



MicroRNA miR-214 Inhibits Snakehead Vesiculovirus Replication by Promoting IFN- α Expression *via* Targeting Host Adenosine 5'-Monophosphate-Activated Protein Kinase

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Background: Snakehead vesiculovirus (SHV), a new rhabdovirus isolated from diseased hybrid snakehead, has emerged as an important pathogen during the past few years in China with great economical losses in snakehead fish cultures. However, little is known about the mechanism of its pathogenicity. MicroRNAs are small noncoding RNAs that posttranscriptionally modulate gene expression and have been indicated to regulate almost all cellular processes. Our previous study has revealed that miR-214 was downregulated upon SHV infection.

Results: The overexpression of miR-214 in striped snakehead (SSN-1) cells inhibited SHV replication and promoted IFN- α expression, while miR-214 inhibitor facilitated SHV replication and reduced IFN- α expression. These findings suggested that miR-214 negatively regulated SHV replication probably through positively regulating IFN- α expression. Further investigation revealed that adenosine 5'-monophosphate-activated protein kinase (AMPK) was a target gene of miR-214. Knockdown of AMPK by siRNA inhibited SHV replication and promoted IFN- α expression, suggesting that cellular AMPK positively regulated SHV replication and negatively regulated IFN- α expression. Moreover, we found that siAMPK-mediated inhibition of SHV replication could be partially restored by miR-214 inhibitor, indicating that miR-214 inhibited SHV replication at least partially *via* targeting AMPK.

Conclusion: The findings of this study complemented our early study, and provide insights for the mechanism of SHV pathogenicity. SHV infection downregulated

miR-214, and in turn, the downregulated miR-214 increased the expression of its target gene AMPK, which promoted SHVV replication via reducing IFN- α expression. It can therefore assume that cellular circumstance with low level of miR-214 is beneficial for SHVV replication and that SHVV evades host antiviral innate immunity through decreasing IFN- α expression *via* regulating cellular miR-214 expression.

Keywords: snakehead vesiculovirus, microRNA, miR-214, interferon, replication, adenosine 5'-monophosphate-activated protein kinase

INTRODUCTION

MicroRNAs (miRNAs) are a class of small (~22 nt) noncoding RNAs that posttranscriptionally degrade and/or suppress translation of target mRNAs through base pairing between the “seed sequences” (2–8 nt at the 5' end) of miRNAs and the target transcripts (1–5). Host miRNAs, typically binding to the 3' untranslated regions (UTRs) of target transcripts (5–10), have been reported to play important roles in the regulation of virus replication (1, 5, 7, 10, 11). Moreover, the regulatory roles of miRNAs in virus replication were even utilized by viruses to promote their replication (6, 9). Therefore, understanding the roles of miRNAs in virus infection is helpful for understanding the mechanisms of virus pathogenesis.

Snakehead vesiculovirus (SHVV) is a fish rhabdovirus isolated from diseased hybrid snakehead in 2014 in China (12). It has caused high mortality to cultured snakehead fish these years. Up to now, the study about the mechanism of its pathogenicity is limited. SHVV belongs to the genus *Perhabdovirus*, family *Rhabdoviridae* (13). Its genome is an ~11 kb negative-sense RNA molecule that encodes five proteins: nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and RNA-dependent RNA polymerase protein (L) (12). Our previous study has revealed that SHVV infection downregulated miR-214 (14), and in turn, miR-214 could inhibit SHVV production by targeting viral N and P (15). However, it is unclear whether miR-214 can regulate SHVV replication *via* targeting host factors that are required for SHVV replication. MiR-214 has recently been observed to be upregulated by *Vibrio harveyi*, and the upregulated miR-214 inhibited the production of inflammatory cytokines by targeting host myd88 (16). Consequently, miR-214 played important roles in regulating pathogens infection.

Adenosine 5'-monophosphate-activated protein kinase (AMPK) is a heterotrimeric serine/threonine kinase (17), which is considered as pivotal regulator of host cellular metabolism *via* sensing cellular energy status (18). When the energy levels in cells decrease, AMPK is activated through phosphorylation by an upstream kinase (18). Activated AMPK thereby downregulates anabolic processes that consume ATP and upregulates catabolic processes that synthesize ATP (18). Given the role of sensing changes of cellular energy status, it is not surprising that AMPK plays an important role in virus infection (19). However, growing evidences have revealed that viruses can modulate the activity of AMPK, and in turn, AMPK affects virus infection by regulating cellular autophagy or innate immunity (20, 21). Here, we reported that AMPK was a target gene of miR-214, negative regulator of

IFN- α expression, and positive regulator of SHVV replication. Moreover, we determined that miR-214 could inhibit SHVV replication by promoting IFN- α expression *via* reducing AMPK expression. This study provided information for understanding the molecular mechanism of SHVV pathogenicity and a potential antiviral strategy against SHVV infection.

MATERIALS AND METHODS

Cells and Viruses

Striped snakehead (SSN)-1 cells were maintained at 25°C in minimum essential medium (MEM) (HyClone, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco, New Zealand), penicillin (100 μ g/ml), and streptomycin (100 μ g/ml). SHVV was isolated from diseased hybrid snakehead fish and stored at -80°C.

