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Inhibition of STAT1 methylation is involved in the resistance of hepatitis B virus to Interferon alpha

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ABSTRACT

As a major therapy for hepatitis B virus (HBV) infection, Interferon alpha (IFN-alpha) triggers intracellular signal transduction including JAK-STAT pathway to produce various antiviral effector mechanisms. However, patients with chronic hepatitis B usually show low response to IFN-alpha treatment and the underlying mechanism remains unclear. In the present study, HepG2 and HepG2.2.15 cells were used to examine the Type I IFN receptors expression, phosphorylation and methylation of STAT1. STAT1–PIAS1 interaction in cells was tested by protein co-immunoprecipitation. The potential improvement of S-adenosylmethionine (SAM) in the antiviral effect of IFN-alpha was also investigated. Our data demonstrated that both chains of the Type I IFN receptors were expressed for a much higher extent in HepG2.2.15 cells than in HepG2 cells. HBV inhibited dramatically the methylation rather than the phosphorylation of STAT1, which was consistent with an increased STAT1–PIAS1 interaction. Combined with IFN-alpha, SAM treatment effectively improved STAT1 methylation and attenuated STAT1–PIAS1 binding, followed by increased PKR and 2′,5′-OAS mRNA expression, thus significantly reducing the HBsAg, HBeAg protein levels and HBV DNA load in the supernatant of HepG2.2.15 cells. Less STAT1 methylation and subsequent increased STAT1–PIAS1 interaction are involved in the mechanism of the IFN-alpha-antagonistic activity of HBV. By improving STAT1 methylation, SAM can enhance the antiviral effect of IFN-alpha.

1. Introduction

Hepatitis B virus (HBV) is the most common hepatitis virus that causes chronic infection of human liver (Lavanchy, 2005). More than 400 million people worldwide have been infected and many of them develop chronic liver disease, evolving eventually into cirrhosis and hepatocellular carcinoma (HCC). An estimated one million people die annually by HBV infection (McMahon, 2005). Currently, treatment with Interferon alpha (IFN-alpha) is one of the major therapies that have been approved for chronic hepatitis B (CHB) patients. But even treated with the pegylated IFN-alpha, only 30% of the CHB patients proved to be sensitive as indicated by clearance of HBeAg and HBV DNA together with a normal ALT level (Férir et al., 2008). The underlying reason for the low response to IFN-alpha treatment in CHB patients remains unclear, although it has been considered to involve multiple causes including both virus-related and host-specific factors.

Previous studies have demonstrated that nucleocapsid HBV proteins could be involved in a deficient IFN-alpha response (Foster et al., 1993; Rosmorduc et al., 1999). The main and most important signal pathway activated by IFNs is the JAK–STAT pathway (Der et al., 1998; Nikol'skiĭ and Vasilenko, 2000). By binding to Type I IFN receptors, IFN-alpha triggers the oligomerization and tyrosine phosphorylation of the receptors followed by the activation of receptor-associated Janus tyrosine kinase (JAK). The activated JAK subsequently phosphorylates STAT2 and STAT1, respectively. Phosphorylated STATs then form hetero-dimers and translocate into the nucleus where they bind to specific DNA elements in the promoters of target genes to initiate their transcription. The genes targeted by JAK–STAT signaling include several antiviral genes such as dsRNA-dependent protein kinase, 2′,5′-oligoadenylate synthetase, P56 and MxA (O'Shea and Visconti, 2000; Pestka, 2000).

The JAK–STAT pathway is subjected to down-regulation by a variety of negative regulators including protein tyrosine phosphatases (PTPs), suppressor of cytokine signaling (SOCS) proteins and protein inhibitor of activated STAT (PIAS) (Starr and Hilton, 1999). PIAS1 is the main member of PIAS family, which specifically associates with STAT1 and only inhibits the binding of the dimeric form of STAT1 to the promoters of STAT1 target genes, but not with

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other STAT proteins. Recent studies have demonstrated that STAT1 can be catalyzed by protein arginine methyltransferase 1 (PRMT1) and methylation of STAT1 is functionally essential for STAT1 since it prevents the binding of PIAS1. In contrast, the demethylation of STAT1 enhances its association with PIAS1 (Liao et al., 2000; Liu et al., 2004; Mowen et al., 2001; Shuai and Liu, 2003).

Viruses, including DNA and RNA viruses, have consequently evolved ways to interfere with action of IFN-alpha through encoding proteins that impair the methylation modification of STAT1. It has been reported that expression of hepatitis C virus (HCV) proteins in liver cells of transgenic mice could decrease STAT1 methylation and consequently increase association of STAT1 with PIAS1 (Blindenbacher et al., 2003). In addition, reduced methylation of STAT1 and enhanced PIAS1–STAT1 binding have also been observed in liver biopsies from HCV infected patients and cells that express HBV proteins (Christen et al., 2007; Duong et al., 2004).

