Molecular Immunology 109 (2019) 134–139

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Over expression of bmo-miR-2819 suppresses BmNPV replication by regulating the BmNPV *ie-1* gene in *Bombyx mori*

Ping Wu*, Qi Shang, Owusu Amanfo Dweteh, Haoling Huang, Shaolun Zhang, Jinbo Zhong, Qirui Hou, Xijie Guo*

Sericultural Research Institute, Jiangsu University of Science and Technology, Zhenjiang, Jiangsu, 212018, China

ARTICLE INFO

Keywords:
Silkworm
BmNPV
miR-2819
Host-pathogen interaction
Down-regulation

ABSTRACT

Bombyx mori nucleopolyhedrovirus (BmNPV) is a major pathogen that threatens the growth and sustainability of the sericulture industry. Accumulating studies in recent years suggest that insect viruses infection can change the host microRNAs (miRNAs) expression profile and both cellular and viral miRNAs play roles in host-pathogen interactions. Until now, the functional analysis of miRNA encoded by silkworm for host-virus interaction is limited. In this study, we validate the down-regulation of bmo-miR-2819 upon BmNPV infection by qRT-PCR and confirm the BmNPV immediately early 1 gene, *ie-1* is one of the targets for bmo-miR-2819 based on the results of dual luciferase report assay. Overexpression of bmo-miR-2819 can significantly decline the abundance of IE-1 protein level in BmNPV-infected silkworm larvae. Further, the expression level of polyhedrin gene and VP39 protein of BmNPV in the infected larvae after applying bmo-miR-2819 mimics was significantly decreased comparing with that of larvae with mimic control. Our results suggest that overexpression of bmo-miR-2819 could suppress BmNPV replication by down-regulating the expression of BmNPV *ie-1* gene, which demonstrate that cellular miRNAs could affect virus infection by regulating the expression of virus genes.

1. Introduction

MiRNAs are endogenous small noncoding RNAs, which are now known to be common modulators or fine-tuners of gene expression. MiRNAs can interact with multiple target genes and play roles in various biological processes such as development, metabolism, immune response, detoxification pathway and insecticide resistance (Jain et al., 2015; Lei et al., 2015; Hong et al., 2014; Seong et al., 2018; Lampe and Levashina, 2017)

BmNPV is a circular double-stranded DNA (dsDNA) virus with a DNA genome of 128 kb and belongs to the family Baculoviridae (Rahman and Gopinathan, 2004). It is a major pathogen that threatens the growth and sustainability of the sericulture industry.

The process of virus infection of insects regulates and is regulated by a complex interplay of biomolecules including miRNA (Monsanto-Hearne and Johnson, 2018). Accumulating studies in recent years suggest that miRNAs play crucial roles in host-pathogen interactions (Zhang et al., 2013; Maharaj et al., 2015; Dennison et al., 2015; Cirimotich et al., 2010). Viral miRNAs may regulate virus replication by changing the expression of host or virus gene in *Bombyx mori*. To date, only four BmNPV encoded miRNAs (BmNPV-miR-1, BmNPV-miR-2, BmNPV-miR-3, and BmNPV-miR-4) are confirmed by experiment (Singh et al., 2010), among which, BmNPV-miR-1 was reported to suppress its host miRNA biogenesis by regulating the exportin-5 cofactor Ran, which in turn resulted in enhancing viral proliferation (Singh et al., 2012). BmNPV-miR-3 facilitates BmNPV infection by modulating the expression of viral P6.9 and other late genes (Singh et al., 2014).

On the contrary, host miRNAs also can directly alter the virus replication. Bmo-miR-390 can effectively down-regulate the expression of BmNPV-cg30 in BmNPV-infected BmN cells (Kang et al., 2018). Bmo-miR-274-3p can facilitate BmCPV replication by up-regulating BmCPV NS5 gene expression (Wu et al., 2017). Bmo-miR-278-3p can negatively regulate *Bombyx mori* IBP2 gene, which is highly induced upon BmCPV infection (Wu et al., 2015).

The roles of miRNAs in virus infection of animal and plant hosts are already extensive(Cullen, 2013; Guo and Steitz, 2014; Trobaugh and Klimstra, 2017; Bruscella et al., 2017; Liu et al., 2017), while studies on

Abbreviations: BmNPV, Bombyx mori nucleopolyhedrovirus; miRNAs, microRNAs; dsDNA, double-stranded DNA; qRT-PCR, quantitative real-time PCR; mfe, the minimum free energy

* Corresponding authors.
E-mail addresses: wp4114@163.com (P. Wu), guoxijie@126.com (X. Guo).

https://doi.org/10.1016/j.molimm.2019.03.013
Received 12 February 2019; Received in revised form 22 March 2019; Accepted 26 March 2019
Available online 01 April 2019

the functional roles of miRNA in silkworm-virus interaction have been comparatively seldom. The possible involvement of miRNAs in the post-transcriptional regulation of genes associated with BmNPV infection in the *Bombyx mori* remains poorly understood.

