Interferon Induces SAMHD1 to Inhibit HBV Replication in Huh7.0 Hepatoma Cells (Post-print)

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Abstract

Objective: To investigate the molecular mechanism by which interferon regulates the expression of the host restriction factor SAMHD1 to inhibit HBV replication. Methods: Huh7.0 cells were treated with different doses of interferon- , - , and - , and the expression levels of SAMHD1 at transcriptional and translational levels were detected by quantitative real-time PCR and Western blot; furthermore, endogenous SAMHD1 was knocked down by siRNA to re-evaluate the inhibitory effects of interferon- and - on viral replication; finally, the cellular localization of interferon- -induced endogenous SAMHD1 was examined by immunofluorescence, and the effect of SAMHD1 cellular localization on viral replication was detected by Southern blot. Results: In Huh7.0 cells, SAMHD1 RNA levels and protein expression were significantly upregulated by interferon- and - ; after SAMHD1 knockdown, the inhibitory effects of interferon- and - on HBV replication disappeared; SAMHD1 was localized in the nucleus, interferon- -induced SAMHD1 was also localized in the nucleus, and SAMHD1 lost its ability to inhibit viral replication after deletion of the nuclear localization signal. Conclusion: In Huh7 cells, interferon- and - can induce upregulation of SAMHD1 expression to inhibit HBV replication, and the antiviral effect of SAMHD1 is dependent on its cellular localization.

Full Text

Interferon Induces SAMHD1 to Inhibit HBV Replication in Hepatocellular Carcinoma Huh7.0 Cells

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Abstract

Objective: To investigate the mechanism by which interferons inhibit hepatitis B virus (HBV) replication through SAMHD1 induction in hepatocellular carcinoma Huh7.0 cells. **Methods:** Huh7.0 cells were treated with varying doses of interferon- , , and , and SAMHD1 expression at both transcriptional and translational levels was detected by quantitative real-time PCR and Western blot. Endogenous SAMHD1 was subsequently knocked down via siRNA to evaluate the antiviral effects of interferon- and . Finally, immunofluorescence was employed to examine the cellular localization of interferon- -induced endogenous SAMHD1, and Southern blot was used to assess how this localization affects viral replication. **Results:** In Huh7.0 cells, both RNA and protein levels of SAMHD1 were significantly upregulated by interferon- and treatment. Knockdown of SAMHD1 abolished the inhibitory effects of interferon- and on HBV replication. SAMHD1 localized exclusively to the nucleus, and interferon-induced SAMHD1 similarly localized within the nucleus. Deletion of the nuclear localization signal resulted in loss of antiviral activity. **Conclusion:** In Huh7 cells, interferon- and induce upregulated SAMHD1 expression to inhibit HBV replication, and the antiviral function of SAMHD1 is dependent on its cellular localization.

Keywords: Hepatitis B virus; interferons; SAMHD1; inhibition

Introduction

Hepatitis B virus (HBV) infection represents a major global health challenge, with approximately 240 million people chronically infected worldwide. Chronic HBV infection can lead to cirrhosis, liver failure, and hepatocellular carcinoma. Current therapeutic options for chronic hepatitis B primarily include nucleos(t)ide analogues and interferons (IFNs). While nucleos(t)ide analogues often fail to produce durable responses due to the emergence of drug-resistant HBV mutants, interferons serve as a common antiviral treatment that induces expression of multiple antiviral proteins. Therefore, identifying interferon-stimulated effector genes and elucidating their antiviral mechanisms holds significant clinical importance.

SAMHD1 (Sterile alpha motif and histidine/aspartic acid domain-containing protein 1) is a crucial intracellular innate immune factor that restricts replication of retroviruses such as HIV-1 and DNA viruses such as HSV-1, primarily by depleting intracellular dNTP pools required for viral replication. Recent reports indicate that HBV, as a pararetrovirus, is also inhibited by SAMHD1 through its dNTPase activity. While interferon- has been shown to induce SAMHD1 protein upregulation in U87-MG, HEK293T, HeLa cells, and primary monocytes, few studies have reported SAMHD1 regulation by interferons and its inhibition of HBV replication in hepatocellular carcinoma cells. This study therefore explores the antiviral effects of interferon-induced SAMHD1 and whether its antiviral function depends on cellular localization.

Materials and Methods

1.1 Materials Huh7.0 hepatocellular carcinoma cells were maintained in our laboratory. Fetal bovine serum was obtained from BI, while DMEM medium, sodium pyruvate, and penicillin-streptomycin were purchased from Hyclone. Reverse transcription kits were from Takara. siRNAs were synthesized by Shanghai GenePharma. SAMHD1 antibody was purchased from Cell Signaling Technology, while HA antibody, Alexa Fluor® 488, and Alexa Fluor® 647 were from Thermo Fisher Scientific. SDS-PAGE gel preparation kits, goat serum, GAPDH antibody, DAPI staining solution, and anti-fluorescence quenching mounting medium were from Beyotime. -Actin antibody was from SANTA CRUZ, mouse and rabbit secondary antibodies from Zhongshan Jinqiao, HBc antibody from Dako, DIG probe labeling and detection kits and PCR purification kits from Roche, and Nylon membranes from Invitrogen. Lipofectamine 3000 transfection reagent was purchased from Invitrogen, PVDF membranes and Western blotting substrates from Millipore, and film, developer, and fixer from Kodak.