In the present study, we have analyzed the IFN-alpha signaling through the JAK–STAT pathway by using two different liver cell lines, one of them is stably transfected with HBV genomes. Our results suggest that less STAT1 methylation contributes at least in part to the resistance of HBV-infected liver cells to IFN-alpha. S-adenosylmethionine (SAM), as the most commonly used methyl donor, could be used to enhance the antiviral effect of IFN-alpha by improving STAT1 methylation in HBV-infected liver cells.

2. Materials and methods

2.1. Reagents

Human IFN-alpha and S-adenosylmethionine were obtained from Sigma (Sigma–Aldrich Quimica, Madrid, Spain). Anti-STAT1 antibody was purchased from Cell Signaling Technology (Bio-Concept, Allschwil, Switzerland). Anti-phospho-STAT1 (Tyr 701) antibody, anti-PIAS1 antibody, together with the monoclonal antibodies to methyl- and dimethylarginine were purchased from Abcam (Abcam Limited, Cambridge, United Kingdom). Anti-IFNAR1 antibody and anti-β-actin antibody were purchased from Santa Cruz (Santa Cruz Biotech, Heidelberg, Germany). Anti-IFNAR2 antibody was purchased from PBL Biomedical Labs (PBL Biomedical Labs Inc., NJ).

2.2. Cell lines and cell culture

HepG2 cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Thermo Electron, Waltham, MA) containing 10% fetal bovine serum (GIBCO, GrandIsland, NY) and antibiotics (50 IU/mL penicillin, 50 mg/mL streptomycin). HepG2.2.15 cells, which derived by transfecting HepG2 cells with a plasmid containing HBV DNA, were maintained in DMEM supplemented with 2 mol/L L-glutamine, 50 IU/mL of penicillin, 50 mg/mL of streptomycin (GIBCO, GrandIsland, NY), 500 μg/mL of G418 (Sigma–Aldrich Quimica, Madrid, Spain), 10% fetal bovine serum, at 37 °C in an humidified incubator at 5% CO_2. HepG2.2.15 cells constitutively express HBsAg, HBeAg, HBcAg and support full HBV replication (Sells et al., 1987). All these cells were seeded at a density of 1×10^6 cells/well and maintained in a confluent state for 2–3 days before being treated with antiviral compounds.

2.3. Cell extracts, immunoprecipitation and immunoblotting

For both immunoblotting and immunoprecipitation, the cells were washed with ice-cold phosphate-buffered saline and lysed in lysis buffer containing 50 mM Tris–HCl pH 8.0, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 5 μg/mL aprotinin, 3 μg/mL pepstatin, and 5 μg/mL leupeptin. Cell lysates were then clarified at 12,000 rpm for 20 min at 4 °C, and the protein concentration in the supernatant was determined by the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific Inc., Rockford). For immunoblotting, the cell lysates were boiled in 2× SDS sample buffer (0.125 M Tris–HCl pH 6.8, 4% SDS, 20% glycerol, 2% mercaptoethanol, and 0.02% bromphenol blue). For immunoprecipitation, the lysates were incubated with antibody and protein A/G-Sepharose (Santa Cruz Biotech, Heidelberg, Germany) overnight at 4 °C. The immunocomplexes were washed five times with lysis buffer, and the immunoprecipitated proteins were removed from protein A/G-Sepharose by boiling in 2× SDS sample buffer and loaded onto SDS-PAGE. After SDS-PAGE and transfer onto polyvinylidene difluoride membranes, proteins were treated with a primary antibody and then with a horseradish peroxidase-conjugated secondary antibody. Bound antibody was detected by enhanced chemiluminescence (Thermo Fisher Scientific Inc., Rockford) and exposed to X-ray film. Densitometric analyses were performed using Glyko BandScan software (Glyko, Inc.).

2.4. RNA isolation, reverse transcription, and real-time PCR analysis

Total RNA was isolated from cultured cells according to Manufacturer's instructions (Invitrogen, Carlsbad, CA). cDNA was synthesized using random hexamer primers and RNase H-reverse transcriptase (Promega, Madison, WI). Relative quantitative real-time PCR was performed using SYBR-green I Premix ExTaq on the ABI Prism 7900 (Applied Bio systems, Foster, CA) following the Manufacturer's instructions. The following primer pairs were used: 2′,5′-OAS (139 bp) F: 5′-ACTCCCAGTTCAACATGG-3′ R: 5′-TGAAGCTGCTGTTTCAGG-3′, PKR (250 bp) F: 5′-AGGCAGTTAGTCCTTTAT-3′ R: 5′-AGATATGCAAGTTTAGCG-3′, GAPDH (80 bp) F: 5′-GCTCCTCCTGTTCGACAGTCA-3′ R: 5′-ACCTTCCCCATGGTGTCTGA-3′. All PCR assays were performed in duplicate, and data were analyzed with the ABI Prism Detection system using the comparative threshold cycle method as previously described (Tang et al., 2007).

