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MiR-101 regulates HSV-1 replication by targeting ATP5B

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ABSTRACT

MicroRNAs (miRNAs) are short non-coding RNAs that negatively modulate gene expression at the post-transcriptional level and are known to be involved in the cross-talk between the host and virus. Using a standard plaque assay and real-time PCR method, we found that ectopic expression of miR-101 could significantly suppress herpes simplex virus-1 (HSV-1) replication, and that blocking endogenous miR-101 could increase viral progeny without affecting cell viability. Bioinformatics analysis indicates the 3′ untranslated region (UTR) of mitochondrial ATP synthase subunit beta (ATP5B) has a putative binding site for miR-101. MiR-101 can directly bind to ATP5B 3′UTR and negatively regulate ATP5B expression. Using a RNA interference technique, knockdown of ATP5B significantly inhibited HSV-1 replication, indicating that ATP5B functions as a pro-viral factor. The ectopic expression of ATP5B lacking the 3′UTR could override the suppressive effect of miR-101 on HSV-1 replication. A concordant inverse correlation between miR-101 and ATP5B was observed in HSV-1-infected HeLa cells. Up-regulation of miR-101 expression may play a role in repressing productive HSV-1 replication by targeting ATP5B. Exploring the role of host-encoded miRNA in the regulation of viral infection would enable us to better understand the intricate networks of host–pathogen interactions.

1. Introduction

MicroRNAs (MiRNAs) have been described as a highly conserved family of 22-nucleotide (nt) noncoding RNAs that can bind to specific target mRNAs and negatively regulate their expression (Bartel, 2009). Increasing evidence suggests that miRNAs not only participate in maintenance of normal cell functions, but are also involved in host–virus interactions and play a key role in the regulation of viral replication (Umbach and Cullen, 2009). Human miR-32 has been shown to limit the replication of primate foamy virus type 1 (PFV-1) (Lecellier et al., 2005). MiR-17-5p and miR-20, components of miR-17/92 cluster, downregulate the histone acetyltransferase Tat cofactor (PCAF) and inhibit HIV-1 replication (Triboulet et al., 2007). MiR-199a-3p and miR-210 were recently reported to suppress HBV replication by targeting HBs genes (Zhang et al., 2010). Besides acting as innate antiviral defenders, some miRNAs function as pro-viral factors and contribute to viral tropism. A typical example is human liver-specific miR-122, which targets the 5′ end of the HCV genome and promotes viral replication (Jopling et al., 2005).

Viruses are intracellular parasites that attempt to create a cellular environment favorable to their replication or survival and to establish a lifelong latent infection. While miRNA-mediated RNA silencing pathways serve as innate antiviral mechanisms, viruses have devised methods to overcome, and even subvert, these host-initiated antiviral responses. Many studies have reported that miRNAs can be used as regulatory molecules by viruses, and many viruses can encode their own miRNAs (Umbach and Cullen, 2009). Thus far, nearly all herpesviruses have been found to encode viral miRNAs, as have human adenovirus, human immunodeficiency virus type 1 (HIV-1), heliothis virescens ascovirus (HvAc), and several members of the polyomavirus family (Aparicio et al., 2006; Hussain et al., 2008; Jurak et al., 2010; Ouellet et al., 2008; Sullivan et al., 2005). These virus-encoded miRNAs regulate expression of their own genes, host genes, or both, and contribute to the pathogenic properties of viruses. Taking HSV-1 as an example, miR-H1, the first discovered miRNA encoded by HSV-1, is expressed relatively abundantly as a late gene in productively infected cells (Cui et al., 2006). Then, 15 other HSV-1 miRNAs (HSV-1 miR-H2 to miR-H18) within, or proximal to, the latency associated transcript region were identified in productive and latent infections (Jurak et al., 2010; Umbach et al., 2008). Many of these HSV-1 miRNAs

Abbreviations: miRNA, microRNA; HSV-1, herpes simplex virus-1; UTR, untranslated region; ATP5B, ATP synthase subunit beta; ASO, antisense oligonucleotide; CPE, cytopathogenic effect; MOI, multiplicity of infection; siRNA, small interference RNA; EGFP, enhanced green fluorescence protein; MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide); RFP, red fluorescent protein; CT, threshold cycle; ORF, open reading frame; Vhs, virion host shutoff.

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are perfectly complementary to HSV mRNAs encoded on the opposite strands, suggesting that they can post-translationally repress HSV-1 gene expression. Repression of viral transcriptional activators ICP0 and ICP4, by miR-H2 and miR-H6, may be important for repression of productive-cycle gene expression and, thus, result in latency (Jurak et al., 2010; Umbach et al., 2008).

Besides encoding miRNAs, viruses can also trigger changes in host miRNAs expression. The adenovirus VA1 non-coding RNA inhibits nuclear export of miRNA precursors (Lu and Cullen, 2004). Infection of HeLa cells with HIV-1 alters the expression of cellular miRNAs, many of which are downregulated (Yeung et al., 2005). Suppression of the expression of the miR-17/92 cluster by HIV-1 is required for efficient viral replication (Triboulet et al., 2007). HSV-1 infection of human brain cells induces upregulation of a brain-enriched miR-146a that is associated with proinflammatory signaling in stressed brain cells and Alzheimer's disease (Hill et al., 2009). MiR-132 is also found to be highly upregulated after HSV-1 and human cytomegalovirus (HCMV) infection and has a negative effect on the expression of interferon-stimulated genes, facilitating viral replication (Lagos et al., 2010).