1.2 Experimental Procedures 1.2.1 Plasmid Construction and Cell Culture

SAMHD1 expression plasmids were constructed by Sino Biological Inc., with the cDNA sequence (NM_015474.3) cloned into the pcDNA3.1 vector with a C-terminal HA tag. Huh7 and HEK293T cells were cultured in DMEM medium supplemented with 10% fetal bovine serum at 37°C with 5% CO . Plasmid transfection and siRNA interference were performed according to the Lipofectamine 3000 protocol.

1.2.2 Immunofluorescence Assay

Sterilized 10×10 mm coverslips were placed in 6-well plates and washed twice with PBS. Cells were seeded and allowed to adhere. The first group was treated with IFN- to induce endogenous SAMHD1 expression, while the second group was transfected with HA-SAMHD1 and HA-SAMHD1 nuclear localization-deficient (D11-14) plasmids. After 48 hours, cells were fixed with 4% paraformaldehyde for 25 minutes, permeabilized with 0.15% Triton X-100 in PBS for 10 minutes, and blocked with goat serum for 30 minutes at room temperature. The IFN- -induced group was incubated with SAMHD1 antibody (1:100) diluted in goat serum, while the overexpression groups were incubated with HA antibody (1:100) overnight at 4°C in a humidified chamber. The IFN- group was then incubated with Alexa Fluor 647-conjugated goat anti-rabbit IgG (1:500), and the overexpression groups with Alexa Fluor 488-conjugated goat anti-mouse IgG (1:500) for 2 hours at room temperature. After DAPI nuclear staining and mounting with anti-fluorescence quenching medium, samples were observed under a confocal microscope.

1.2.3 Quantitative Real-Time PCR

Cells were treated with interferon- , , or for 2 days, after which total

mRNA was extracted using TRIzol reagent and reverse-transcribed into cDNA. SAMHD1 mRNA levels were then quantified by real-time PCR.

1.2.4 Western Blot Analysis

After 3 days of treatment with interferon- , , or , siRNA-mediated SAMHD1 knockdown, or SAMHD1 overexpression, cells were lysed with RIPA buffer containing 10% protease inhibitor cocktail. Proteins were separated by 12% SDS-PAGE, transferred to PVDF membranes, and blocked with 5% non-fat milk for 2 hours. Membranes were incubated overnight at 4°C with SAMHD1 T592 phosphorylation antibody (#15038, CST), anti-HA mouse antibody (AH158, Beyotime), or -actin (TA-09, Zhongshan Jinqiao). After four washes with TBST (5 minutes each), membranes were incubated with goat anti-rabbit or anti-mouse secondary antibodies for 1 hour, followed by ECL detection and signal capture on Kodak film.

1.2.5 Extraction of Viral Core Particle DNA

HBV DNA from viral core particles was extracted using sucrose density gradient centrifugation. Three days post-transfection, cells were lysed with CPLB buffer (10 mM Tris-HCl, 1 mM EDTA, 1% NP-40, 2% sucrose) and centrifuged at 10,000 rpm for 5 minutes. The supernatant was digested with micrococcal nuclease at 37°C for 1 hour, then ultracentrifuged at 45,000 rpm for 2 hours through a 30% sucrose cushion to pellet viral particles. After overnight proteinase K digestion, HBV DNA was extracted with phenol/chloroform/isoamyl alcohol and ethanol precipitation.

1.2.6 Southern Blot Detection of HBV Replication

HBV DNA was separated by 1.2% agarose gel electrophoresis for 70 minutes, denatured with alkali, and transferred to a Nylon membrane. After UV cross-linking, DNA was hybridized using a digoxigenin-labeled DNA hybridization kit and detected with digoxigenin antibody.

1.3 Statistical Analysis All statistical data were processed using SPSS 19.0 software. Data were analyzed by one-way ANOVA, with statistical significance defined as $P < 0.05$ (two-tailed).