2.5. Virological assessment

HBsAg, HBeAg levels of the supernatants were determined by time-resolved fluorescent immuno assay kit (TRFIA) according to the Manufacturer's instructions. HBV DNA was extracted from supernatants of culture cells and quantified by using a commercial polymerase chain reaction (PCR) diagnostic kit with a detect limit of 500 copies/mL (PG Biotech, Shenzhen, China). The decreased percentage was calculated according to the following formula: decreased percentage (%) = (C control − C tester)/C control × 100%.

2.6. Statistical interpretation

Data were analyzed with SPSS 12.0 for Windows (SPSS Inc., Chicago, IL). Statistical analysis was performed using the ANOVA and *T*-test to assess differences between groups. A probable value of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. High-level expression of IFNAR in HBV-infected liver cells

Type I IFN receptor consists of two subunits, IFNAR1 and IFNAR2. To test whether the presence of HBV could affect the IFN-alpha signaling, we examined firstly the IFNAR1 and IFNAR2 expression in HepG2 and HepG2.2.15 cells stimulated with or without IFN-alpha. As shown in Fig. 1, both IFNAR1 and IFNAR2 were abundantly expressed in HepaG2 and HepG2.2.15 cells. Densitometric

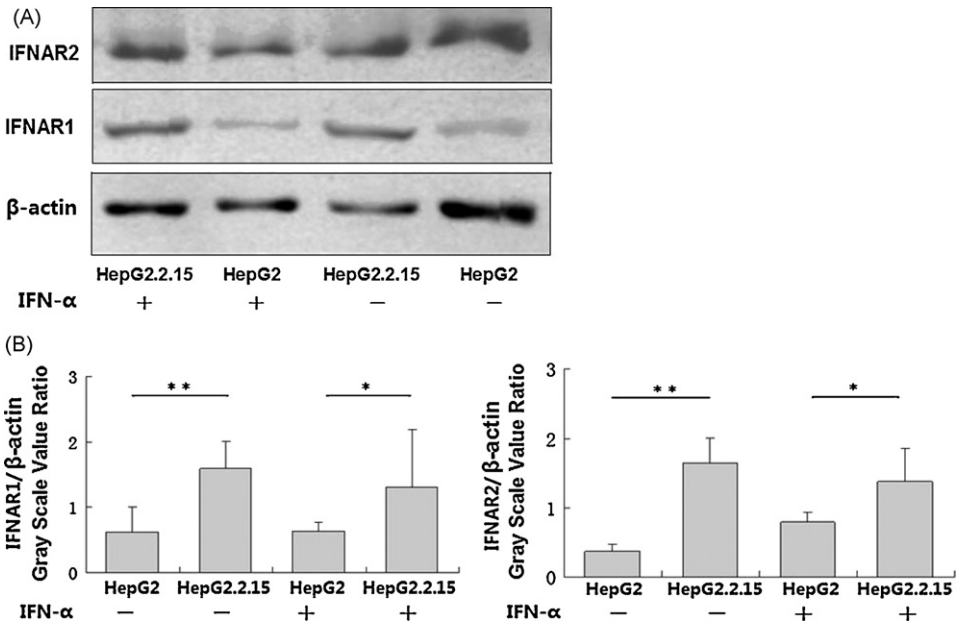

Fig. 1. High-level expression of IFNAR in HBV-infected liver cells. (A) HepG2 and HepG2.2.15 cells were treated with or without 1000 IU/mL IFN-alpha for 30 min respectively. Equal amounts of total cell protein extracts were immunoblotted with either anti-IFNAR1 or anti-IFNAR2 antibody. An anti-β-actin antibody was used to verify loading of protein per lane. The results were representative of three independent experiments. (B) Both IFNAR2 and IFNAR1 intensities in western blots were quantified by densitometry using software Glyko Bandscan 5.0. Protein levels were normalized against those of β-actin respectively. Data are expressed as mean ± S.D. Error bars were calculated from three independent experiments. The *P* values were obtained using the *T*-test (**P* < 0.05, ***P* < 0.01).

analyses indicated that with or without IFN-alpha treatment, the expression levels of IFNAR1 and IFNAR2 were evidently higher in HepG2.2.15 cells than in HepG2 cells. Furthermore, IFN-alpha treatment changed neither in HepG2 cells nor in HepG2.2.15 cells the expression level of either of the two subunits.