In this study, we carried out a functional analysis of bmo-miR-2819 and found that bmo-miR-2819 regulates BmNPV replication by interacting with BmNPV *ie-1* gene. Our finding may provide insights into the understanding of the interaction mechanism between silkworm and BmNPV infection.

2. Materials and methods

2.1. Cells, viruses and silkworm variety

The *Bombyx mori* cell line BmN was cultured at 27℃ in TC-100 medium (United States Biological, USA) supplemented with 10% (V/V) fetal bovine serum (FBS) (Gibco, USA), 100 μg/ml of penicillin and 30 μg/ml of streptomycin. Recombinant BmNPV BVs containing an EGFP marker gene was a gift from Dr. Xudong Tang, Jiangsu University of Science and Technology. Viruses were cultured in BmN cells, and viral titers were determined by 50% tissue culture infective doses (TCID50) assay. BmN cells were infected with BmNPV BVs at a MOI of 5 $TCID_{50}$/cell.

The P50 silkworm strain was utilized in this study. The larvae were reared with fresh mulberry leaves at 25℃, 75% relative humidity up to the fourth molting for virus inoculation. Each larva was inoculated with 2 μL BmNPV viral stock. The control larvae were treated with the same amount of 0.9% NaC1 solution.

2.2. RNA and DNA extraction

Total RNA was isolated from BmN cells and different tissues of silkworm larvae by using Trizol reagent (Invitrogen, USA) according to the manufacturer's protocol and quantified by spectrophotometer before the further experiments. Total DNA was extracted from the silkworm midguts in the different treated groups using a Wizard Genomic DNA extraction kit (Promega).

2.3. Stem-loop RT-PCR

Specific stem-loop primer for miR-2819 was designed based on the method of Chen (Chen et al., 2005) and the sequences were listed in Table 1. RT-PCR was performed under the following conditions: denaturation for 2 min at 94 ˚C, 30 cycles of 94 ˚C for 40 s, 58 ˚C for 40 s and 72 ˚C for 40 s, and then 72 ˚C for 5 min. The PCR products were analyzed on 2% agarose gels.

2.4. MiR-2819 target gene prediction

RNAHybrid (Rehmsmeier et al., 2004) was used in combination with NCBI BLAST (http://www.ncbi.nih.gov/BLAST) to predict the potential target genes of miR-2819 in BmNPV genome. Seed region (2–8 nt from the 5′ end of miRNA) complementarity and minimum free energy (mfe) of −20 kcal/mol were taken as the screening criteria.

2.5. Target validation by dual luciferase report assay

Dual luciferase report assay was performed as our previous study (Wu et al., 2015, 2017). Simply, *ie-1* 3′UTR with miR-2819 potential target-binding sequences and mutated 3′UTR sequences were cloned downstream of firefly luciferase in the PmirGLO vector (Promega) to construct PmirGLO-ie-1, PmirGLO-ie-1-mut vectors. 293 cells were cultured in antibiotic-free PRMI 1640 media (Life Technology, USA) to a confluency of 80% for further transfection. 100 nM miR-2819 mimics/mimic controls (negative control, NC) and 500 ng PmirGLO-ie-1/PmirGLO-ie-1-mut vectors were co-transfected with the cells. Cells were harvested 48 h after transfection and analyzed for Firefly and Renilla luciferase activities using the Dual-Luciferase Reporter Assay System (Promega). Activities were normalized to Renilla luciferase. Three independent experiments were performed in triplicate. The sequences of the mimic and mimic control were listed in Table 1.

2.6. Overexpression and inhibition of miR-2819 in silkworm larvae

The miR-2819 mimic and inhibitor were synthesized and modified by GenePharma (Shanghai, China) with methylation at 3′−OH of oligo. The microinjecting process of miR-2819 mimics and inhibitors into silkworm larvae (5th instar, 1nd day) was described as our previous study(Wu et al., 2015). After 72 h of infection, the mimic or inhibitor injected and control midguts were collected and quickly stored in liquid nitrogen for further experiments. 5 larvae were pooled as 1 sample. Three independent experiments were carried out.