Reagents and Antibodies

The miR-214 mimic, miR-214 inhibitor, negative control (NC) mimic, and NC inhibitor were purchased from GenePharma (Shanghai, China). Their sequences were previously described (15). Two siRNAs for AMPK (accession number: MF989224) were synthesized from GenePharma (Shanghai, China). The sequences of the first one were: 5'-CCUCCAGUAUCAAGAUCUUTT-3' (forward) and 5'-AAGAUCUUGAUACUGGAGGTT-3' (reverse); the sequences of the second one were: 5'-GGACACGCCCAUUAUAAATT-3' (forward) and 5'-UUUAAUAAUGGGCGUGU CCTT-3' (reverse).

The antibodies against G protein of SHVV and AMPK were produced and stored in our laboratory. The antibody against β -actin was purchased from Bioss Biotechnology Co., LTD. (Beijing, China). The secondary antibody donkey anti-rabbit IgG antibody was purchased from Gene Co., LTD. (Shanghai, China).

Plasmids

The luciferase reporter plasmid pmirGLO-AMPK was constructed by amplifying the miR-214 target sequence (~200 nt) in the 3' UTR of AMPK and cloning into vector pmirGLO with primers listed in **Table 1**. The plasmids pmirGLO-AMPK-MUT1 and pmirGLO-AMPK-MUT2 were generated by PCR mediated mutations into plasmid pmirGLO-AMPK using primers listed in **Table 1**. The expression plasmid p3XFLAG-CMV-14-AMPK was constructed by amplifying the open reading frame of AMPK gene and cloning into vector p3XFLAG-CMV-14 using primers listed in **Table 1**.

TABLE 1 | Primers used in this study.

Application	Primer	Sequence (5'-3')
qRT-PCR	SHVV-G-FW	ACACCATACATGCCAGAGGC
	SHVV-G-BW	GCCTCGCTGGGTATCCAAT
	AMPK-FW	GCAGGAAGGAGGATAGAA
	AMPK-BW	GCAACTGAGCCCGTAAAA
	IFN- α -FW	TGTACCTCGGCCTTCTCGAT
	IFN- α -BW	CGAAGCCTGCAACTGGATGA
	β -actin-FW	CACTGTGCCCATCTACGAG
	β -actin-BW	CCATCTCCTGCTCGAAGTC
	miR-214-F	CGGACAGCAGGCACAGACAGGCAAAAA
5SrRNA-F	GGAGACCGCCTGGGAATA	
Reporter plasmids	AMPK-FW	CTAGCTAGC GTTGCTGCTCCGTTTC
	AMPK-BW	GCTCTAGA CGAGTCCTTCTCACCC
	AMPK-MUT1-FW	AGTCGTCTAAGGCGGAAAGGACGACAAATTTAAGGCAGAAA
	AMPK-MUT1-BW	TC GTCGTCCTTTCCGCTTAGACGACG
	AMPK-MUT2-FW	TTAAATTTTTTATAGATGTTTCAAACATCGGACGACAAAAA
	AMPK-MUT2-BW	AACTAAATATAGTCGGTCTCGGATGTTGAAACCTTTTAA
Expression plasmid	AMPK-FW-1	GGGGTACCAATGGGCAGCACGGCGGC
	AMPK-BW-1	GCTCTAGA CGTCTCTCTCTGCCTTCTTTT

Transfection

The mimics, inhibitors, or plasmids were incubated with TransIntro™ EL Transfection Reagent (TransGen Biotech, China) in 500 μ l Opti-MEM medium (Invitrogen, USA) for 30 min at room temperature. The incubated samples were then put onto the SSN-1 cells. At 6 h post of transfection, the medium was replaced by 1 ml of MEM and continued incubation at 25°C.

Dual-Luciferase Reporter Assay

The dual-luciferase reporter assay was performed as described previously (15). In brief, SSN-1 cells were co-transfected with NC mimic, miR-214 mimic, NC inhibitor, or miR-214 inhibitor, together with the luciferase reporter plasmids using TransIntro™ EL Transfection Reagent (TransGen Biotech, China). At 24 h post of transfection, the *Renilla* and firefly luciferase activities were measured, and the data were expressed as relative firefly luciferase activity normalized to *Renilla* luciferase activity.

Virus Infection and Titration

Virus infection and titration experiments were performed as previously described (15). In brief, SSN-1 cells were incubated with SHVV for 2 h, the inoculum was then removed and the cells were washed twice with PBS followed by adding MEM medium with 5% FBS. At 24 h post of infection (poi), the supernatants were collected for virus titration by 50% tissue culture infectious dose (TCID₅₀), and the cells were harvested for the detection of viral mRNA or host miR-214 by qRT-PCR with primers listed in Table 1.

Quantitative RT-PCR of Viral mRNA, Host IFN- α mRNA, and miR-214

Total RNAs were extracted from cells with TRIzol reagent (Invitrogen) according to manufacturer's instructions. The detection of viral mRNA, host IFN- α (accession number: MF989225) mRNA, and miR-214 was performed by qRT-PCR as previously

described (15). Two sets of data were normalized using the 2^{- $\Delta\Delta$ Ct} method. For the detection of viral mRNA, data was normalized to the level of β -actin in each sample, while for the miR-214 detection, the expression level of miR-214 was calculated after normalization to 5S rRNA.

Western Blotting

Western blotting was performed as previously described (15). In brief, the extracted proteins were transferred onto a nitrocellulose membrane (Biosharp, China), which were blocked with 5% skim milk in tris-buffered saline with tween 20 (TBST) at 4°C overnight, followed by incubation with the primary antibody of SHVV protein (1:1,000) or β -actin (1:1,000) for 2 h at room temperature. The membranes were then washed three times with TBST and then incubated with IRDye 800CW conjugated donkey anti-rabbit antibody (1:10,000) for 1 h at room temperature. The signal intensity was then determined using Odyssey CLx (LI-COR, USA).