3.2. IFN-alpha dependent STAT1 phosphorylation is intact in HBV-infected liver cells

In view of the crucial role of STAT1 phosphorylation in IFN-alpha signaling, we decided to check whether HBV could potentially influence the phosphorylation of STAT1 responding to IFN-alpha. We treated HepG2.2.15 and HepG2 cells with different doses of IFN-alpha, and the phosphorylated STAT1 was detected by western blot using a specific anti-phospho-STAT1 antibody. As shown in Fig. 2A, no detectable STAT1 phosphorylation was found in IFN-alpha untreated HepG2.2.15 and HepG2 cells. In contrast, addition of IFN-alpha stimulated the phosphorylation of STAT1, in a time- and concentration-dependent manner (Fig. 2B and C). Withing the same tested times and with the same concentrations, IFN-alpha led completely to a parallel generation of STAT1 phosphorylation in HepG2 and HepG2.2.15 cells (Fig. 2B).

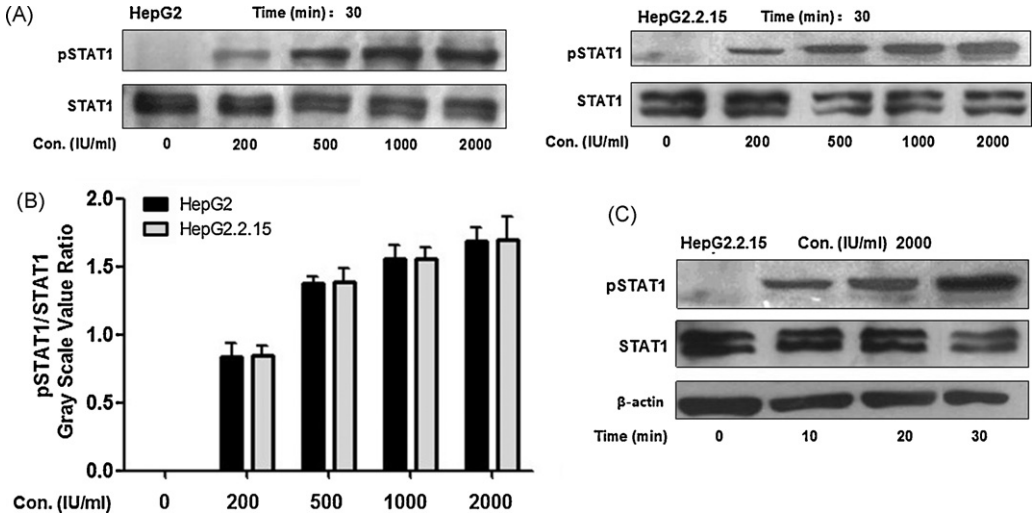

Fig. 2. IFN-alpha dependent STAT1 phosphorylation in HBV-infected liver cells. (A) HepG2 and HepG2.2.15 cells were treated with varying concentrations (0–2000 IU/mL) of IFN-alpha for 30 min. Equal amounts of total cell protein extracts were immunoblotted with anti-phospho-STAT1 antibody. The membrane was reblotted for STAT1 as a loading control. Shown is a representative of results from three independent experiments. (B) The densities of STAT1 and phosphorylated STAT1 were quantified by densitometry using software Glyko Bandscan 5.0. There were significant differences among various dose groups (both $P < 0.05$). Data are expressed as mean ± S.D. Error bars were calculated from three independent experiments. The *P* values were obtained using the ANOVA test. (C) Time course of STAT1 phosphorylation after stimulation with IFN-alpha. HepG2.2.15 cells were stimulated with IFN-alpha for the indicated time points. The membrane was immunoblotted with anti-phospho-STAT1 antibody and anti-STAT1 antibody. An anti-β-actin antibody was used to verify loading of protein per lane. Total STAT1 and phosphorylated STAT1 expression were determined by immunoblotting. Shown is a representative of results from three independent experiments.

