Inhibition of HBV Replication by VPS4B and Its Dominant Negative Mutant VPS4B-K180Q In Vivo*

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Summary: This study examined the anti-hepatitis B virus (HBV) effect of wild-type (WT) vacuolar protein sorting 4B (VPS4B) and its dominant negative (DN) mutant VPS4B-K180Q in vivo in order to further explore the relationship between HBV and the host cellular factor VPS4. VPS4B gene was amplified from Huh7 cells by RT-PCR and cloned into the eukaryotic expression vector pXF3H. Then, the VPS4B plasmid and the VPS4B-K180Q mutation plasmid were constructed by using the overlap extension PCR site-directed mutagenesis technique. VPS4B and HBV vectors were co-delivered into mice by the hydrodynamic tail-vein injection to establish HBV vector-based models. Quantities of HBsAg and HBeAg in the mouse sera were determined by ElectroChemiLuminescence (ECL). HBV DNA in sera was measured by real-time quantitative PCR. Southern blot analysis was used to assay the intracellular HBV nuclear capsid-related DNA, real-time quantitative PCR to detect the HBV-related mRNA and immunohistochemical staining to observe the HBcAg expression in the mouse liver tissues. Our results showed that VPS4B and its mutant VPS4B-K180Q could decrease the levels of serum HBsAg, HBeAg and HBV-DNA. In addition, the HBV DNA replication and the mRNA level of HBV in the liver tissues of treated mice could be suppressed by VPS4B and VPS4B-K180Q. It was also found that VPS4B and VPS4B-K180Q had an ability to inhibit core antigen expression in the infected mouse liver. Furthermore, the anti-HBV effect of mutant VPS4B-K180Q was more potent than that of wild-type VPS4B. Taken together, it was concluded that VPS4B and its DN mutant VPS4B-K180Q have anti-HBV effect in vivo, which helps develop molecular therapeutic strategies for HBV infection.

Key words: hepatitis B virus; vacuolar protein sorting; AAA ATPase

Hepatitis B virus (HBV) infection is a major threat to public health. The number of HBV carriers worldwide is estimated to be 350 million. Approximately 15%–40% of HBV carriers progresses to cirrhosis, liver failure, and even hepatocellular carcinoma[1]. Although HBV therapeutic vaccine has been available and proven effective in some researches[2, 3], treatment of HBV remains a challenge. Interferon and nucleotide analogs are currently the conventional drugs for HBV treatment, effectiveness of which is not desirable. HBV tends to undergo rapid mutagenesis because its reverse-transcriptase lacks the proof-reading function, which brings a large number of variants. Some of the variants develop resistance to antiviral treatments[4, 5]. Therefore, the development of some new anti-HBV agents has been clinically desired. Vacular protein sorting (VPS4) is a member of the AAA family and it has been reported to be involved in lysosomal/endosomal membrane trafficking[6]. HBV is an enveloped DNA virus that utilizes endosomal membranes as assembly sites and traverses through endosomes during release from hepatocytes. Therefore, VPS4 might be involved in HBV replication and secretion. In the present study, we examined the anti-HBV effect of wild-type VPS4B and its dominant negative (DN) mutant VPS4B-K180Q in HBV mouse model to further explore the relationship between HBV and the host cellular factor VPS4.

1 MATERIALS AND METHODS

1.1 Plasmids

Total RNAs were extracted from Huh7 cells to construct the eukaryotic expression vector pXF3H-VPS4B coding for human wild-type VPS4B. RT-PCR amplification of VPS4B sequence involved the forward

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*This project was supported by a grant from the National Mega Research Program of China (No. 2008ZX10002-011).
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mice were co-injected with 10 μg pVPS4B-K180Q; (4) NT group, in which the mice were randomly divided into 4 groups (6 mice in each): (1) infected group, in which the mice were co-injected with 10 μg HBV1.3 and 10 μg pXF3H; (2) WT-VPS4B group, in which the mice were co-injected with 10 μg pHBV1.3 and 10 μg pVPS4B; (3) DN-VPS4B group, in which the mice were co-injected with 10 μg pHBV1.3 and 10 μg pVPS4B-K180Q; (4) NT group, in which the mice were injected with 2 mL phosphate buffered saline (PBS).