Statistical Analysis

All statistical analyses were performed using Graphpad Prism 5.0 (GraphPad Software, CA, USA). The statistical significance of the data was determined by Student's *t* test, and *P* < 0.05 was considered statistically significant. For data sets in which multiple comparisons were being made, the Student's *t*-test was corrected by using false discovery rate.

RESULTS

The Effect of miR-214 on the Transcription, Translation, and Production of SHVV

MiR-214 has been indicated to inhibit the replication of several human and mammalian viruses, including human cytomegalovirus (HCMV), murine cytomegalovirus (MCMV), and herpes

simplex virus 1 (HSV-1) (22). In the same vein, our previous study has suggested that miR-214 inhibited SHVV replication (15). In this study, we further evaluated the effect of miR-214 on the transcription, translation, and production of SHVV at different time point poi. SSN-1 cells were transfected with miR-214 mimic, NC mimic, miR-214 inhibitor, or NC inhibitor, followed by SHVV infection. At 3, 12, and 24 h poi, the cells and supernatants were collected in order to detect viral G mRNA, G protein, and viral titer by qRT-PCR, western blot, and TCID₅₀, respectively. As shown in **Figure 1**, viral G mRNA expression was not significantly altered at 3 h poi. However, at 12 and 24 h poi, overexpression of miR-214 significantly reduced, whereas miR-214 inhibitor increased, G mRNA level (**Figures 1A,D**). The expression of viral G protein was under detection at 3 and 12 h poi. At 24 h poi, it was apparent from **Figures 1B,E** that G protein expression was decreased by about 50% or increased to about 2.5-fold when the cells were transfected with miR-214 mimic or miR-214 inhibitor, respectively. Similar to G mRNA and G protein, the viral titers were reduced by transfection of miR-214 mimic and increased by transfection of miR-214 inhibitor (**Figures 1C,F**). Taken together, these findings demonstrate that miR-214 inhibits SHVV replication.

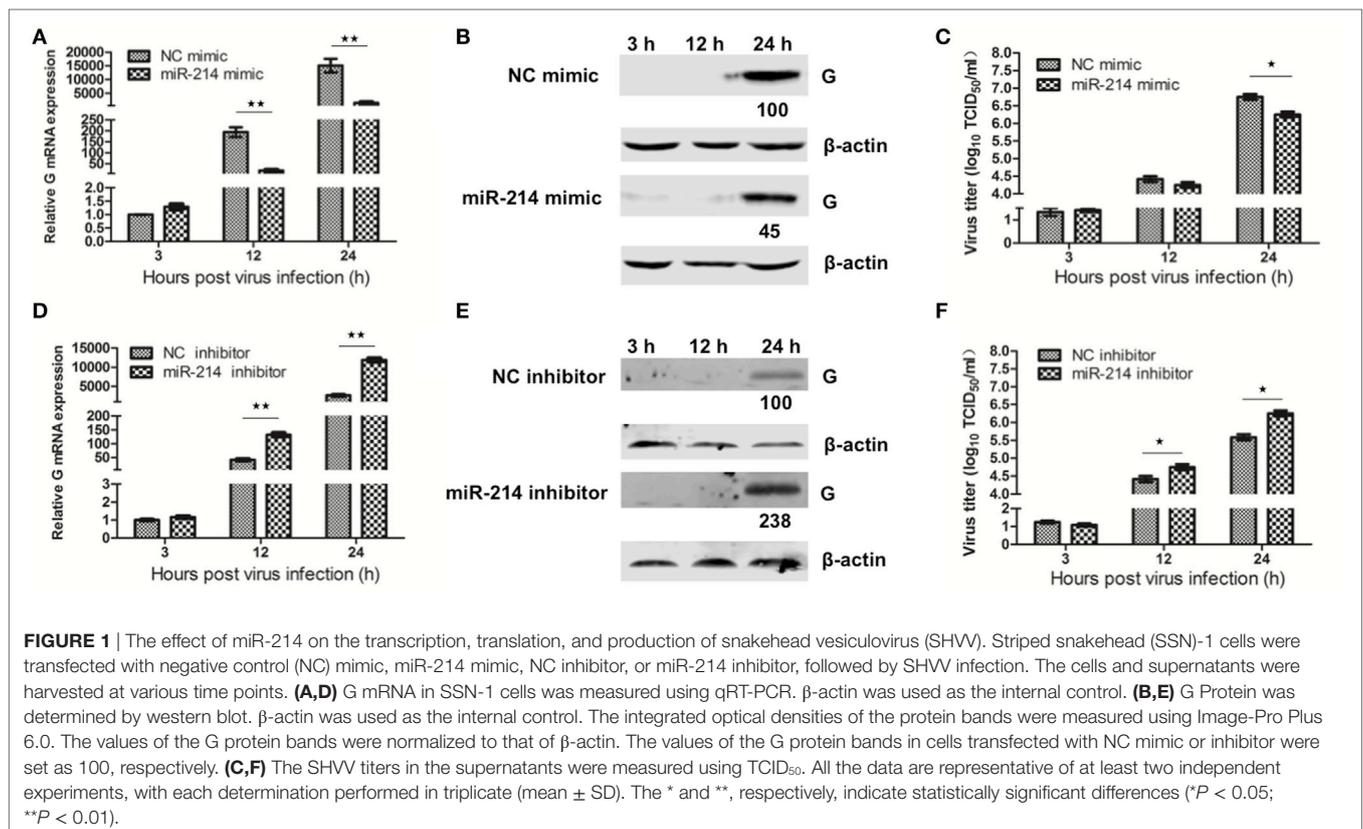
miR-214 Promotes IFN- α Expression

Our previous study has revealed that miR-214 promoted IFN- α expression during SHVV infection by targeting SHVV P protein, an IFN- α antagonist (15). In this study, we further investigated