Although increasing evidence indicates that miRNAs have important functions in host–virus interactions, the molecular mechanisms of many of these are yet to be understood. To explore the effect of host miRNAs on HSV-1 replication, a loss-off-function screening assay were performed as described in Zhang et al. (2010). MiR-101 was found to be related with HSV-1 replication. Here, we report that human miR-101 can suppress HSV-1 replication by targeting a subunit of mitochondrial ATP synthase (ATP5B). HSV-1-induced miR-101 overexpression may have a role in regulating viral replication.

2. Material and methods

2.1. Cell culture and transfection

HeLa cell lines were propagated and maintained in RPMI 1640 medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum and antibiotics, in a humidified atmosphere at 37 °C with 5% CO_2. Plasmids or antisense oligonucleotides (ASO) were transfected in antibiotic-free Opti-MEM medium (Invitrogen, Carlsbad, CA) with lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA), following the manufacturer's protocol.

2.2. Virus infection

HSV-1 Stocker strain was propagated on HeLa cells. At the peak of the cytopathogenic effect (CPE), viruses were harvested by three cycles of freezing and thawing. After centrifugation, the supernatant was aliquoted, titrated by plaque assays, and stored at −80 °C. At 24 h post-transfection, HeLa cells were infected with HSV-1 at a multiplicity of infection (MOI) of 0.01. HSV-1 yields were determined by standard plaque assays after a 2-day incubation (Bhuyan et al., 2004).

2.3. Vector construction

To construct the miR-101 expression plasmid (pcDNA3/pri-miR-101), the DNA fragment carrying pre-miR-101 was amplified from genomic DNA by PCR using specific primers (miR-101-S, 5′-CGC GGA TCC TCA CGT CTC CAA CCA GAA G-3′; miR-101-AS, 5′-CGG AAT TCG CAG AAT AAC TCT CCC TAT GCC-3′). The amplified fragment was cloned into pcDNA3.1 (Invitrogen, Carlsbad, CA) at BamH I and EcoR I sites.

To construct the ATP5B expression vector, the coding sequence of ATP5B without the 3′UTR was obtained by PCR using primers (ATP5B-S, 5′-CGG AAT TCA CCA TGT TGG GGT TTG TGG GTC-3′; ATP5B-AS, 5′-AGG ACC TCG AGC CCT CAC GAT GAA TGC TCT TC-3′), and then cloned into the pcDNA3.1 vector. The plasmid expressing small interference RNA (siRNA) targeting ATP5B was constructed using the following sequences of oligonucleotides: shATP5B-top, 5′-GAT CCC GGC AGA ATC ATG AAT GTC ATT CGA AGA AAT GAC ATT CAT GAT TCT GCC TTT TTT GA-3′; shATP5B-bot, 5′-AGC TTC AAA AAA GGC AGA ATC ATG AAT GTC ATT TCT TCG AAT GAC ATT CAT GAT TCT GCC GG-3′. The annealing products were ligated into the HindIII and BamHI restriction sites of the pSilencer 2.1-U6 neo vector (Ambion, Austin, TX).

The EGFP (enhanced green fluorescence protein) reporter vectors were constructed as previously described (Zhang et al., 2010). The 3′UTR of ATP5B containing the miR-101 binding site was cloned into pcDNA3/EGFP. Similarly, the fragment of the ATP5B 3′UTR mutant, which contained a triple point mutation in miR-101 binding site, was also cloned into pcDNA3/EGFP.

2.4. Cell viability assay

To determine relative cell viability, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed at 24 h post-transfection in 96-well plates according to standard procedures (Liu et al., 2009). The absorbance at 570 nm was detected using a μQuant Universal Microplate Spectrophotometer (Bio-Rad, Hercules, CA).

2.5. Fluorescent report assays

HeLa cells were transfected in 48-well plates with pcDNA3/pri-miR-101, control vector, miR-101-ASO, or control ASO, and then with the reporter vector pcDNA3/EGFP-ATP5B-UTR or pcDNA3/EGFP-ATP5B-MUT the following day. The vector pDsRed2-N1 (Clontech, Mountain View, CA), expressing RFP (red fluorescent protein), was spiked in and used for normalization. 72 h later, the cells were lysed with RIPA lysis buffer (150 mM NaCl, 50 mM Tris–HCl, pH 7.2, 1% Triton X-100, 0.1% SDS). The intensities of EGFP and RFP fluorescence were detected with a Fluorescence Spectrophotometer F-4500 (Hitachi, Tokyo, Japan).

2.6. DNA extraction

DNA was extracted from HSV-1 infected cells. 500 μl of lysis buffer (10 mM Tris–HCl pH 7.4, 0.1 mM EDTA, 0.5% sodium dodecyl sulfate, 20 μg/ml RNase A) was added to 1× PBS-washed 1×10^5 cells and incubated for 1 h at 37 °C. Proteinase K was added to cell lysates at a final concentration of 100 μg/ml and incubated overnight at 50 °C. The samples were then extracted twice with phenol–chloroform–isoamyl alcohol (25/24/1, vol/vol). The DNA was precipitated with 100% alcohol in the presence of 0.3 M sodium acetate. The DNA pellets were washed with 70% alcohol, dried, and suspended in 100 μl of sterile TE buffer. The resulting DNA preparations were stored at −60 °C.