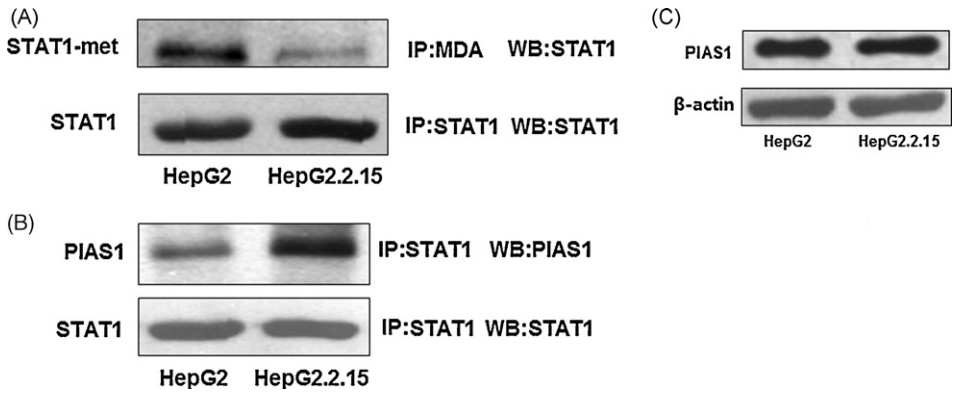

Fig. 3. Less STAT1 methylation and association increased STAT1–PIAS1 binding in HBV-infected liver cells. (A) HepG2 and HepG2.2.15 cells were treated with 1000 IU/mL of IFN-alpha for 30 min, respectively. In these cells, both STAT1 methylation (immunoprecipitation with antibody to methyl- and dimethylarginine (MDA), western blot with anti-STAT1 antibody) and the binding of PIAS1 to STAT1 (immunoprecipitation with anti-STAT1 antibody, western blot with anti-PIAS1 antibody) were detected by Co-IP analysis. STAT1 protein was used as a loading control. (B) HepG2 and HepG2.2.15 cells were treated with 1000 IU/mL of IFN-alpha for 30 min, respectively. PIAS1 expression was determined by immunoblotting with an anti-PIAS1 antibody. An anti-β-actin antibody was also used to verify loading of protein per lane. Shown is a representative of results from three independent experiments.

3.3. Less STAT1 methylation and association increased STAT1–PIAS1 binding in HBV-infected liver cells

Members of the PIAS family can negatively regulate the cytokine-activated JAK–STAT pathway in vitro (O'Shea and Watford, 2004). Cells lacking PIAS1 have shown enhanced antiviral responses (Liu et al., 2004). Studies have demonstrated that the methylation of STAT1 on Arg-31 appears to be involved in STAT1–PIAS1 association as unmethylated STAT1 can be bound and inactivated by PIAS1 (Duong et al., 2006). To test the possibility that HBV could down regulate the methylation of STAT1 thereby inhibiting the expression of antiviral effectors due to an increased STAT1–PIAS1 interaction, we carried out experiments to measure the STAT1 methylation and to determine STAT1–PIAS1 association. In HepG2 and HepG2.2.15 cells, we immunoprecipitated the methylated proteins with a specific anti-methyl- and dimethylarginine antibody, then stained the proteins with an anti-STAT1 antibody. As shown in Fig. 3A, compared to HepG2 cells in which marked methylated STAT1 was represented, in HepG2.2.15 cells, less detectable STAT1 methylation was revealed. Co-immunoprecipitation (Co-IP) experiment was then performed in HepG2.2.15 cells, and the result demonstrated an enhanced association of PIAS1 with STAT1 (Fig. 3B). Also no difference of the PIAS1 expression was observed in these two cells (Fig. 3C). These results indicate that HBV induces less STAT1 methylation and up-regulates STAT1–PIAS1 binding in Hep2.2.15 cells.

3.4. S-adenosylmethionine addition reverses STAT1 methylation

S-adenosylmethionine (SAM) is available in many countries as a drug used for the treatment of liver diseases such as alcohol-induced liver damage. Its supplementation can restore hepatic glutathione deposits and attenuate liver injury (Lieber et al., 1990). SAM has been used in many reactions to transfer methyl group to the nitrogen or oxygen residues in many different compounds (Grillo and Colombatto, 2005). PRMT1 uses SAM as the methyl group donor for STAT1 methylation. To test if addition of SAM can improve HBV-induced decrease in methylation of STAT1, we treated HepG2.2.15 cells with SAM and quantified the methylation level of STAT1. As shown in Fig. 4, compared to HepG2 cells, less STAT1 methylation was detected in HepG2.2.15 cells. Strikingly, SAM addition effectively increased STAT1 methylaion which was very close to the level in HepG2 cells. Consistently with this change in STAT1 methylation, a decreased STAT1–PIAS1 association was also detected.