the role of miR-214 in IFN- α expression at different time point post of SHVV infection. SSN-1 cells were transfected with miR-214 mimic, NC mimic, miR-214 inhibitor, or NC inhibitor, followed by SHVV infection. At 3, 12, and 24 h poi, IFN- α mRNA was detected using qRT-PCR. As shown in **Figures 2A,B**, overexpression of miR-214 significantly increased, whereas miR-214 inhibitor reduced, IFN- α mRNA at 12 and 24 h poi. Moreover, we found that overexpression of miR-214 inhibited SHVV replication and increased IFN- α mRNA in a dose-dependent manner (**Figure 2C**). In addition, we found that overexpression of miR-214 promoted poly (I:C)-induced IFN- α mRNA (Figure S1 in Supplementary Material). Our data suggest that the promotion of IFN- α expression by miR-214 could be the cause of its inhibition of SHVV replication.

miR-214 Targets the 3' UTR of AMPK mRNA

In addition to targeting viral P gene, it's speculated that miR-214-mediated inhibition of SHVV replication and promotion of IFN- α expression could also be caused by targeting host genes. To determine host target genes of miR-214, high throughput transcriptomic sequencing of SSN-1 cells transfected with miR-214 mimic or NC mimic has been performed. The results showed that overexpression of miR-214 resulted in 1,301 upregulated genes and 1,613 downregulated genes (data not shown). Based on association possibility with virus replication, six downregulated genes were selected for further validation



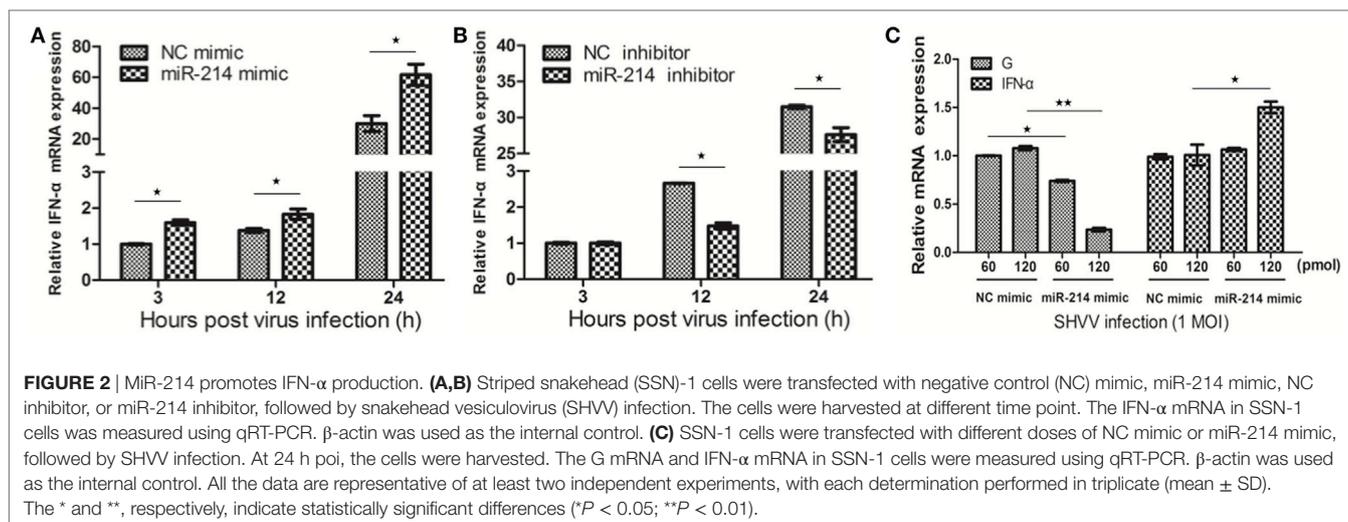


TABLE 2 | Six downregulated genes from transcriptomic sequencing data.

Gene	Signaling pathway	Fold change	P value
AMPK	AMPK signaling pathway	0.72	0.049
STAT	STAT signaling pathway	0.87	0.047
MMP9	MTOR signaling pathway	0.78	0.003
EIF4E	TNF signaling pathway	0.79	0.020
NLK	FOXO signaling pathway	0.81	0.048
STAT3	HIF-1 signaling pathway	0.86	0.047

AMPK, adenosine 5'-monophosphate (AMP)-activated protein kinase; STAT, signal transducers and activators of transcription; MMP9, matrix metalloproteinase 9; EIF4E, eukaryotic translation initiation factor 4E; NLK, nemo-like kinase; STAT3, signal transducer and activator of transcription 3.

using qRT-PCR as follows (Table 2): signal transducers and activators of transcription (STAT), matrix metalloproteinase 9 (MMP9), eukaryotic translation initiation factor 4E (EIF4E), nemo Like Kinase (NLK), signal transducer and activator of transcription 3 (STAT3), and AMPK. Among them, AMPK was the most downregulated gene (Figure 3A). To further determine the effect of miR-214 on AMPK expression, SSN-1 cells were transfected with 60 and 120 pmol of miR-214 mimic, NC mimic, miR-214 inhibitor, or NC inhibitor, followed by the detection of cellular AMPK protein using specific AMPK antibody (Figure S2 in Supplementary Material). We found that transfection of 120 pmol of miR-214 mimic significantly reduced, while miR-214 inhibitor increased, cellular AMPK protein expression (Figure 3B), suggesting that AMPK was probably a target gene of miR-214.