2.7. Quantitative real-time PCR analysis

For detecting the relative expression of miR-2819 in different groups, relative quantitative real-time PCR (qRT-PCR) was performed as descried in our previous study (Wu et al., 2015). As to polyhedrin gene copies, absolute qRT-PCR was carried out. In brief, the positive recombinant plasmid containing polyhedrin gene fragment (previous constructed in our lab) was diluted to 1.0×10^7,1.0×10^6,1.0×10^5,1.0×10^4,1.0×10^3 copies /μL to form gradient standard plasmid used for absolute qRT-PCR. RNA extracted from miR-2819 mimic and mimic control injected midguts was

Table 1
the sequences of primers.

Primers/Mimics/Inhibitors	Sequences
miR-2819 mimic	5'-UCAAUGCCUGCUCUAUCGGUUC -3'
mimic control	5'-ACCGAUAGAGCAGGCAUUGAUU -3'
miR-2819 inhibitor	5'-UUCUCCGAACGUGUCACGUTT-3'
Inhibitor control	5'-ACGUGACACGUUCGGAGAATT-3'
miR-2819 (Stem-loop RT)	5'-GAACCGAUAGAGCAGGCAUUGA -3'
	5'-CAGUACUUUUGUGUAGUACAA-3'
	5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGGAACCGAT-3'
miR-2819 (Forward)	5' - ACACTCCAGCTGGGTCAATGCCTGCTCT - 3'
Universal reverse primer	5' - TGGTGTCGTGGAGTCG- 3'
ie-1 (Forward)	5'- CGACTACAATTCCAACAGGT -3'
ie-1 (Reverse)	5'- ATTTCAAACGGCTTTACTTC - 3'
U6 (Forward)	5'- CGTATACTAAAATTGGAACGATACAG -3'
U6 (Reverse)	5'- ATTTTGCGTGTCATCCTTGC -3'
Polyhedrin (Forward)	5'- TTTAGGGTGGTATGTTAGAG -3'
Polyhedrin (Reverse)	5' - AAGATTTGGCAAGTCGTG -3'

transcribed into cDNA, respectively. The cDNAs and gradient standard plasmids were as templates for absolute qRT-PCR. PCR reactions were run with a thermal cycling at 95 °C for 30 s followed by 40 cycles of 95 °C for 5 s, 60 °C for 31 s. Following the amplification, melting curves were constructed. Three independent experiments with three technical replicates were analyzed. All data were represented by the mean ± SD. The unpaired Student's *t*-test was used to compare the difference in means. P value < 0.05 is set for statistically significant. The sequences of the primers were listed in Table 1.

2.8. Western blotting

Protein samples from silkworm midguts were dissociated by RIPA lysis buffer (Beyotime Shanghai). A total of 30 μg protein sample was loaded on each lane and separated on SDS-PAGE before transferred onto a nitrocellulose membrane. The membrane was incubated with rabbit IE1 (1:2000) and rabbit VP39 (1:2000), which are kindly provided by professor Pan, Southwest University. Then, the membrane was further incubated with HRP labeled goat anti-rabbit IgG (1:20,000; Beyotime, China). The same blots were re-probed with α-Tubulin antibody (1:5000; Sigma, USA) to confirm equal loading of samples.

3. Results

3.1. Down-regulation of miR-2819 upon BmNPV infection

In our previous study, we constructed two small RNA libraries from BmNPV-infected 72 h larvae and the control larvae. The results of the small RNA solexa sequencing revealed the significant down-regulation of miR-2819 in infected larvae (Wu et al. 2016). In this study, we detected the expression profile of miR-2819 in different tissues of silkworm larvae and found that miR-2819 has relatively high abundance in several tissues including malpighian tubule, fat body, hemolymph, silk gland and midgut (Fig. 1A). Further, we compared the abundance of miR-2819 of infected cells with that of control cells at different time-point by qRT-PCR. The results showed that during the 6–12 hours post infection (hpi), the expression level of miR-2819 was increased while during 24–72 hpi, the expression level of miR-2819 was significantly declined. The down-regulation of miR-2819 in BmNPV infected cells at 72 h is consonant with the results of solexa sequencing.

3.2. Validating the interaction of miR-2819 with BmNPV ie-1 gene

By using RNAHybrid online software (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid), miR-2819 was predicted to target on the 3′ UTR of BmNPV *ie-1* (GenBank: AY048770.1) based on the two main screening criteria, the seed region and the minimum free energy (mfe) of -20 kcal/mol (Fig. 2A). The target site is located at 3012 ~ 3036 bp of AY048770.1 and the target sequence is CGCCGGTTCTATTGATGGCGT TGAT. To further validate the interaction between miR-2819 and *ie-1*, the *ie-1* 3′UTR target sequences containing the binding site with miR-2819 and the mutated sequences lack of the binding site were cloned downstream of the firefly luciferase gene in the PmirGLO vector (Fig. 2B). Each of the constructs, PmirGLO-ie-1/PmirGLO-ie-1-mut was co-transfected along with the miR-2819 mimic or the mimic control into 293 cells. As shown in Fig. 2C, the luciferase activity declined significantly in the 293 cells co-transfected with PmirGLO-ie-1 and miR-2819 mimic as compared with the mimic control. However, the luciferase activity demonstrated no change in the PmirGLO-ie-1-mut and miR-2819 mimic co-transfected cells. These results implied that *ie-1* are one of the target genes for miR-2819.