2.7. Quantitative real-time PCR

Quantitation of miRNAs was carried out using SYBR Green-based real-time RT-PCR as previously described (Zhang et al., 2010). To detect the relative level of ATP5B transcription, quantitative RT real-time PCR was performed. To detect the relative level of HSV-1 DNA, the primers amplified a generic HSV 146 bp product from the HSV DNA polymerase I gene (Aryee et al., 2005). 18S rRNA was used as the internal control gene (Nyström et al., 2004). All PCR was performed under the following conditions: 94 °C for 4 min followed by 40 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s. SYBR Premix Ex Taq™ kit (TaKaRa, Madison, WI) was used according to the manufacturer's instructions, and the real-time

PCR was performed on an iQ5 Real-Time PCR system (Bio-rad). The real-time PCR results were analyzed and expressed as relative expression of CT (threshold cycle) value using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

2.8. Western blot

Total proteins from transfected HeLa cells were extracted 48 h post-transfection using RIPA buffer, and protein expression was analyzed by Western blot. GAPDH served as a loading control. The following antibodies were used: rabbit anti-ATP5B, rabbit anti-GAPDH, and goat anti-rabbit (Tianjin Saier Biotech, China). Bands were quantified with Labworks 4.0 software.

2.9. Statistics and data analysis

Statistical significance was determined using the Student's *t* test. In all figures, values are expressed as mean ± standard deviation (SD), and statistical significance ($P < 0.05$) is indicated by a single asterisk. The data generated *in vitro* are representative of at least three separate experiments conducted in triplicate.

3. Results

3.1. MiR-101 suppresses HSV-1 replication in HeLa cells

To study the role of miR-101 in host–virus interaction, we constructed plasmids expressing the miR-101 precursor (pcDNA3/pri-miR-101) and synthesized 2′-OMe miR-101 ASO. The efficiency of either overexpression or suppression of miR-101 in HeLa cells was validated using real-time PCR. PCR results showed that transfection with pcDNA3/pri-miR-101 could result in an 70% increase of miR-101 levels in HeLa cells, whereas miR-101 2′-OMe ASOs could result in an 50% reduction of miR-101 levels compared to the control group (Fig. 1A). Transfection efficiency was over 90%, as determined using pDsRed2-N1 or Cy5-oligomer (data not shown). To explore the effect of human miR-101 on HSV-1 replication in HeLa cells, we first used the MTT assay to determine whether miR-101 affects cell viability. As shown in Fig. 1B, regardless of whether miR-101 expression levels were upregulated or downregulated, the viability of HeLa cells remained unchanged.

Next, we performed a simultaneous gain-of-function and loss-of-function assay by transfecting HeLa cells with either the miR-101