3.5. S-adenosylmethionine improves the antiviral effect of IFN-alpha

dsRNA-dependent protein kinase (PKR), an IFN-inducible serine/threonine kinase, is a key mediator of IFN-alpha inducible antiviral activity (Clemens and Elia, 1997). Like PKR, 2′,5′-oligoadenylate synthetase (OAS) is synthesized in an inactive form and utilizes dsRNA as a cofactor. The 2′,5′-OAS binds to and activates RNase L, which degrades cellular and viral RNAs (Randall and Goodbourn, 2008). We then tested if SAM could improve the antiviral effect of IFN-alpha by detecting the PKR and 2′,5′-OAS transcription levels. We first compared the IFN-alpha induced 2′,5′-OAS, PKR mRNA levels in HepG2 and HepG2.2.15 cells after pretreatment of these cells with or without SAM. Real-time PCR analysis showed that the induction of 2′,5′-OAS and PKR mRNA was impaired in HepG2.2.15 cells compared to HepG2 cells, but both could be enhanced by pretreating the cells with SAM (Fig. 5A).

Furthermore, we hypothesized that the HBV replication in HepG2.2.15 cells could also be changed by SAM treatment. To test this hypothesis, we quantified HBsAg and HBeAg protein levels together with HBV genomic DNA in the culture medium of HepG2.2.15 cells. As shown in Fig. 5B, treatment with SAM and IFN-alpha together led to a significant reduction of medium HBsAg and HBeAg. Consistently, the HBV DNA load in the culture medium also decreased (Fig. 5C). These results indicate that SAM can improve the antiviral effect of IFN-alpha in HBV-infected cells.

4. Discussion

In the present study, we applied HepG2 and HepG2.2.15 cells to analyze the HBV-caused low response of hepatocytes to IFN-alpha stimulation. Our results showed that HBV infection downregulates methylation rather than the phosphorylation of STAT1, which was consistent with an increased STAT1–PIAS1 interaction. Combined with IFN-alpha, S-adenosylmethionine effectively improved the STAT1 methylation and attenuated STAT1–PIAS1 binding, followed by increased mRNA expression of PKR, 2′,5′-OAS in HBV-infected liver cells. Thus we suggested that less STAT1 methylation and subsequent increased STAT1–PIAS1 interaction is involved in the mechanism of IFN-alpha antagonistic activity of HBV. S-adenosylmethionine can sensitize in a great degree the antiviral effect of IFN-alpha by improving STAT1 methylation.

IFN-alpha is the first substance licensed to treat CHB patients and has been used for many decades. Only approximately 30% of treated patients develop a sustained virologic response. The