Using Miranda software, two putative binding sites of miR-214 were identified at the 3' UTR of AMPK mRNA (Figure 3C). To further confirm whether AMPK was the target gene of miR-214, we first constructed a dual-luciferase reporter plasmid pmirGLO-AMPK containing the wild-type sequence of the 3' UTR of AMPK. Based on the plasmid pmirGLO-AMPK, we generated two mutant plasmids pmirGLO-AMPK-MUT1 and pmirGLO-AMPK-MUT2, in which the miR-214-targeted sequences were mutated (Figure 3C). These plasmids were subsequently transfected into SSN-1 cells with miR-214 mimic, NC mimic, miR-214 inhibitor, or NC inhibitor, respectively.

Significant reduction in luciferase activity was observed in cells co-transfected with miR-214 mimic and the plasmid with wild-type AMPK 3' UTR, whereas significantly increased luciferase activity was detected when transfected with miR-214 inhibitor (Figure 3D). However, the luciferase activity was not significantly altered when miR-214 mimic or inhibitor was co-transfected with the mutant plasmids harboring miR-214 seed-region mutated sequences (Figure 3D). These results indicate that AMPK is a target gene of miR-214.

SHVV Infection Upregulates AMPK

To study the effect of SHVV infection on AMPK, SSN-1 cells were infected with SHVV and the cells were harvested at 3, 12, and 24 h poi. The G mRNA, AMPK mRNA, and miR-214 were determined by qRT-PCR. Along with the increase of G mRNA, miR-214 was steadily decreased at 12 and 24 h poi (Figure 4). This result was consistent with our previous study, in which SHVV infection downregulated miR-214 (14). In addition, AMPK mRNA was significantly increased at 12 and 24 h poi (Figure 4), suggesting that SHVV infection upregulated AMPK possibly via downregulating miR-214.

Knockdown of AMPK Inhibits SHVV Replication and Promotes IFN- α Expression

In order to understand the role of AMPK in SHVV infection, SSN-1 cells were transfected with siAMPK or siNC, followed by SHVV infection. Transfection of siAMPK significantly reduced the mRNA and protein levels of AMPK compared to that transfected with siNC (Figures 5A,B). The effect of AMPK on SHVV replication was further evaluated by detecting viral G mRNA, G protein, and viral titer at 24 h poi. As shown in Figures 5C,D, the viral G mRNA and protein were reduced to less than 10% in siAMPK transfected cells than in siNC transfected cells. Similarly, the viral titer was decreased more than 10-fold in siAMPK group than in siNC group (Figure 5E). It can thus be suggested that knockdown of AMPK, similar to the overexpression of miR-214, inhibited SHVV replication.