3.3. Overexpression of miR-2819 suppresses BmNPV reproduction

In order to further confirm the negative regulation of *ie-1* by miR-2819, overexpression and inhibition of miR-2819 assay were performed

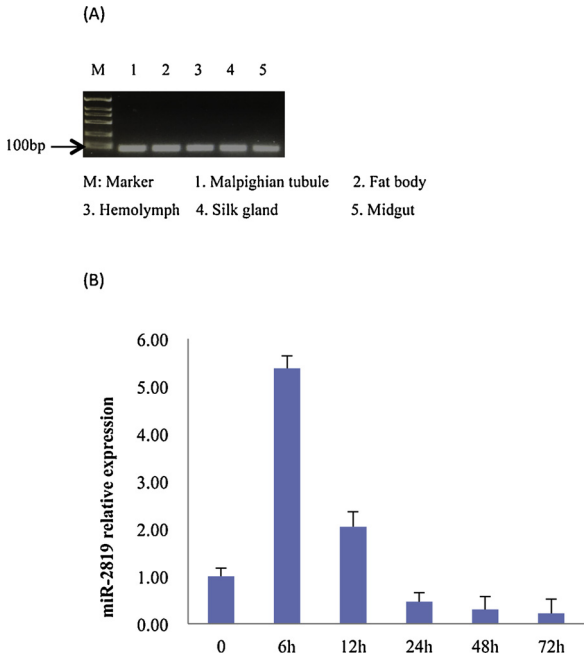

Fig. 1. Expression level of miR-2819 in *Bombyx mori*. (A) RT-PCR analysis of RNA extracted from the different tissues of normal silkworm. M: Marker; 1. Malpighian tubule; 2. Fat body; 3. Hemolymph; 4. Silk gland; 5. Midgut. (B) qRT-PCR results of miR-2819 in BmNPV infected and control cells at different time-points. U6 was taken as the endogenous control. The data represent the relative transcript level of miR-2819 in the infected cells to that of control cells for three independent samples carried out in triplicate. The error bars indicate standard deviations.

in vivo. MiR-2819 mimic or inhibitor and their correspondingly negative control were microinjected into *B. mori* larvae (5th instar, 1nd day) with BmNPV-infected. After infection 72 h, RNA was extracted from the transfected midguts and used for qRT-PCR analysis. The results showed significantly higher transcript level of miR-2819 in mimics transfected midguts comparing with negative control midguts, which suggested that overexpression of miR-2819 is effective but the inhibition effect is not significant (Fig. 3A).

Since miRNAs has been recognized to play roles in the post-transcriptional regulation of gene expression, western blotting assay was performed to detect the protein level change of IE-1 in mimics transfected midguts. The results revealed that overexpression of miR-2819 can significantly decrease IE-1 expression in infected midguts (Fig. 3B, C).

In order to explore the potential role of miR-2819 on BmNPV replication, the DNA level of polyhedrin gene of BmNPV was observed. The results showed that overexpression of miR-2819 inhibited the polyhedrin gene expression in infected midguts (Fig. 4A). Subsequently, western blotting for VP39 of BmNPV was performed to assess the effects of miR-2819 on BmNPV replication. As shown in Fig. 4B, upon the overexpression of miR-2819, the abundance of VP39 protein is significantly lower than that of mimic control treated samples, indicating that miR-2819 could suppress BmNPV replication by regulating the expression of *ie-1* gene.