1.3 Injection of Naked Plasmids DNA

Plasmid DNA was prepared by using an EndoFree plasmid system (Qiagen, Germany) according to the manufacturer’s instructions. Plasmid DNA was diluted with 2 mL PBS and injected via the tail vein within 5 s. The sera of the treated mice were collected at indicated time after hydrodynamic injection, and then the HBsAg, HBeAg and HBV DNA that were secreted were measured. Five days post-injection (p.i.), three mice of each group were sacrificed, and then liver tissues were collected and preserved for later Southern blotting, HBV mRNA assay and immunohistochemical staining.

1.4 Detection of HBsAg, HBeAg and HBV-DNA in the Sera

HBsAg and HBeAg in the sera of the treated mice were quantitatively assayed by using ElectroChemiluminescence (ECL) on an E170 modular immunoassay analyzer (Roche, Germany), according to the manufacturer’s instructions. HBV-DNA in the sera of the mice was measured by real-time quantitative PCR as follows: 100 μL mouse sera was mixed with 6 mmol/L magnesium acetate and incubated for 2 h at 37°C to pellet nuclei. The supernatant was mixed with 6 mmol/L magnesium acetate and incubated for 2 h at 37°C with 200 μg/mL of DNase I and 100 μg/mL of RNase A. After digestion, the lysate was centrifuged at 14000 r/min for 1 min to pellet nuclei. The supernatant was incubated at 55°C for 1 h after addition of 10 mmol/L EDTA, 1% SDS, 100 mmol/L NaCl and 200 μg/mL proteinase K. Finally, the sample was extracted twice with phenol/chloroform, precipitated with 0.7 volume of isopropanol, and resuspended in TE pH 8.0 (10 mmol/L Tris-HCl pH 7.5, 1 mmol/L EDTA) and digested with 100 ng/μL of RNase A for 30 min at 37°C. Purified DNA was subjected to Southern blot analysis. DNA samples were loaded onto 1.3% agarose gels, blotted onto nylon membranes, and probed for a Dig-labeled full-length HBV genome in the EasyHyb hybridization solution (Roche, Germany).

1.6 RT-PCR Analysis of the Transcriptional Level of HBV mRNA

For quantitative RT-PCR, approximately 20 mg of liver tissue was obtained from mice for total RNA extraction with the RNeasy total RNA kit (Qiagen, Germany), according to the manufacturer’s protocol. cDNA was synthesized from 2 μg of total RNA with oligo(dT)15 primer in a total volume of 20 μL. Quantitative RT-PCR was performed with the Light Cycle Real-time PCR system (Roche, Germany). The PCR primers used were as follows: GAPDH, 5'-TTGGCTAAGTAGGACTG-3' (forward), 5'-TTGGCTAAGTAGGACTG-3' (reverse); HBV, 5'-TCACAACTCCGCAAGTCTC-3' (nt231-248, forward), 5'-AGCAACAGGAGGATACA-3' (nt569-552, reverse). The pHBV1.0 vector containing the full-length HBV genome was used to establish a standard curve to calculate HBV copies per milliliter of sera[10].

1.7 Immunohistochemistry for Liver HBcAg

HBV core protein was visualized by immunohistochemical staining of the mouse liver tissue fixed in 4% neutral buffered formalin. The primary antibody used was polyclonal rabbit anti-HBc antibodies. Dako-Cytomation EnVision System labelledPolymer-HRP anti-rabbit antibody (DAKO, Denmark) was used according to the manufacturer’s instructions. Immunostaining was performed under the same conditions