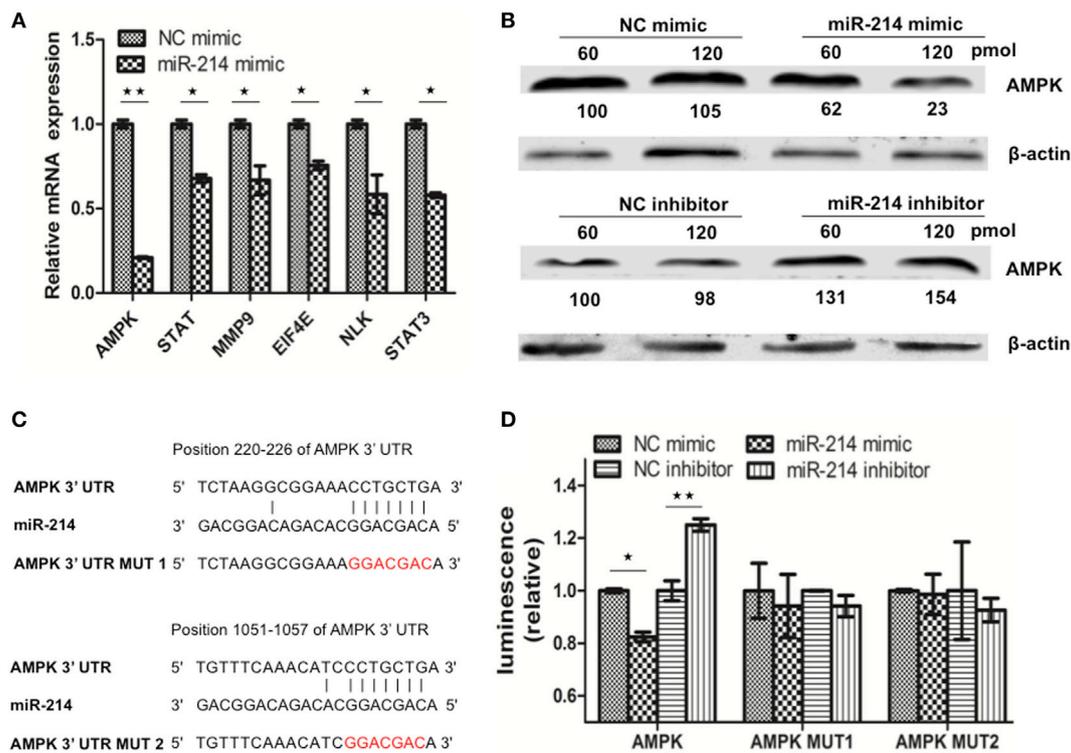


FIGURE 3 | miR-214 targets to the 3' UTR of adenosine 5'-monophosphate-activated protein kinase (AMPK) mRNA. **(A)** qRT-PCR was used to evaluate the expression of six candidate target genes of miR-214. **(B)** Striped snakehead (SSN)-1 cells were transfected with different doses of negative control (NC) mimic, miR-214 mimic, NC inhibitor, or miR-214 inhibitor. At 24 h post of transfection, AMPK protein was determined by western blot. β -actin was used as the internal control. The integrated optical densities of the protein bands were measured using Image-Pro Plus 6.0. The values of the AMPK protein bands were normalized to that of β -actin. The values of the AMPK protein bands in cells transfected with 60 pmol of NC mimic or inhibitor were set as 100, respectively **(C)** Alignment of miR-214 with the predicted target sequences in the 3' UTR of AMPK mRNA. **(D)** SSN-1 cells were transfected with pmirGLO-AMPK, pmirGLO-AMPK-MUT1, or pmirGLO-AMPK-MUT2, together with NC mimic, miR-214 mimic, NC inhibitor, or miR-214 inhibitor. Luciferase activity was measured at 24 h post of transfection, and the data were expressed as relative firefly luciferase activity normalized to Renilla luciferase activity. All the data are representative of at least two independent experiments, with each determination performed in triplicate (mean \pm SD). The * and **, respectively, indicate statistically significant differences (* P < 0.05; ** P < 0.01).

In addition to affecting SHVV replication, siAMPK increased IFN- α mRNA about 10-fold (Figure S3A in Supplementary Material). To further confirm the effects of AMPK on IFN- α expression, SSN-1 cells were transfected with plasmid p3XFLAG-CMV-14 or p3XFLAG-CMV-14-AMPK, followed by SHVV infection. At 24 h poi, the cellular IFN- α mRNA was measured by qRT-PCR. The results showed that overexpression of AMPK reduced IFN- α mRNA level (Figure S3B,C in Supplementary Material). These findings suggest that AMPK negatively regulates IFN- α expression.

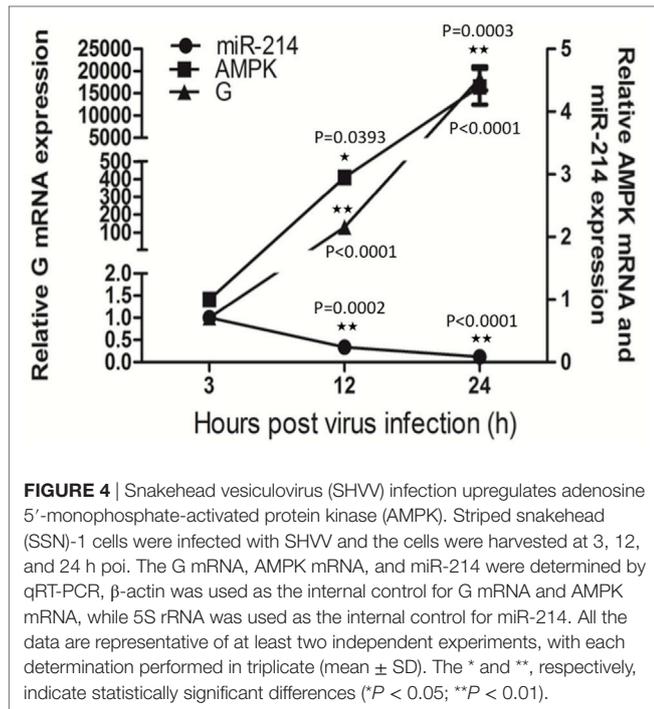
Suppression of Cellular miR-214 Can Restore siAMPK-Mediated Inhibition of SHVV Replication

In order to figure out whether miR-214-mediated inhibition of SHVV replication was caused by targeting AMPK, SSN-1 cells were transfected with siNC, siAMPK, or siAMPK with miR-214 inhibitor, followed by SHVV infection. The cells and supernatants were collected at 24 h poi. The viral G protein and viral titer were determined. As shown in Figure 6A, siAMPK reduced G protein level to about 1% compared to that in siNC group. However,

addition of miR-214 inhibitor restored the G protein level to 15%. Similarly, the viral titer was significantly decreased by siAMPK, which was partially restored by the transfection with miR-214 inhibitor (Figure 6B). Overall, these findings indicate that miR-214 inhibits SHVV replication at least partially due to its targeting AMPK.

