Hepatitis B and D Viruses Exploit Sodium Taurocholate Co-transporting Polypeptide for Species-Specific Entry nto Hepatocytes

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BACKGROUND & AIMS: Hepatitis B and D viruses (HBV and HDV) are human pathogens with restricted host ranges and high selectivity for hepatocytes; the HBV L-envelope protein interacts specifically with a receptor on these cells. We aimed to identify this receptor and analyze whether it is the recently described sodium-taurocholate co-transporter polypeptide (NTCP), encoded by the *SLC10A1* gene. **METHODS:** To identify receptor candidates, we compared gene expression patterns between differentiated HepaRG cells, which express the receptor, and naïve cells, which do not. Receptor candidates were evaluated by small hairpin RNA silencing in HepaRG cells; the ability of receptor expression to confer binding and infection were tested in transduced hepatoma cell lines. We used interspecies domain swapping to identify motifs for receptor-mediated host discrimination of HBV and HDV binding and infection. **RESULTS:** Bioinformatic analyses of comparative expression arrays confirmed that NTCP, which was previously identified through a biochemical approach is a bona fide receptor for HBV and HDV. NTCPs from rat, mouse, and human bound Myrcludex B, a peptide ligand derived from the HBV L-protein. Myrcludex B blocked NTCP transport of bile salts; small hairpin RNA-mediated knockdown of NTCP in HepaRG cells prevented their infection by HBV or HDV. Expression of human but not mouse NTCP in HepG2 and HuH7 cells conferred a limited cell-type–related and virus-dependent susceptibility to infection; these limitations were overcome when cells were cultured with dimethyl sulfoxide. We identified 2 short-sequence motifs in human NTCP that were required for species-specific binding and infection by HBV and HDV. **CONCLUSIONS:** Human NTCP is a specific receptor for HBV and HDV. NTCP-expressing cell lines can be efficiently infected with these viruses, and might be used in basic research and high-throughput screening studies. Mapping of motifs in NTCPs have increased our understanding of the species specificities of HBV and HDV, and could lead to small animal models for studies of viral infection and replication.

Keywords: Species Specificity; Virology; Virus Entry; Myrcludex B.

Cell entry of enveloped viruses is mediated through specific interactions of viral membrane proteins with cellular receptors. Subsequently, viral fusion proteins merge the membranes, a process induced on endocytosis or co-receptor binding. Evolutionary adaptation of viral envelope proteins to receptors are major determinants of host specificity and tissue tropism.[1] For many human pathogenic viruses, receptors are known, however, the identity of the hepatitis B virus (HBV)/hepatitis D virus (HDV) receptor remained elusive[2] until recently.[3] HBV is a small, enveloped DNA-virus replicating through reverse transcription of an RNA intermediate.[4] HDV is an RNA-virusoid depending on HBV because it requires envelopment by HBV surface proteins.[5] Both viruses exhibit remarkable hepatotropism and idiosyncratic host specificities, infecting humans and human primates and *tupaia belangeri*. Mice resist infection, although HBV/HDV replicates after artificial genome transfer.[2] One reason for these preferences is receptor recognition.[6]

Only primary human hepatocytes (PHH) and tupaia hepatocytes are susceptible to HBV/HDV infection; primary mouse hepatocytes and rat hepatocytes are resistant. Attempts to infect nonhepatic cells failed, reflecting absence of receptor(s) or other host-dependency factors. Remarkably, HepG2 and HuH7 hepatoma cell lines are resistant as well. One reason is their loss of receptor expression.[7] The only established susceptible cell line is HepaRG; however, dimethyl sulfoxide (DMSO)-induced differentiation is required.[8] Using peptidic ligands from the large HBV envelope protein, we demonstrated an induced expression of a specific receptor on differentiation.[7]

HBV/HDV are enveloped by the L-(large), M-(middle), and S-(small) HBV surface proteins.[4] Important infectivity

Abbreviations used in this paper: cccDNA, covalently closed circular DNA; DMSO, dimethyl sulfoxide; h, human; HBcAg, hepatitis B core antigen; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HDV, hepatitis D virus; m, mouse; MyrB, Myrcludex B; NTCP, sodium taurocholate co-transporter polypeptide; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PHH, primary human hepatocyte; shRNA, small hairpin RNA.

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