4. Discussion

Based on accumulating reports, it has been well accepted that miRNAs play a key role in host-pathogen interactions (Asgari, 2013, 2015; Dykxhoorn 2007; Scaria 2006). The changes of cellular miRNAs pattern induced by viral infection have been reported in different viruses (Cullen, 2006; Trobaugh 2017; Pedersen, 2007; Maharaj, 2015). Generally, identifying the differentially expressed miRNAs during viral

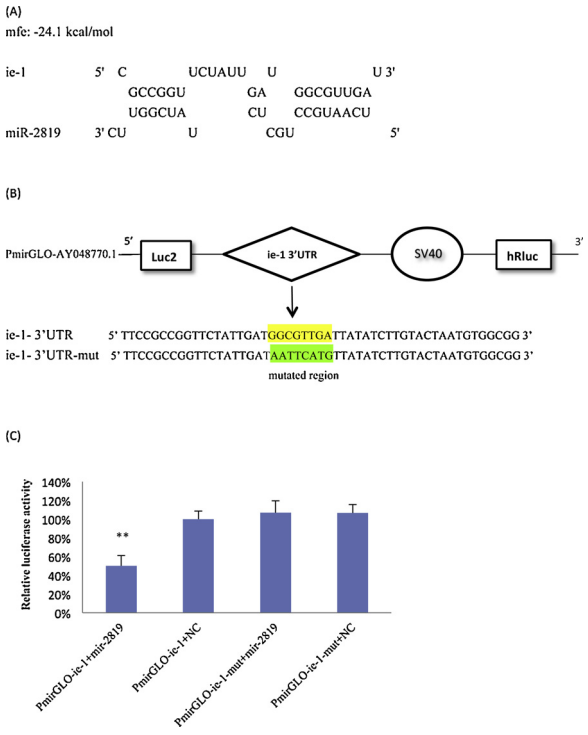

Fig. 2. Target validation of miR-2819. (A) Bioinformatics prediction of miR-2819 targeting *ie-1*. mfe, minimum free energy. (B) Schematic diagram showing the cloning strategy of *ie-1* target sequence downstream of the firefly luciferase gene in the PmirGLO vector. The mutated nucleotides including the seed region of PmirGLO-ie-1 and PmirGLO-ie-1-mut construct were highlighted in yellow and green, respectively. (C) Luciferase reporter assays were carried out in 293 cells, and Renilla luciferase was used as the endogenous control. Data were generated from three independent experiments performed in triplicate and represented as mean ± SD (**p < 0.01) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

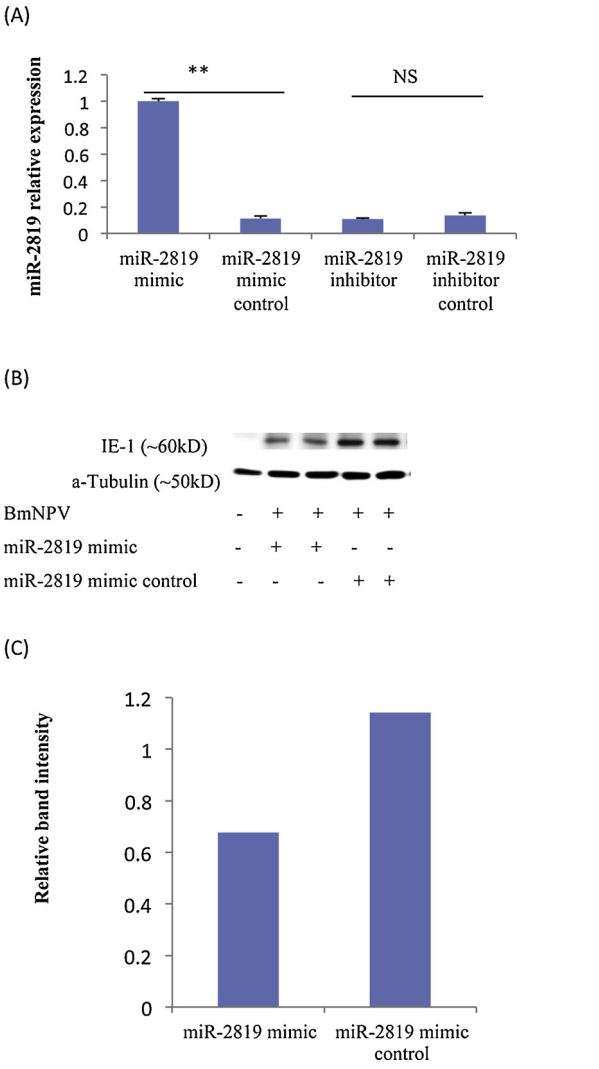

Fig. 3. Overexpression of miR-2819 suppresses IE-1 expression. (A) qRT-PCR analysis of expression of miR-2819 gene. RNAs were extracted from BmNPV-infected *Bombyx mori* co-transfected with miR-2819 mimic, mimic control, inhibitor and inhibitor control. The data were represented as mean ± SD. The error bars indicate standard deviations of averages from three independent experiments with three technical replicates (**p < 0.01). (B) Western blotting analysis of IE-1. Samples were from uninfected larvae, BmNPV-infected larvae co-transfected with miR-2819 mimic or mimic control, respectively. a-Tubulin was served as the loading control. Two biological replicates were performed for infected larvae. (C) The changes in the band intensity were quantified by normalizing a-Tubulin and the relative band intensity has been shown as bar diagram.