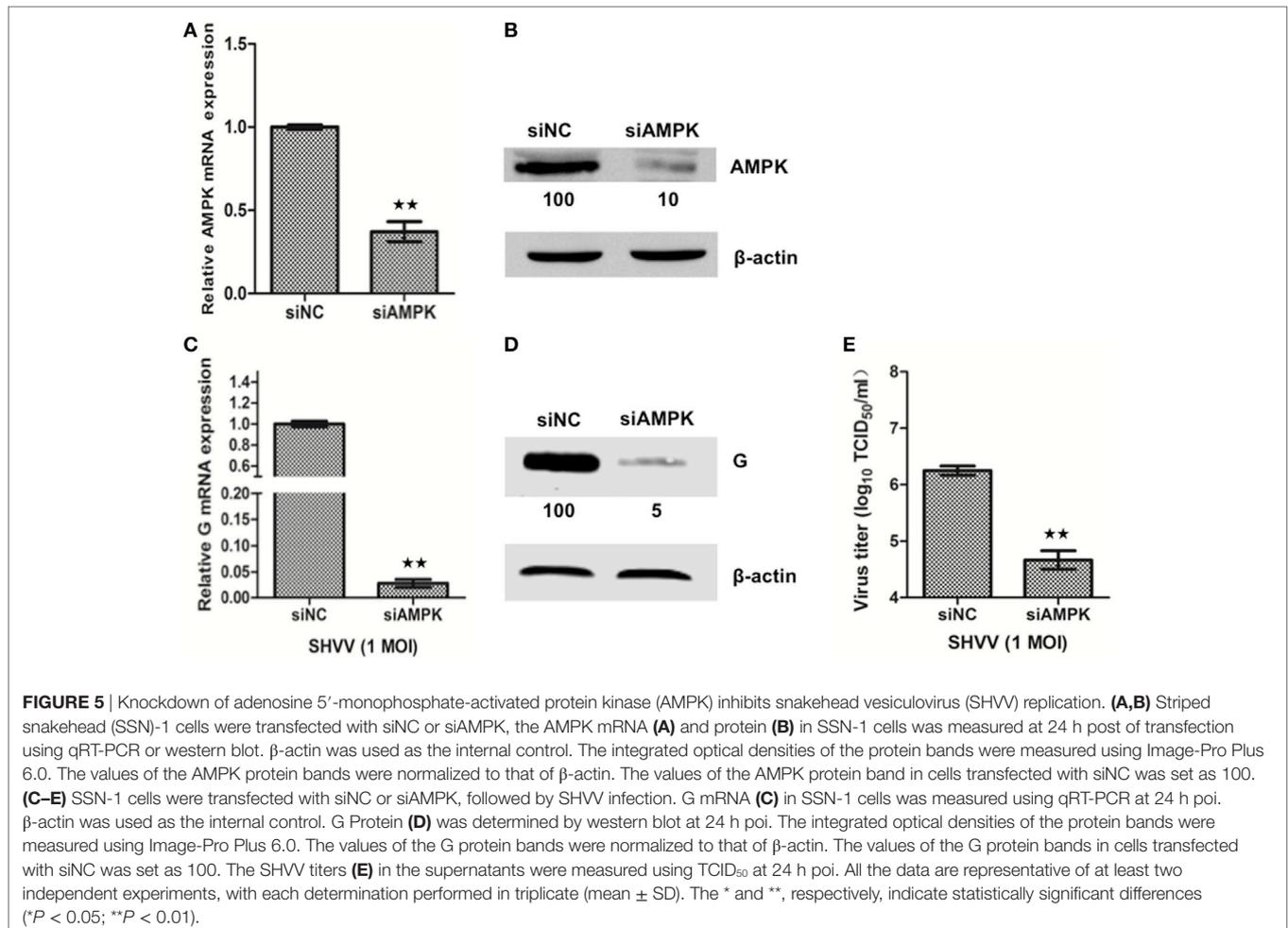
DISCUSSION

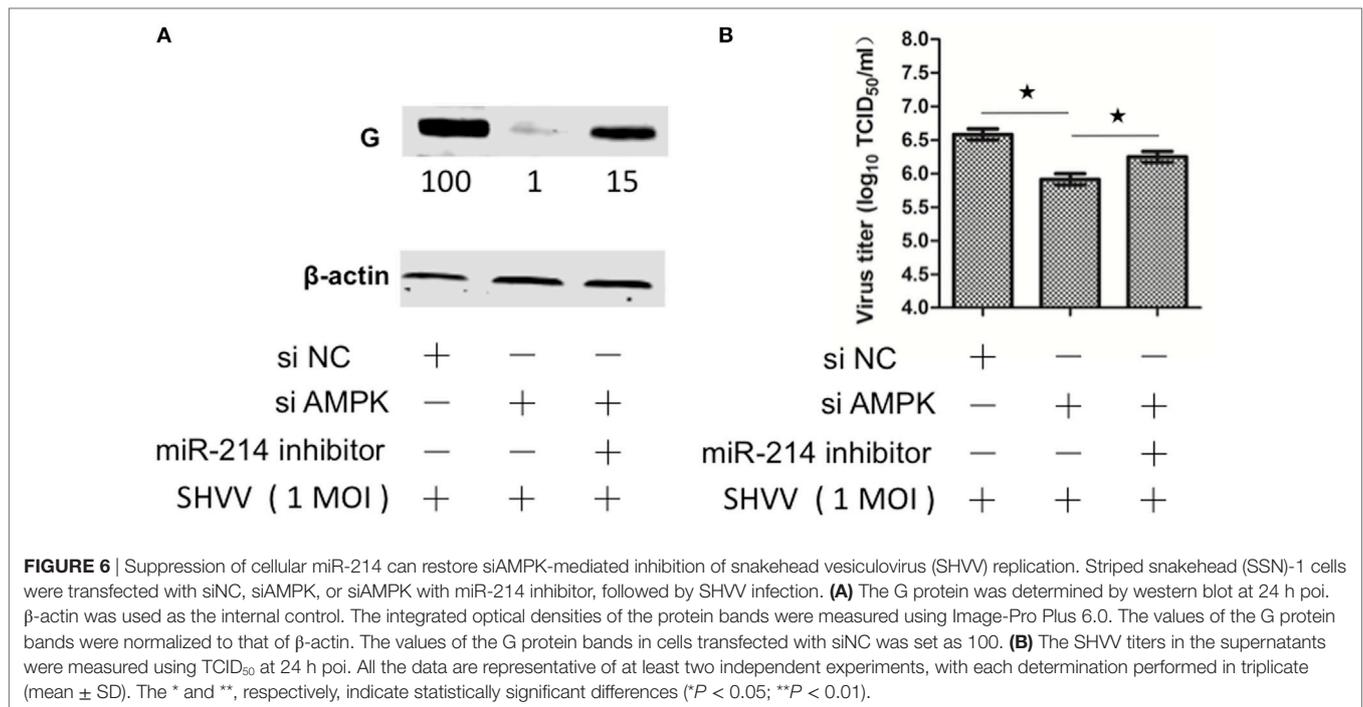
Host miRNA has emerged as both responsive factor and modulator of virus infection. In detail, host miRNA expression is commonly altered in response to virus infection, and the altered miRNA in turn modulate virus infection (1, 6, 9–11, 22). Santhakumar et al. have revealed that miR-214 was downregulated upon several human and mammalian viruses infection, and in turn, miR-214 inhibited the replication of these viruses, suggesting that miR-214 acted as a broad antiviral miRNA (22). Our previous study has revealed that miR-214 was downregulated upon a fish rhabdovirus SHVV infection (14), and overexpression of miR-214 inhibited SHVV replication *via* targeting N and P genes of SHVV (15). As the genomes of these miR-214-inhibited viruses share little sequence similarity, it is speculated that miR-214 might target