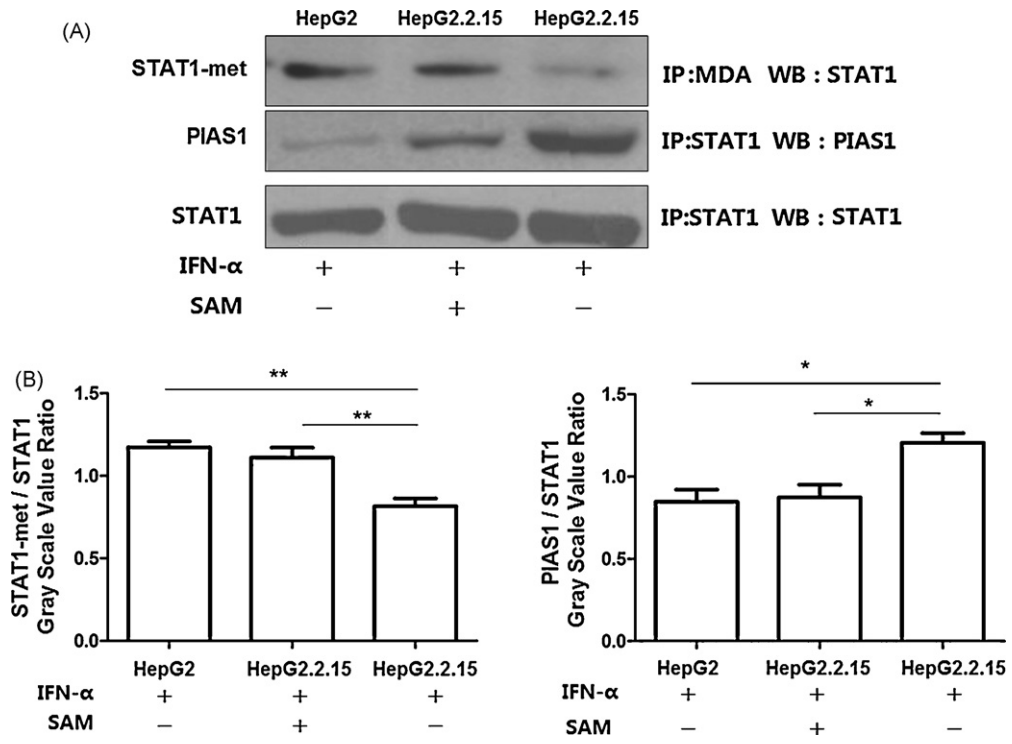

Fig. 4. S-adenosylmethionine addition reverses STAT1 methylation. HepG2 cells were treated with 1000 IU/mL of IFN-alpha for 30 min. HepG2.2.15 cells were stimulated with IFN-alpha (1000 IU/mL) for 30 min with or without pretreatment with SAM (263.66 nmol/L) for 3 h. (A) In these cells, STAT1 methylation and the binding of PIAS1 to STAT1 were detected by Co-IP analysis. STAT1 protein level was used as a loading control. Shown is a representative of results from three independent experiments. (B) The intensities of STAT1-associated PIAS1, methylated STAT1 and STAT1 in western blots were quantified by densitometry using software Glyko Bandscan 5.0. Data are expressed as mean ± S.D. Error bars were calculated from three independent experiments. The *P* values were obtained using the ANOVA test (***P* < 0.01, **P* < 0.05).

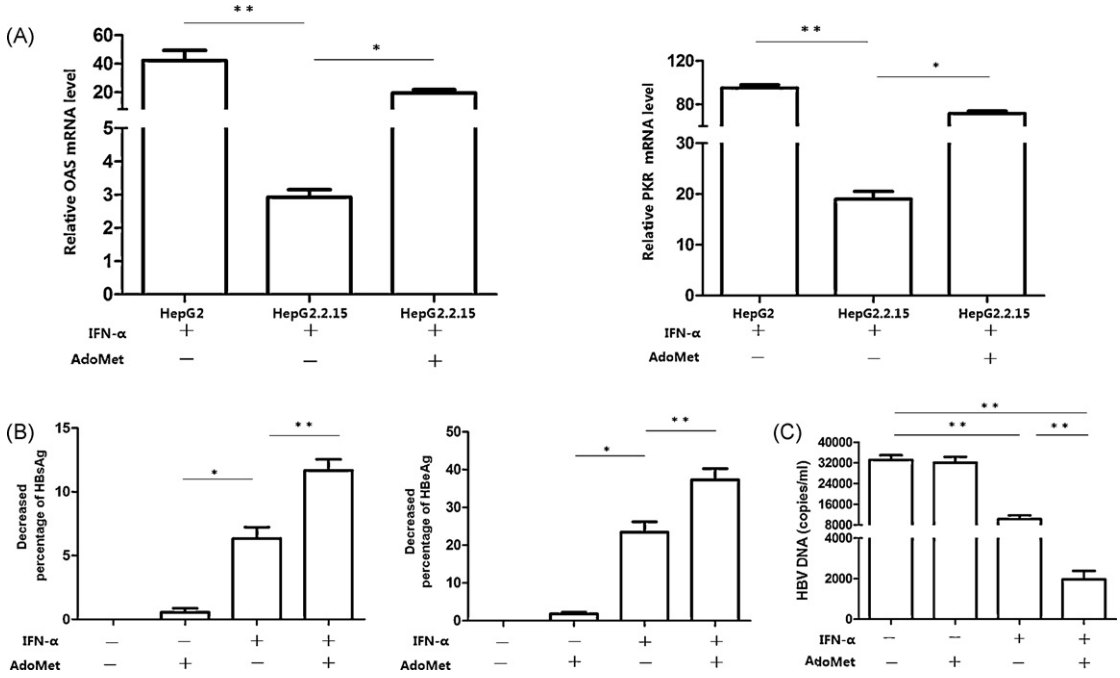

Fig. 5. S-adenosylmethionine improves the antiviral effect of IFN-alpha. (A) HepG2 cells were treated with 1000 IU/mL of IFN-alpha for 6 h. HepG2.2.15 cells were stimulated with IFN-alpha (1000 IU/mL) for 6 h with or without treatment with SAM (263.66 nmol/L) for 18 h. The induction of the interferon target gene was measured with real-time PCR. The fold decreases of target gene mRNA was calculated as several fold increase of the mRNA amounts in IFN-alpha-treated samples versus that in untreated samples. (B) HepG2.2.15 cells were stimulated with IFN-alpha (1000 IU/mL) for 10 days with or without a treatment with SAM (263.66 nmol/L) for 18 h. Then, supernatants were collected and assayed for the presence of HBsAg and HBeAg by TRFIA. (C) Extracellular HBV DNA was analyzed by using a commercial polymerase chain reaction (PCR) diagnostic kit. Data are expressed as mean ± S.D. Error bars were calculated from three independent experiments. Samples were done at least in triplicate. The *P* values were obtained using the ANOVA test (**P* < 0.05, ***P* < 0.01).