infection is most often used as the initial step to explore the miRNAs possible roles in host-virus interaction (Tang et al., 2018). In our previous study, we reported that BmNPV infection of silkworm resulted in 38 differentially expressed miRNAs compared with that of normal silkworm. Fragmentary, these varied expression miRNAs may play potential role in silkworm and BmNPV interaction (Wu et al., 2016). Functional analysis of these miRNAs will make more meaningful use of them. MiRNA of both viral and host origin plays important roles in the regulation of the pathogen-host interaction (Zhumur et al., 2009; Gottwein and Cullen, 2008). It is reported that BmNPV-miR-1 and BmNPV-miR-3 affect BmNPV replication by regulating the expression of host and viral gene, respectively(Singh et al., 2014, 2012). Until now, functional studies of cellular miRNAs on BmNPV reproduction are limited. We predicted the target genes from BmNPV for all of these differentially expressed miRNAs and the results showed that miR-2819 could interact with *ie-1* gene of BmNPV. *Ie-1* is one of essential genes required for BmNPV DNA replication, and the encoded product of *ie-1* is the principal transcriptional regulator of baculovirus(Kool et al., 1994). Silencing *ie-1* could improve the resistance of transgenic silkworm to BmNPV (Zhang et al., 2013). Therefore, we carried out the functional research of miR-2819 on silkworm-BmNPV interaction.

Firstly, we conducted the spatio-temporal expression profile assay of miR-2819 and found that miR-2819 can expressed in several tissues (Fig. 1A). Upon BmNPV infection, the expression level of miR-2819 is varied at different time-points. It was up-regulated at 6–12 hpi, but down-regulated at 24–72 hpi (Fig. 1B). The down-regulation of miR-2819 at 72 hpi observed by solexa sequencing was confirmed by qRT-PCR in this study. MiRNAs can have opposite directions of regulation depending on the course of infection (Maharaj 2015; Saldana, 2017), which suggests the invasion and counter-invasion mechanisms occur between the insects and virus during the progression of infection (Monsanto-Hearne and Johnson, 2018).

Secondly, by dual luciferase reporter assay, we confirmed the positive interaction between miR-2819 and *ie-1* (Fig. 2). In order to provide further evidence in support of the interaction between *ie-1* gene and miR-2819, the miR-2819 mimic and inhibitor were typically injected into silkworm larvae. The success of mimic injection was validated by qRT-PCR results, which showed that the transcript level of miR-2819 was highly increased in mimic injected silkworm. However, the inhibitor injection has no significant effects on the regulation of miR-2819 expression (Fig. 3A). Because perfect binding of even just the 2nd to the 8th molecule from 5′ end of miRNA (seed region) may be sufficient for miRNA function, a single miRNA can include target sites

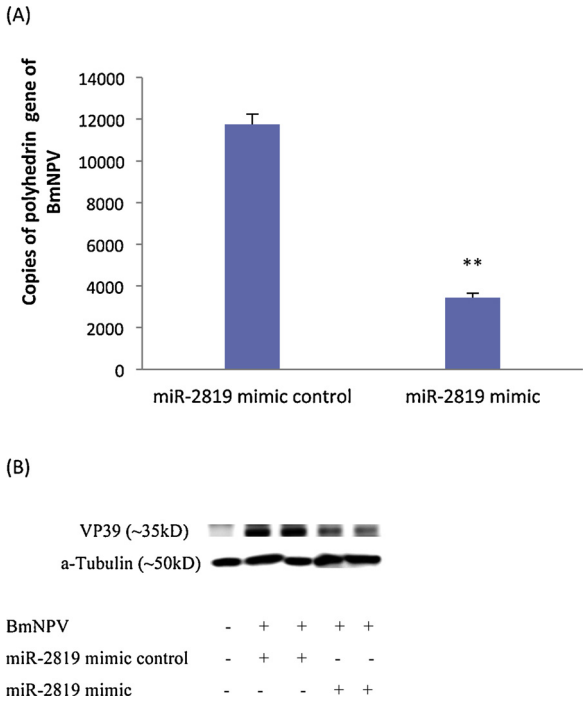

Fig. 4. Overexpression of miR-2819 suppresses BmNPV replication. (A) qRT-PCR analysis of expression of polyhedrin gene. RNAs were extracted from BmNPV-infected *Bombyx mori* co-transfected with miR-2819 mimic and mimic control. The data were represented as mean ± SD. The error bars indicate standard deviations of averages from three independent experiments with three technical replicates (**$p < 0.01$). (B) Western blotting analysis of VP39. Samples were from uninfected larvae, BmNPV-infected larvae co-transfected with miR-2819 mimic or mimic control, respectively. a-Tubulin was served as the loading control. Two biological replicates were performed for infected larvae.

for multiple mRNAs. It speculates that the miR-2819 inhibitor' effects may be diluted by other potential target sites. Subsequently, we found the abundance of IE-1 was significantly decreased in the infected larvae with miR-2819 mimics based on the results of western blotting (Fig. 3B), which validated that *ie-1* gene was exactly regulated by miR-2819.