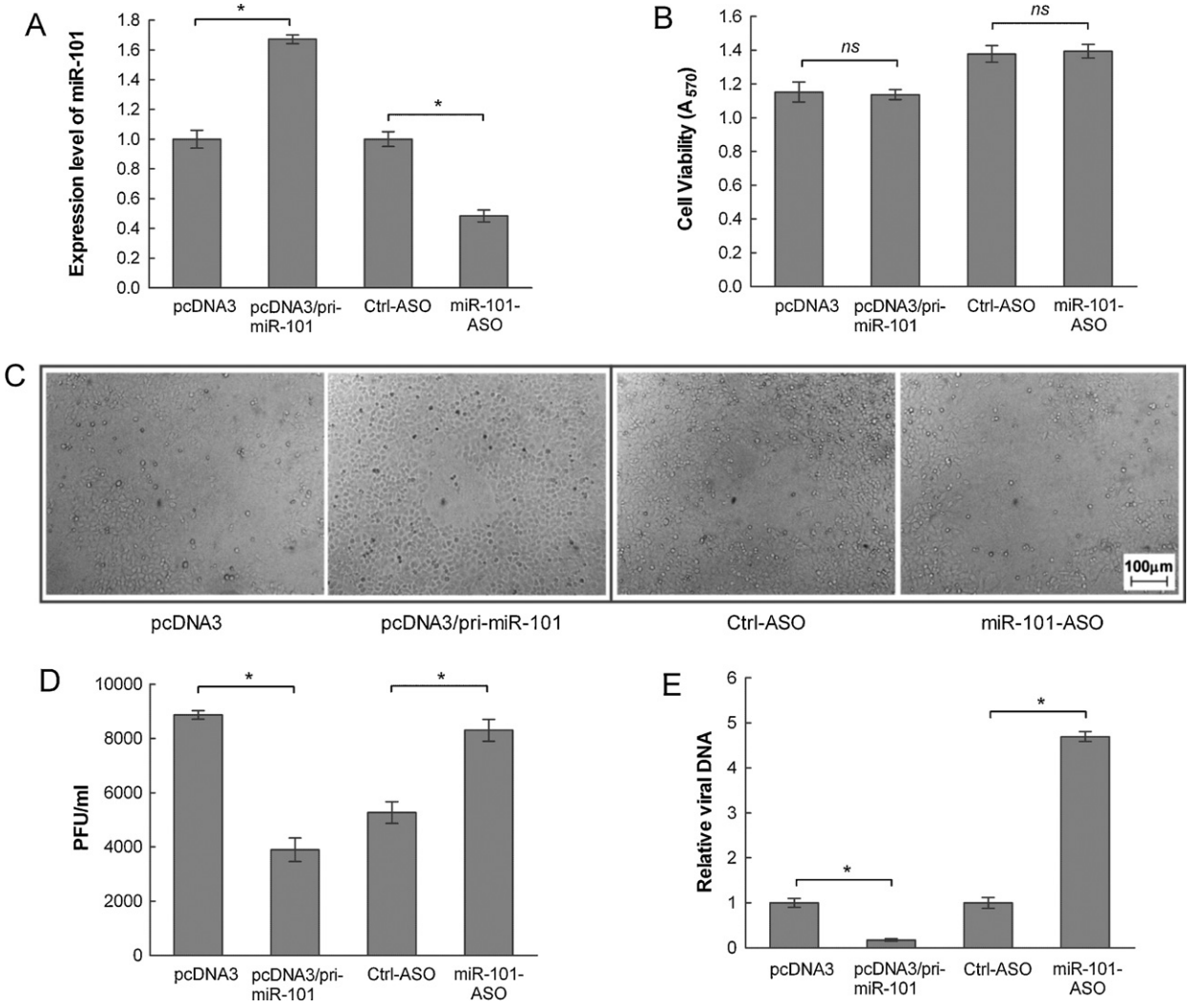

Fig. 1. miR-101 inhibits the production of HSV-1 in HeLa cells. (A) HeLa cells were transfected with pcDNA3/pri-miR-101, control vector, miR-101-ASO or control ASO as indicated. The expression level of miR-101 was assessed by real-time RT-PCR. Expression levels were normalized to the small nuclear RNA U6. $^*P < 0.05$. (B) Cell viability was measured by the MTT assay 24 h post-transfection. The histogram shows mean values (±SD) of A_{570} from three independent experiments. *ns* indicates $P > 0.05$. (C) At 24 h post-transfection, cells were infected with HSV-1 at 0.01 PFU/cell. Individual plaques are shown at a magnification of 400×. (D) Viral yields were determined by standard plaque assays at a 2-day incubation for HSV-1. $^*P < 0.05$. (E) DNA was extracted from HSV-1 infected cells. Relative HSV-1 DNA content was detected using real-time the PCR technique with primers targeting a sequence from the HSV DNA polymerase gene. Values were normalized to endogenous GAPDH. $^*P < 0.05$.

expression plasmid or 2′-OMe ASO, respectively. At 24 h post-transfection, the cells were inoculated with HSV-1 at 0.01 PFU/cell, and after 48 h the concentration of viral progeny were determined by a standard plaque assay. The results showed that, compared to HeLa cells that were transfected with the control vector, HSV-1 infection of HeLa cells transfected with pcDNA3/pri-miR-101 produced small plaques and, conversely, blocking miR-101 produced large plaques (Fig. 1C). Consistent with the plaque phenotype, ectopic expression of miR-101 could significantly reduce HSV-1 virions in the supernatants, while blocking endogenous miR-101 could increase viral progeny (Fig. 1D).

Our viral yield results validate the antiviral activity of miR-101 in HeLa cells. Because DNA amplification using PCR techniques is reported to be a sensitive method for estimating the HSV load in cells (Aryee et al., 2005), we also used real-time PCR technique with primers targeting a sequence from the HSV DNA polymerase gene for viral quantification. The quantity of HSV in HeLa cell was expressed as viral DNA copies/cell. As shown in Fig. 1E, highly enriching miR-101 could result in an 80% reduction of viral DNA in cells, while blocking miR-101 could result in an 5-fold increase of viral DNA. Together, these findings indicated that miR-101 could suppress HSV-1 replication *in vitro*.

3.2. ATP5B is the target gene of miR-101

To elucidate the mechanism of miR-101 antiviral activity, it is necessary to identify the target genes of miR-101. The question that is to be addressed is whether miR-101 directly targets HSV-1 genes and/or negatively regulates host genes that are involved in the regulation of HSV-1 replication. Because no complementary sequences of miR-101 were found in HSV-1 transcripts, two algorithm programs (PicTar and TargetScan Release 5.1) were used to predict the cellular targets of miR-101. ATP synthase subunit beta (ATP5B) was found to have a putative miR-101 binding site within its 3′UTR (Fig. 2A). To confirm whether miR-101 could directly bind to the ATP5B 3′UTR and negatively regulate gene expression, the 3′UTR of ATP5B was cloned into the pcDNA3 vector, downstream of an EGFP gene. The reporter vector, or a pcDNA3/EGFP control vector, was transfected into HeLa cells. As shown in Fig. 2B, the intensity of EGFP fluorescence in reporter-vector-transfected cells is lower than that in the control group, indicating that endogenous miRNAs could negatively regulate EGFP expression by targeting its 3′UTR. Then HeLa cells were transfected with the reporter vector along with the miR-101 expression vector or ASO. The results showed that ectopic expression of miR-101 could reduce the intensity of EGFP fluorescence, and miR-101 blocking could enhance EGFP expression (Fig. 2C). However, when using a mutant vector containing a triple point mutation in the miR-101 binding site, the intensity of EGFP fluorescence was not significantly changed by either overexpression or blocking of miR-101 (Fig. 2C). These results suggested that miR-101 could directly bind to the 3′UTR of ATP5B mRNA and specifically suppress target gene expression.

To further confirm this relationship, we used real-time RT-PCR and Western blot assays to determine the effect of miR-101 on endogenous ATP5B gene expression. When miR-101 was overexpressed, the levels of ATP5B mRNA (Fig. 3A) and protein (Fig. 3B) were decreased nearly 60%, relative to the control groups. Conversely, blocking miR-101 resulted in a 40–60% increase of ATP5B mRNA (Fig. 3A) and protein (Fig. 3B) levels. This indicated that miR-101 negatively regulates ATP5B expression at a post-transcriptional level, which is consistent with the results of the EGFP reporter assay.