cause of treatment failure in non-responders is not fully understood, but recently interference of HBV with IFN-alpha-induced JAK–STAT signaling has emerged as a possible escape strategy of HBV contributing to viral persistence and disease progression. HBV polymerase protein has been demonstrated to inhibit the STAT1 nuclear translocation induced by IFN-alpha (Wu et al., 2007). SOCS-1, which can inhibit the catalytic activity of JAK, is highly expressed in liver tissues of CHB patients (Vlotides et al., 2004; Zhao et al., 2008). Our research demonstrated that HBV-induced inhibition occurred not in the recognition level as both IFNAR1 and IFNAR2 expression is even higher in HBV-infected liver cells (Fig. 1A). In addition, consistent with previous opinion that viral infection do not affect IFN-alpha-induced tyrosine phosphorylation of STAT1 (Heim et al., 1999), STAT1 phosphorylation remains intact when the hepatocytes constitutively express HBV proteins (Fig. 2C). Thus, resistance to IFN-alpha in HBV-infected liver cells must occur downstream.

Furthermore, we detected less STAT1 methylation and increased STAT1–PIAS1 binding, followed by reduced mRNA expression of PKR and 2′,5′-OAS in HBV-infected liver cells. It has been proposed that STAT1 can be catalyzed by PRMT1 at Arg-31 within the N-terminal region. Interestingly, the interaction of PIAS1 with STAT1 occurs just at the N terminus of STAT1 (aa1–191) where Arg-31 is located. Thus, methylation is functionally essential for STAT1 as unmethylated STAT1 can be bound and inactivated by PIAS1. Removal of PIAS1 can enhance the binding of STAT1 to the promoter of IFN-alpha-sensitive gene, such as PKR, P56, MxA and 2′,5′-OAS (Liao et al., 2000; Mowen et al., 2001). However, whether STAT1 protein can be methylated by PRMT1 is still controversial. Some groups do not support the proposed model and suggest that PRMT1 does not affect STAT1 function with respect to transcriptional regulation (Komyod et al., 2005; Meissner et al., 2004; Weber et al., 2009). In accordance with the report by Christen et al. (2007), we consistently confirmed that STAT1 is involved in the impairment of IFN-alpha signaling by regulating the interaction with PIAS1 in a methylation-dependent manner during HBV infection. Although it has been proposed that HBV proteins can enhance expression of PP2Ac which interacts directly with PRMT1 thus inhibiting PRMT1 enzymatic activity (Christen et al., 2007), the underlying reason for the low level of STAT1 methylation in HBV-infected cells is not yet clear.

Subsequently, we found that SAM can sensitize to a great degree the antiviral effect of IFN-alpha by improving STAT1 methylation. SAM is one of the most frequently used enzyme substrates. Its sulfonium group enables it to be recognized as the major methyl donor in all living organisms. PRMT1 always uses SAM for STAT1 methylation (Grillo and Colombatto, 2005). Combined with IFN-alpha, SAM treatment significantly reduced the HBsAg and HBeAg protein levels and the HBV DNA in the supernatant of HBV-infected cells. Consistent with our results, addition of SAM and betaine significantly improved the antiviral efficacy of IFN-alpha in HCV replicon cells (Duong et al., 2006). Also the clinical trial named “Chronic hepatitis C non-responder study with AdoMet and Betaine” (NCT00310336) is currently recruiting participants. It will also be interesting to test if SAM can improve IFN-alpha efficacy in the treatment of CHB patients.

More interestingly, recent research suggested that PRMT1 methylates PIAS1 at Arg-303 in vitro or vivo. PIAS1 methylation can trigger the interaction between STAT1–PIAS1 and enforce the release of STAT1 from its target gene promoters, which subsequently results in the turn off of STAT1-activated transcription (Weber et al., 2009). This mechanism seems to be contradictive to the one proposed by our study. Furthermore, it cannot be ruled out that PRMT1 has a regulatory effect on both proteins and could cause same gene expression in response to different stimulus. Indeed, the role of PRMT1 in the regulation of PIAS1 or STAT1 methylation during IFN-alpha stimulation remains unclear. Also whether knockdown of PIAS1 expression could modulate the methylation of STAT1 needs to be investigated in the future.

Taken together, our data demonstrate that less STAT1 methylation and subsequent increased STAT1–PIAS1 interaction is involved in the mechanism of IFN-alpha antagonistic activity to HBV. SAM can sensitize to a great degree the antiviral effect of IFN-alpha by improving STAT1 methylation. Combination of IFN-alpha and S-adenosylmethionine could be a new potential therapeutic strategy for patients with chronic HBV infection.

Acknowledgments

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