Further, to investigate the potential role of miR-2819 in the BmNPV multiplication, based on the results of qRT-PCR and western blotting, we detected the lower abundance of polyhedrin gene and VP39 protein of BmNPV in miRNA-2819 mimic injected larvae (Fig. 4), which demonstrated that miR-2819 can affect BmNPV replication by regulating *ie-1* gene.

Viruses have evolved to developed multiple strategies to escape the host's immune system (Tang et al., 2018). Among these strategies, miRNAs were reported to be exploited by virus to take as an effective means for their own propagation. Viral infection usually resulted in the changes of cellular miRNA pattern. Some cellular target genes which are involved in different antiviral host defense were predicted to be targets of virus or host miRNAs, which suggests the complicated mechanisms of miRNAs in antiviral response by regulating immune-related genes (Tang et al., 2018; Wu et al., 2015).

Here we confirmed the BmNPV *ie-1* gene is one of the target genes of miR-2819. BmNPV infection declined the expression level of miR-2819, which resulted in the up-regulation of *ie-1* and consequently facilitate the virus replication. Our results suggest that BmNPV can indirectly regulate the expression of virus genes by changing the silkworm miRNA abundances to create the favorable environment for their reproduction. This data will expand our understanding of silkworm-virus interactions and provide potential avenues to develop new strategy to resist virus infection.

Conflict of interest

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

Funding

This work was supported by the National Nature Science Foundation of China [grant No.31502016, 31572463]; the Six Talent Peak Project of Jiangsu Province [grant No.NY-079]; and the First Level Young Scholar Program of Jiangsu University.

Acknowledgements

We thank Prof. Minghui Pan of Southwest University for providing the IE-1 and VP39 antibody.