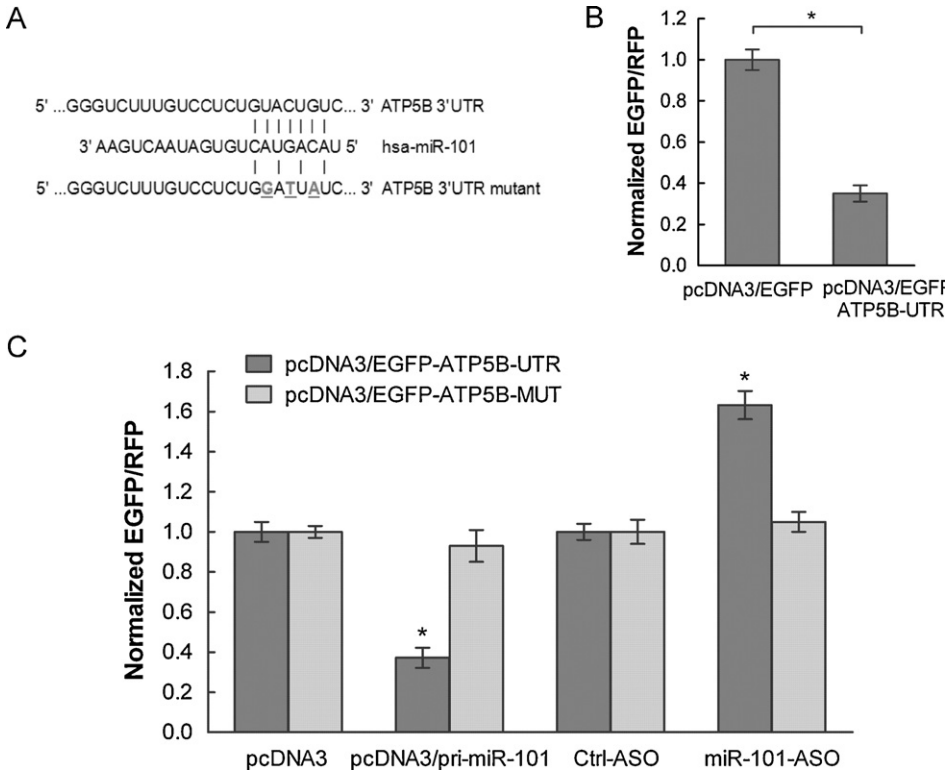

Fig. 2. ATP5B is the direct target gene of miR-101. (A) As is predicted in the TargetScan database, the ATP5B 3′UTR carries a miR-101 binding site. The ATP5B 3′UTR mutant, containing a mutated miR-101 'seed region' binding site, is shown. The underlined area indicates the mutated nucleotides. (B) HeLa cells were transfected with either pcDNA3/EGFP or pcDNA3/EGFP-ATP5B-UTR. pDsRed2-N1 expressing RFP was also spiked in for normalization. The fluorescence value in the control group was set to 1. $^*P < 0.05$. (C) HeLa cells were transfected with either pcDNA3/EGFP-ATP5B-UTR reporter vector or pcDNA3/EGFP-ATP5B-MUT mutant vector, along with pcDNA3/pri-miR-101, control vector, miR-101-ASO or control ASO as indicated. The fluorescence value in the control group was set to 1. $^*P < 0.05$.

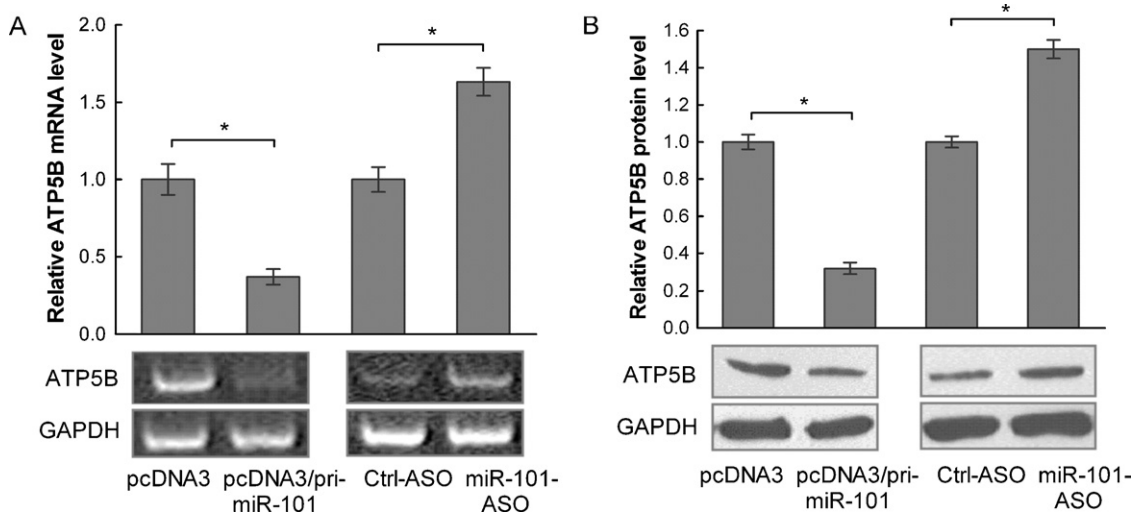

Fig. 3. miR-101 negatively regulates endogenous ATP5B level. (A) RNA was extracted from transfected HeLa cells, and the ATP5B mRNA level was measured by quantitative RT-PCR. Values were normalized to GAPDH mRNA, and the level of ATP5B mRNA in control group was set to 1. $^{*}P < 0.05$. (B) The protein level of ATP5B was measured by Western blot. $^{*}P < 0.05$.