host factors that are required by multiple viruses. Thereby, the aim of this study is to identify miR-214-targeted host factors and the related antiviral mechanism.

The mechanisms of miRNA-mediated regulation of virus replication have attracted much attention these years. Growing evidences have demonstrated that miRNAs could inhibit virus replication by targeting host factors that were critically important for virus replication (23–32). For example, host eukaryotic translation elongation factor 1A1 (EEF1A1) can interact with NS3 and NS5 proteins of Japanese encephalitis (JEV) to form a complex that is essential for JEV replication, miR-33a can target EEF1A1 and reduce its expression, thus suppressing JEV replication (31). In addition, miRNAs can also target host factors that positively or negatively regulate type I interferon expression or the following signaling (6, 9, 33–44). In the current study, we found that miR-214, the important cancer development regulator (45, 46), inhibited SHVV replication by regulating host IFN- α expression (15) (Figure 2). Many miRNAs have been identified as type I interferon regulators, including miR-373 (6), miR-4661 (34), miR-155 (36), miR-15b (38), miR-526a (39), miR-223 (41), and miR-146a (42–44). Here, miR-214 was identified as a novel type I interferon regulator.





Adenosine 5'-monophosphate-activated protein kinase has been extensively studied as a pivotal regulator of cellular energy metabolism (47). Recent studies have revealed that AMPK was involved in the regulation of virus replication (18). Inhibition of AMPK severely attenuated HCMV replication, suggesting that AMPK was required for HCMV replication (48, 49). In the current study, knockdown of AMPK inhibited SHVV replication, suggesting that AMPK was beneficial for SHVV replication (Figure 5). However, activation of AMPK has been reported to restrict hepatitis B virus (HBV) production, suggesting that AMPK was disadvantageous for HBV replication (50). Therefore, AMPK played different roles in different viruses infection. Although our study demonstrated that miR-214-mediated inhibition of SHVV replication was at least partially due to targeting its target gene AMPK, the broad antiviral property of miR-214 was probably not caused by its targeting AMPK because AMPK not only promoted but also restricted some viruses replication (50). In the current study, in addition to AMPK, five other host factors, including STAT, MMP9, EIF4E, NLK, and STAT3, have also been identified as potential target genes of miR-214 (Figure 3A). Therefore, identifying the host target gene of miR-214 that was responsible for the broad antiviral property of miR-214 needed to be investigated further.

Recently, AMPK has been indicated to regulate type I interferon expression. Inhibition of AMPK was observed to suppress IFN-β induction (51). In the current study, knockdown of AMPK promoted, whereas overexpression of AMPK inhibited, IFN-α expression (Figure S3 in Supplementary Material). Moreover, our study revealed that knockdown of AMPK promoted poly (I:C)-induced IFN-α expression (Figure S4 in Supplementary Material). These findings suggested that AMPK could regulate

type I interferon expression. Our previous study has indicated that viral P protein of SHVV inhibited IFN-α expression, and miR-214 could target the P gene and thus suppressed P-mediated inhibition of IFN-α expression (15). As AMPK was also identified as a target gene of miR-214, It's speculated that miR-214-mediated regulation of IFN-α expression might also be due to targeting AMPK. Taken together, our studies suggested that miR-214 promoted IFN-α expression by targeting not only viral P gene but also host AMPK gene. Despite these promising results, further studies are needed to investigate how P and AMPK regulated host IFN-α expression.

AUTHOR CONTRIBUTIONS

JT and LL designed the research. CZ, SF, WZ, NC, AH, WC, and LZ performed the experiments, contributed to the data collection and statistical analysis. JT, LL, XL, and JL finalized the paper writing.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at <http://www.frontiersin.org/articles/10.3389/fimmu.2017.01775/full#supplementary-material>.

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