References

Asgari, S., 2013. MicroRNA functions in insects. Insect Biochem. Mol. Biol. 43, 388e397.
Asgari, S., 2015. Regulatory role of cellular and viral microRNAs in insect-virus interactions. Curr. Opin. Insect Sci. 8, 104–110.
Bruscella, P., Bottini, S., Baudesson, C., Pawlotsky, J.M., Feray, C., Trabucchi, M., 2017. Viruses and miRNAs: more friends than foes. Front. Microbiol. 8, 824.
Chen, C., Ridzon, D.A., Broomer, A.J., Zhou, Z., Lee, D.H., Nguyen, J.T., Barbisin, M., Xu, N.L., Mahuvakar, V.R., Andersen, M.R., Lao, K.Q., Livak, K.J., Guegler, K.J., 2005. Real-time quantification of microRNAs by stem-loop RT-PCR. Nucleic Acids Res. 33, e179.
Cirimotich, C.M., Dong, Y., Garver, L.S., Sim, S., Dimopoulos, G., 2010. Mosquito immune defenses against Plasmodium infection. Dev. Comp. Immunol. 34, 387–395.
Cullen, B.R., 2013. MicroRNAs as mediators of viral evasion of the immune system. Nat. Immunol. 14, 205–210.
Dennison, N.J., Benmarzouk-Hidalgo, O.J., Dimopoulos, G., 2015. MicroRNA - regulation of Anopheles gambiae immunity to Plasmodium falciparum infection and midgut microbiota. Dev. Comp. Immunol. 49, 170–178.
Gottwein, E., Cullen, B.R., 2008. Viral and cellular MicroRNAs as determinants of viral pathogenesis and immunity. Cell Host Microbe 3, 375–387.
Guo, Y.E., Steitz, J.A., 2014. Virus meets host microRNA: the destroyer, the booster, the hijacker. Mol. Cell. Biol. 34, 3780.
Hong, S., Guo, Q., Wang, W., Hu, S., Fang, F., Lv, Y., Yu, J., Zou, F., Lei, Z., Ma, K., 2014. Identification of differentially expressed microRNAs in Culex pipiens and their potential roles in pyrethroid resistance. Insect Biochem. Mol. Biol. 55, 39–50.
Jain, S., Rana, V., Tridibes, A., Sunil, S., Bhatnagar, R.K., 2015. Dynamic expression of miRNAs across immature and adult stages of the malaria mosquito Anopheles stephensi. Parasit. Vectors 8, 179.
Kang, L., Wang, M., Cao, X., Tang, S., Xia, D., Shen, X., Zhao, Q., 2018. Inhibition of expression of BmNPV cg30 by bmo-miRNA-390 is a host response to baculovirus invasion. Arch. Virol. 163, 2719–2725.
Kool, M., Ahrens, C.H., Goldbach, R.W., Rohrmann, G.F., Vlak, J.M., 1994. Identification of genes involved in DNA replication of the Autographa californica baculovirus. Proc. Natl. Acad. Sci. U. S. A. 91, 11212–11216.
Lampe, L., Levashina, E.A., 2017. The role of microRNAs in Anopheles biology-an emerging research field. Parasite Immunol. 39.
Lei, Z., Yuan, L., Wang, W., Qin, G., Zou, F., Hu, S., Fang, F., Tian, M., Liu, B., Liu, X., 2015. MiR-278-3p regulates pyrethroid resistance in Culex pipiens pallens. Parasitol. Res. 114, 699–706.
Liu, S.R., Zhou, J.J., Hu, C.G., Wei, C.L., Zhang, J.Z., 2017. MicroRNA-mediated gene silencing in plant defense and viral counter-defense. Front. Microbiol. 8, 1801.
Maharaj, P.D., Widen, S.G., Huang, J., Wood, T.G., Thangamani, S., 2015. Discovery of mosquito saliva microRNAs during CHIKV infection. PLoS Negl. Trop. Dis. 9, e0003386.
Monsanto-Hearne, V., Johnson, K.N., 2018. miRNAs in insects infected by animal and plant viruses. Viruses 10.
Rahman, M.M., Gopinathan, K.P., 2004. Systemic and in vitro infection process of Bombyx mori nucleopolyhedrovirus. Virus Res. 101, 0–118.
Rehmsmeier, M., Steffen, P., Hochsmann, M., Giegerich, R., 2004. Fast and effective prediction of microRNA/target duplexes. Rna 10, 1507–1517.
Seong, K.M., Coates, B.S., Kim, D.H., Hansen, A.K., Pittendrigh, B.R., 2018. Differentially expressed microRNAs associated with changes of transcript levels in detoxification pathways and DDT-resistance in the Drosophila melanogaster strain 91-R. PLoS One 13, e0196518.
Singh, J., Singh, C.P., Bhavani, A., Nagaraju, J., 2010. Discovering microRNAs from Bombyx mori nucleopolyhedrosis virus. Virology 407, 120–128.
Singh, C.P., Singh, J., Nagaraju, J., 2012. A baculovirus-encoded MicroRNA (miRNA) suppresses its host miRNA biogenesis by regulating the exportin-5 cofactor Ran. J. Virol. 86, 7867–7879.
Singh, C.P., Singh, J., Nagaraju, J., 2014. bmnpv-miR-3 facilitates BmNPV infection by modulating the expression of viral P6.9 and other late genes in Bombyx mori. Insect Biochem. Mol. Biol. 49, 59–69.

Tang, Q., Qiu, L., Li, G., 2018. Baculovirus-encoded MicroRNAs: a brief overview and future prospects. Curr. Microbiol. 1-6.
Trobaugh, D.W., Klimstra, W.B., 2017. MicroRNA regulation of RNA virus replication and pathogenesis. Trends Mol. Med. 23, 80–93.
Wu, P., Qin, G., Qian, H., Chen, T., Guo, X., 2015. Roles of miR-278-3p in IBP2 regulation and Bombyx mori cytoplasmic polyhedrosis virus replication. Gene 575, 264–269.
Wu, P., Jiang, X., Guo, X., Li, L., Chen, T., 2016. Genome-wide analysis of differentially expressed microRNA in Bombyx mori infected with nucleopolyhedrosis virus. PLoS One 11, e0165865.
Wu, P., Jiang, X., Sang, Q., Annan, E., Cheng, T., Guo, X., 2017. Inhibition of miR-274-3p increases BmCPV replication by regulating the expression of BmCPV NS5 gene in Bombyx mori. Virus Genes 53, 643–649.
Zhang, P., Wang, J., Lu, Y., Hu, Y., Xue, R., Cao, G., Gong, C., 2013. Resistance of transgenic silkworm to BmNPV could be improved by silencing ie-1 and lef-1 genes. Gene Ther. 21, 81–88.
Zhumur, G., Bibekanand, M., Jayprokas, C., 2009. Cellular versus viral microRNAs in host-virus interaction. Nucleic Acids Res. 37, 1035–1048.