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Mini Review

NTCP Transporter as Novel Target for Anti-Hepatitis B Virus Agents

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Abstract

Hepatitis B virus (HBV) constitutes a serious public health problem worldwide, and its chronic infection elevates the risk of developing liver cirrhosis and hepatocellular carcinoma. The current anti-HBV agents are limited to interferon-based regimens and nucleos(t)ide analogs, therefore, the development of new anti-HBV drugs is greatly needed. Recently, sodium taurocholate cotransporting polypeptide (NTCP) was identified as an entry receptor for HBV. This finding enable to establish cell lines that reproduce HBV infection and thus are good tool for analyzing the HBV life cycle. In addition, NTCP potentially serves as a target for the development of anti-HBV agents. In this article, we summarize the recent findings on the role of NTCP in HBV infection.

ABBREVIATIONS

HBV: Hepatitis B Virus; **NTCP:** Sodium Taurocholate Cotransporting Polypeptide; **IFN:** Interferon; **SLC:** Solute Carrier Protein; **PHH:** Primary Human Hepatocytes; **PTH:** Primary Tupaia Hepatocyte; **L:** Large Surface Protein

INTRODUCTION

Hepatitis B virus (HBV) constitutes a serious public health problem, chronically infecting approximately 350 million individuals worldwide. HBV infection causes a wide spectrum of liver diseases ranging from both acute and chronic viral hepatitis, and chronic HBV infection elevates the risk of developing liver cirrhosis and hepatocellular carcinoma; 15-40% of HBV-infected patients are reported to develop liver cirrhosis, liver failure or hepatocellular carcinoma [1]. In the last decade, anti-HBV therapeutics have been developed, and interferon (IFN)-based drugs including IFNα and pegylated-IFNα and nucleos(t)ide analogues including lamivudine, adefovir, entecavir, tenofovir and telbivudine are currently approved for anti-HBV treatment [2]. Although current treatments can significantly improve liver pathogenesis, only a minority of patients treated with IFNα show a clinical response with seroconversion, resulting in a loss of serum HBe antigen and development of anti-HBe antibody [3]. Furthermore, the emergence of drug-resistant viruses against nucleos(t)ide analogs remains unresolved; therefore, the development of new types of anti-HBV agents is required.

Viral entry is an attractive therapeutic target for anti-viral drug development. However, no HBV entry inhibitors have yet been approved for clinical use. Although HBV infection is triggered with a low affinity viral attachment to cell surface mainly mediated by heparan sulfate proteoglycans, the following entry steps involving specific receptor(s) has not been largely understood. Establishment of a cell culture system that efficiently supports HBV infection is necessary for the development of new entry inhibitors and for clarifying the mechanisms underlying the viral entry process. In late 2012, Dr. Wenhui Li and colleagues successfully identified the sodium taurocholate cotransporting polypeptide (NTCP) as a functional receptor for HBV and its satellite virus, hepatitis delta virus (HDV) [4]. NTCP, also designated as solute carrier protein 10A1 (SLC10A1), is a sodium-dependent transporter for taurocholic acid and belongs to the SLC10 family, which consists of seven members (SLC10A1-A7) [5]. In the liver, hepatocytes take up bile salts from the portal blood and secrete them into bile for enterohepatic circulation. Uptake of conjugated bile salts into hepatocytes largely occurs in a sodium-dependent manner by NTCP on the basolateral membrane of hepatocytes [5]. However, NTCP is weakly expressed in the majority of cell lines derived from hepatocytes such as HepG2 and Huh-7, and these cells rarely support HBV infection [4,6]. In contrast, primary human hepatocytes (PHH), primary tupaia hepatocyte (PTH) and differentiated HepaRG cells, which are susceptible to HBV infection, express significant levels of NTCP [4].

NTCP was identified as a binding factor for the preS1 domain of the HBV L envelope protein, which is essentially involved in HBV infection [7], by affinity purification using a synthetic peptide for this region as a probe [4]. PreS1 was confirmed to bind human NTCP (hNTCP) and also tupaia NTCP (tsNTCP), but not crab-eating monkey NTCP (mkNTCP), which correlated with the species tropism of HBV infection. Knockdown of NTCP in PHH, PTH or HepaRG cells significantly reduced the infection

of HBV and HDV. Ectopic expression of hNTCP into HepG2 cells conferred susceptibility to viral infection [4,8,9]. Similarly, Huh-7 and undifferentiated HepaRG cells overexpressing hNTCP were significantly susceptible to infection [10]. These results indicate that hNTCP is required for HBV and HDV infection into human hepatocyte cell lines. In contrast, mouse NTCP (mNTCP), which shares approximately 73.8% homology with hNTCP, did not support HBV infection by overexpression [11]. By comparing and altering the sequences of hNTCP and mNTCP proteins, amino acid residues 84 to 87 of hNTCP were found to be important for HBV infection [11]. In addition, HBV infection was abolished when 157-165 residues of hNTCP were replaced with the corresponding sequence derived from mkNTCP, thus indicating a role for this region in infection [4]. Moreover, tupaia NTCP functions as a receptor for woolly monkey HBV [12]. It remains uncertain whether there is an additional entry receptor that has yet to be identified. Introduction of hNTCP to cell lines originating from mouse hepatocytes such as Hepa1-6 and MMHD3 cells or non-hepatocyte line HeLa cells did not render HBV susceptibility [11], suggesting that additional host entry factor(s) may determine HBV susceptibility. It should be noted that the role of membrane proteins, including endonexin II, asialoglycoprotein receptor, and carboxypeptidase D in the entry process of HBV and duck HBV has been reporter so far [13-15]. Requirement of these or other factors needs to be explored in the future in order to fully clarify the molecular mechanisms underlying HBV infection.

The process of viral entry involving NTCP is a promising therapeutic target. It has been reported that myrcludex-B, a lipopeptide derived from 2-48 aa of the preS1 region of the HBV L protein, strongly inhibited HBV infection both in vitro and in vivo, with approximately 8 nM as IC_{50} in a cell culture system [16]. It interrupted binding between the HBV L and NTCP on the cell surface. As another compound targeting NTCP, cyclosporin A (CsA), which is an immunosuppressant, was recently reported to block HBV infection into cells [6,9]. CsA inhibited HBV entry by directly binding to NTCP and interrupting the interaction between NTCP and the preS1 region. A derivative analysis confirmed that CsA analogs, including those abrogating its immunosuppressive activity, had stronger anti-HBV activity with submicromolar IC_{50} values [6]. In addition, other inhibitors and substrates for NTCP transporter including ursodeoxycholate, bosentan and bromosulfophthalein were also shown to block viral infections [6], indicating that the region responsible for NTCP transporter function is likely to be important for HBV entry [6]. Recently, we also found that a compound modulating NTCP expression levels prevented viral infection [17]. Other compounds were also reported to inhibit infection, including oxysterol and sorafenib, but the modes of action remain unelucidated [8, 18]. A number of FDA-approved drugs that could inhibit NTCP transporter activity have been reported [19], and there is great interest in identifying compounds that efficiently inhibit HBV infection among these drugs.

As summarized above, the identification of NTCP as an HBV entry receptor and the establishment of cell culture systems supporting HBV infection facilitate the analysis of molecular mechanisms underlying the viral entry process, as well as the development of anti-HBV agents inhibiting HBV entry. Identification and evaluation of agents that physically or functionally target NTCP and that inhibit HBV infection are expected to present novel therapeutic choices for preventing and eradicating HBV infection.

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