Results

2.1 Interferon Induction of SAMHD1 Expression We first examined the induction of SAMHD1 by interferon- , , and in Huh7.0 cells. Cells were treated with varying doses of each interferon for 48 hours, and SAMHD1 expression was assessed by quantitative real-time PCR and Western blot. The results demonstrated that interferon- and treatment significantly increased SAMHD1 protein expression in a dose-dependent manner. Compared to control cells, treatment with 2000 U/ml interferon- upregulated SAMHD1 mRNA and protein levels by 6-fold and 17.5-fold, respectively, while 20 U/ml interferon- upregulated them by 10-fold and 7.2-fold. In contrast, interferon- treatment

did not significantly upregulate SAMHD1 expression at either the mRNA or protein level [Figure 1: see original paper]. These findings indicate that SAMHD1 is predominantly induced by type I interferons in Huh7.0 cells.

2.2 SAMHD1 Knockdown Antagonizes Interferon-Mediated HBV Inhibition We next investigated whether SAMHD1 knockdown affects interferon- and inhibition of HBV replication. First, we confirmed that siRNA specifically reduced endogenous SAMHD1 expression by 67% at the RNA level [Figure 2: see original paper]a. In Huh7.0 cells transfected with HBV replication plasmids following SAMHD1 knockdown, treatment with interferon- (1000 U/ml) or interferon- (20 U/ml) was administered, and viral replication was assessed by Southern blot of core particle DNA after 72 hours [Figure 2: see original paper]b. Compared to control cells, SAMHD1 knockdown (65% efficiency) significantly increased HBV replication by 2.3-fold (compare lanes 1 and 2), demonstrating that endogenous SAMHD1 restricts HBV replication. Interferon- or interferon- treatment markedly reduced HBV replication (compare lanes 1 and 3, or 1 and 5). However, this inhibitory effect was substantially attenuated when endogenous SAMHD1 was silenced, indicating that interferon- and suppression of HBV replication depends on the host restriction factor SAMHD1.

2.3 SAMHD1 Inhibition of HBV Is Dependent on Cellular Localization To further clarify the cellular localization of interferon-induced SAMHD1 and whether its antiviral function depends on this localization, we performed immunofluorescence analysis. Both endogenous SAMHD1 induced by interferon and overexpressed SAMHD1 localized exclusively to the nucleus [Figure 3: see original paper]A, B, whereas a mutant lacking the nuclear localization signal (NLS) was found in the cytoplasm [Figure 3: see original paper]C, consistent with previous reports by Rice and Brandariz-Nuñez et al. We further examined how SAMHD1 localization affects its antiviral activity using an HBV replication system. Huh7 cells were co-transfected with HBV replication plasmids and either wild-type or mutant SAMHD1. Southern blot analysis revealed that the NLS-deficient SAMHD1 mutant D11-14 lost its antiviral activity compared to wild-type SAMHD1 [Figure 4: see original paper].

Discussion

Interferons are commonly used antiviral agents in chronic hepatitis B therapy that exert their effects through multiple interferon-stimulated genes targeting various stages of viral replication, though the precise mechanisms remain incompletely understood. SAMHD1 is a host restriction factor that inhibits retroviral replication and has recently been shown to suppress herpes simplex virus and HBV replication by depleting intracellular dNTP pools. Additionally, SAMHD1 expression can be induced by interferons. This study aimed to further characterize the antiviral effects and mechanisms of interferon-induced SAMHD1.

Our findings demonstrate that type I interferons and upregulate both SAMHD1 mRNA and protein levels in Huh7 hepatocellular carcinoma cells, suggesting that type I interferons inhibit HBV replication in hepatocytes, at least in part, through SAMHD1 induction. However, studies on SAMHD1-mediated restriction of HIV-1 have shown that interferon treatment of activated CD4+ T cells, monocyte-derived macrophages, and monocyte-derived dendritic cells induces SAMHD1 dephosphorylation to exert antiviral activity, indicating that the mechanism of interferon-induced SAMHD1 function varies across cell types. Importantly, we found that silencing SAMHD1 expression abolished interferon-mediated HBV inhibition, establishing SAMHD1 as an interferon-stimulated gene that mediates antiviral activity against HBV. Furthermore, our analysis of SAMHD1 nuclear localization revealed that NLS-deficient mutants lose antiviral function, demonstrating that SAMHD1' s restriction of HBV is dependent on its nuclear localization. These findings provide a foundation for further investigation into interferon-mediated HBV suppression mechanisms and for targeted drug development against HBV.

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Table 1
Sequence of primers for site-directed mutagenesis and siRNA target for SAMHD1

Sequence (5' →3')
SAMHD1-D11-14 FP: CGATTCCGAGCAGCCCTCCTGCGATGACA
SAMHD1-D11-14 RP: TGTCATCGCAGGAGGGCTGCTCGGAATCG
SAMHD1-D207N FP: GCTGGACTTTGTCATAATCTCGGTCATGGGCC
SAMHD1-D207N RP: GGCCCATGACCGAGATTATGACAAAGTCCAGC
siSAMHD1 FP: GCAGCCAACAGGACAAAUATT
siSAMHD1 RP: UAUUUGUCCUGUUGGCUGCTT