3.3. Knocking down ATP5B inhibits HSV-1 replication

Next, we constructed an ATP5B siRNA expression vector to explore the role of ATP5B in regulation of HSV-1 replication. The results indicated that the ATP5B siRNA could significantly reduce endogenous ATP5B levels (Fig. 4A), and that ATP5B knockdown resulted in small viral plaques in HeLa cells, without obviously affecting cell viability at this time (Fig. 4B and C). Virus titration, using plaque assays and real-time PCR showed that knockdown of ATP5B significantly inhibited HSV-1 replication (Fig. 4D and E), which is consistent with the results of miR-101 overexpression.

3.4. Introduction of ATP5B lacking the 3′UTR abrogates the inhibition of miR-101 on HSV-1 replication

Because miR-101 directly targets the 3′UTR of ATP5B and down-regulates ATP5B expression, we reasoned that ectopic expression of ATP5B, by transfection with a construct (pcDNA3/ATP5B) that contains the open reading frame (ORF) of ATP5B without the 3′UTR, should result in escape from the regulation by miR-101 and thus attenuate the miR-101 antiviral effects. Western blot assays showed that the protein level of ATP5B was enhanced when HeLa cells were transfected with pcDNA3/ATP5B (Fig. 5A), and that over-expression of ATP5B could promote HSV-1 replication (Fig. 5B). However, the viral yield from HeLa cells that were cotransfected with ATP5B and miR-101 expression vectors showed no significant differences from the cells transfected with pcDNA3 vector (Fig. 5B), indicating that ectopic expression of ATP5B overrode the suppressive effect of miR-101 on HSV-1 replication. Based on these results, we conclude that ATP5B is a major target of miR-101 and largely mediates its antiviral activity.

3.5. A concordant inverse correlation between miR-101 and ATP5B in HSV-1-infected HeLa cells

As a miR-101/ATP5B axis was identified to be involved in the cross-talk between the host and virus, we next investigated the effect of HSV-1 infection on the level of miR-101 and ATP5B. HeLa cells were inoculated with HSV-1 at 1 PFU/cell, and uninfected HeLa cells were used as controls. Total RNAs were extracted by Trizol reagent at 1 h, 4 h and 8 h post-infection. Quantitative RT-PCR was used to detect the mRNA level of ATP5B and miR-101. The results indicated that the expression level of miR-101 increased gradually after viral attachment and penetration, resulting in a 12-fold increase of miR-101 levels 8 h post infection, compared to uninfected cells (Fig. 6A). In contrast, the ATP5B level correspondingly declined during the course of HSV-1 infection, which inversely correlates with the miR-101 levels in HSV-1-infected HeLa cells (Fig. 6B and C).

4. Discussion

MiRNAs are known to be crucial post-transcriptional regulators that also function as host cell defenses against both RNA and DNA viruses. The host- or virus-encoded miRNAs, and their target genes, together form novel regulatory networks between the host and the virus. Here, we explored the effect of host-encoded miRNAs on HSV-1 infection and found that miR-101 has an antiviral effect.

Recent research indicates that miR-101 is involved in physiological and pathological processes such as proliferation, megakaryocytopoiesis, autoimmunity, neurodegenerative diseases and tumorigenesis (Garzon et al., 2006; Lee et al., 2008; Varambally et al., 2008; Yu et al., 2007). One, or both, of the two genomic loci encoding miR-101 were found to be somatically lost in a variety of tumors, which leads to overexpression of histone methyltransferase enhancer of zeste homolog 2 (EZH2) (Varambally et al., 2008). In hepatocellular carcinoma, miR-101 may exert its proapoptotic function via targeting Mcl-1 (Su et al., 2009). Strillacci et al. (2009) demonstrated that miR-101 mediated the direct inhibition of cyclooxygenase-2 (COX-2) mRNA translation in colon cancer cells. In addition, miR-101 is also reported to participate in the regulation of viral infection. Human cytomegalovirus (HCMV) infection negatively regulates miR-100 and miR-101 expression. MiR-101, together with miR100, interacts with components of the mTOR pathway and inhibits HCMV replication (Wang et al., 2008). HCMV selectively suppresses the expression of cellular miRNAs (miR-101) to promote its own replication (Wang et al., 2008). However, in this report, we found that the miR-101-mediated antiviral mechanism is utilized by host cells to defend against HSV-1 infection.

In elucidating the mechanism of miR-101 antiviral activity, ATP5B was identified to be a new target gene of miR-101. Using RNA interference techniques, knockdown of ATP5B could inhibit HSV-1 replication. Furthermore, ectopic expression of ATP5B lacking the 3′UTR overrode the suppressive effect of miR-101 on HSV-1 replication. These results provide further evidence that miR-101 negatively regulates HSV-1 replication by targeting ATP5B.

