COMMENTARY ON:


Abstract: Human hepatitis B virus (HBV) infection and HBV-related diseases remain a major public health problem. Individuals coinfected with its satellite hepatitis D virus (HDV) have more severe disease. Cellular entry of both viruses is mediated by HBV envelope proteins. The pre-S1 domain of the large envelope protein is a key determinant for receptor(s) binding. However, the identity of the receptor(s) is unknown. Here, by using near zero distance photo-cross-linking and tandem affinity purification, we revealed that the receptor-binding region of pre-S1 specifically interacts with sodium taurocholate cotransporting polypeptide (NTCP), a multiple transmembrane transporter predominantly expressed in the liver. Silencing NTCP inhibited HBV and HDV infection, while exogenous NTCP expression rendered nonsusceptible hepatocarcinoma cells susceptible to these viral infections. Moreover, replacing amino acids 157–165 of nonfunctional monkey NTCP with the human counterpart conferred its ability in supporting both viral infections. Our results demonstrate that NTCP is a functional receptor for HBV and HDV. DOI:http://dx.doi.org/10.7554/eLife.00049.001.

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Abbreviations: ASBT, apical sodium-dependent bile acid transporter; DHBV, duck hepatitis B virus; HBV, hepatitis B virus; HDV, hepatitis delta virus; PTH, primary human hepatocytes; uPA/SCID, urokinase-type plasminogen activator/severe combined immunodeficiency; MS, mass spectrometry; NTCP, sodium taurocholate cotransporting polypeptide; SLC10, solute carrier family 10; TM, transmembrane; SOAT, sodium-dependent organic anion transporter; HS, heparan sulfate; cccDNA, covalently closed circular DNA; HNF, hepatocyte nuclear factor.

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 solute carrier family 10 (SLC10), the major bile acid uptake system in human hepatocytes, that localizes to the basolateral hepatocyte membrane (Fig. 1). 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 discoidin domains of tyrosine kinase 2 (DDR2) [18], which is a member of the discoidin domain receptor family. DDR2 regulates NTCP expression by post-translational modifications and through the PI3K/AKT signal pathways. DDR2 expression is increased in liver disease, indicating a possible role in HBV infection.

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 declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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