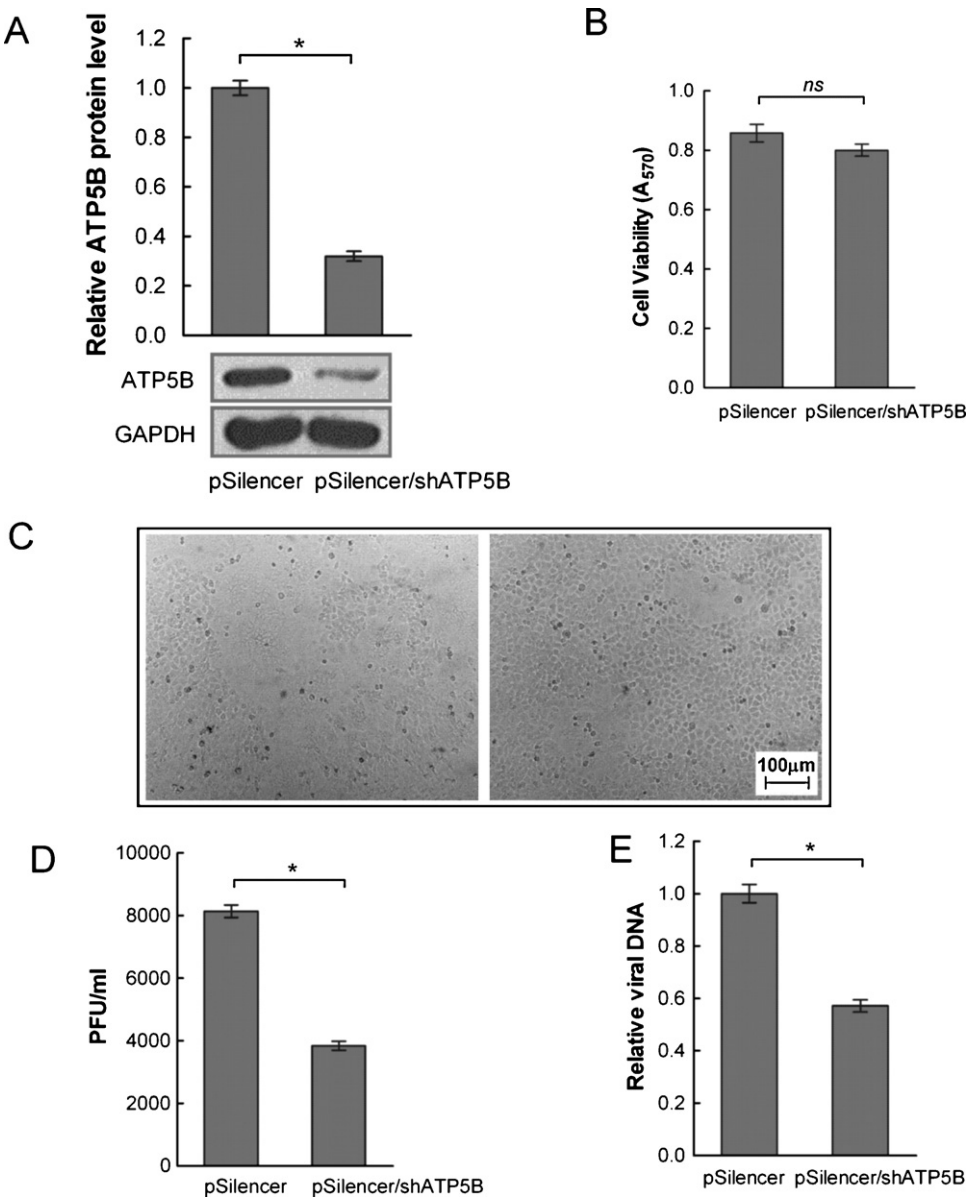

Fig. 4. Knocking down ATP5B suppresses HSV-1 replication. (A) HeLa cells were transfected with either pSliencer/shATP5B or control vector. Western blot was used to detect the expression level of ATP5B. $^{*}P < 0.05$. (B) At 24 h post-transfection, cell viability was tested by MTT. *ns* indicates $P > 0.05$. (C) At 24 h post-transfection, cells were infected with HSV-1 at 0.01 PFU/cell. Individual plaques are shown at a magnification of 400×. (D) Viral yields were determined by standard plaque assays at a 2-day incubation for HSV-1. $^{*}P < 0.05$. (E) DNA was extracted from HSV-1 infected cells. Relative HSV-1 DNA content was detected using real-time PCR. $^{*}P < 0.05$.

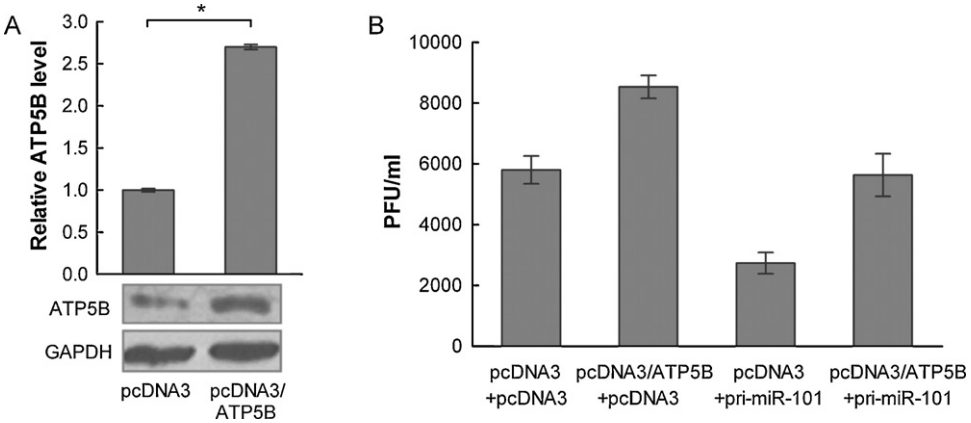

Fig. 5. Transfection of ATP5B cDNA lacking a 3′-UTR overrides the effects of miR-101 on HSV-1 replication. (A) HeLa cells were transfected either pcDNA3/ATP5B or control vector. Western blot was used to detect the expression level of ATP5B. $^{*}P < 0.05$. (B) HeLa cells were transfected with either pcDNA3/ATP5B or control vector, along with pcDNA3/pri-miR-101 or control vector. At 24 h post-transfection, cells were infected with HSV-1 at 0.01 PFU/cell. Viral yields were determined by standard plaque assays at a 2-day incubation for HSV-1.

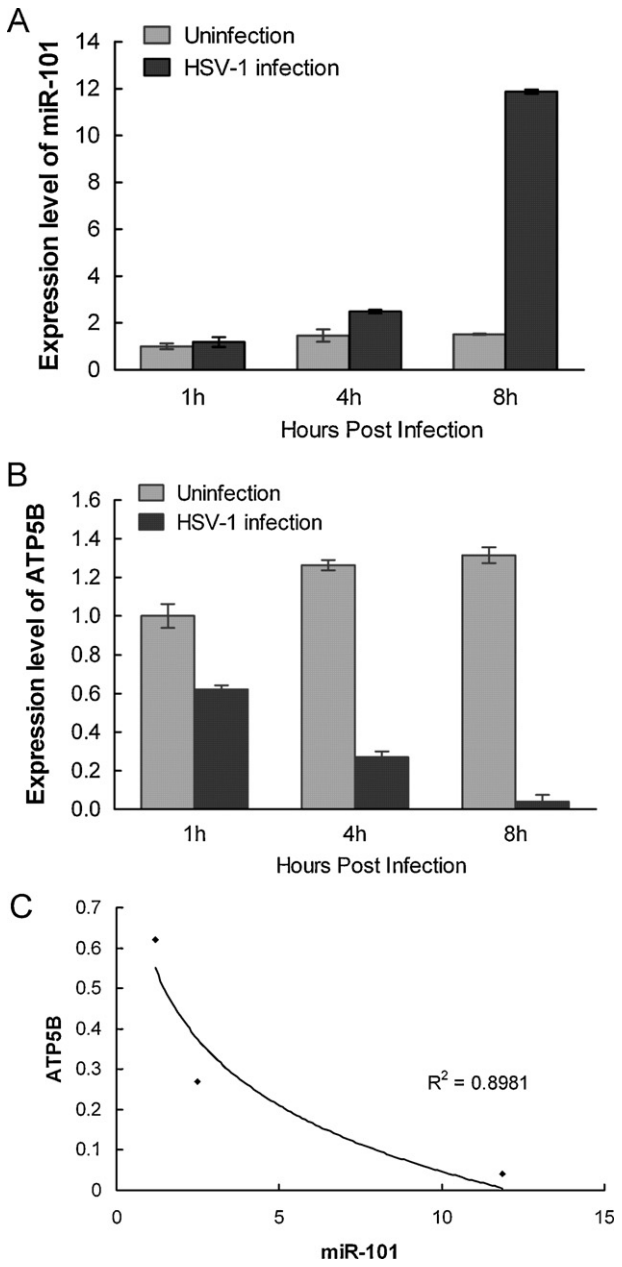

Fig. 6. The effect of HSV-1 infection on the level of miR-101 and ATP5B in HeLa cells. (A and B) HeLa cells were either infected with HSV-1 or left uninfected. Total RNAs were extracted at 1 h, 4 h, and 8 h post-infection. The expression level of miR-101 (A) and ATP5B (B) were detected by real-time RT-PCR. Expression levels of miR-101 were normalized to the small nuclear RNA U6, and ATP5B levels were normalized to 18S rRNA. (C) The coefficients of correlation between miR-101 and ATP5B in HSV-1-infected HeLa cells. The normalized miR-101 expression level is on the abscissa vs. ATP5B levels on the ordinates.

ATPB is a subunit of F1 ATP synthases constitutively expressed in the inner mitochondrial membrane in normal cells and is involved in the regulation of a variety of cellular functions (Maguire et al., 2006). Abnormal expression of ATP5B is found to be involved in cell dysfunction and tumorigenesis. Given that down-regulation of ATP5B is a hallmark of many human tumors, the expression level of ATP5B provides a marker of the prognosis of cancer patients, as well as the tumor response to chemotherapy (López-Ríos et al., 2007; Willers et al., 2010). As yet, there have been no reports of an involvement of ATP5B in the regulation of HSV-1 replication, but some clues indicate that ATP and FoF1 ATP synthase are crucial in supplying the energy that is required for the virus to complete steps in its full replication cycle, such as DNA packaging and capsid maturation (Dasgupta and Wilson, 1999). HSV encoded glycoprotein J (gJ) has been also found to interact with FoF1 ATP synthase subunit 6 and have strong antiapoptotic effects (Aubert et al., 2008). Our results show that ATP5B can promote HSV-1 replication, but characterizing the detailed mechanism remains the subject of further study.

It is well known that HSV-1 can inhibit cellular protein synthesis through the degradation of RNA by the virion host shutoff (Vhs) protein (Smiley et al., 2001). However, the effect of HSV-1 infection on miRNA expression is yet to be understood. Cui et al. (2006) observed a constant level of let-7, a cellular miRNA, during HSV-1 infection. MiR-146a and miR-132 have been found to be highly upregulated after HSV-1 infection (Hill et al., 2009; Lagos et al., 2010). Here, we found that HSV-1 infection could enhance miR-101 expression levels and inversely reduce ATP5B levels, which benefit the host cells by defending against virus infection. Although highly enriching miR-101 resulted in a decline of ATP5B expression, the effect of Vhs protein cannot be excluded.

In conclusion, we provide evidence for an intricate interplay between a cellular miRNA and HSV-1. Host-encoded miR-101 has an antiviral effect on HSV-1 replication via targeting the ATP synthase beta subunit, ATP5B. In HeLa cells, miR-101 expression increases during HSV-1 infection in parallel to a decrease in ATP5B expression. The miR-101/ATP5B axis as a host defense mechanism also may promote long-term HSV-1 latency. The identification of anti-HSV-1 miRNAs and their targets not only enable us to better understand the molecular basis of viral pathogenesis, but also may enable us to develop better therapeutic strategies.

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