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MicroRNA gga-miR-455-5p suppresses Newcastle disease virus replication via targeting cellular suppressors of cytokine signaling 3

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ABSTRACT

Newcastle disease (ND) is an acute and contagious avian disease caused by Newcastle disease virus (NDV). MicroRNAs (miRNAs) play a significant role in host-pathogen interactions and the innate immune response. However, the role of miRNAs in the host response to NDV infection is not clearly understood. In this study, we showed that expression of the cellular miRNA gga-miR-455-5p was downregulated *in vivo* and *in vitro* in response to NDV infection. Next, we found that the transfection of chicken embryonic fibroblasts (CEFs) with gga-miR-455-5p suppressed NDV replication, while the blockade of endogenous gga-miR-455-5p expression with inhibitors enhanced NDV replication. In addition, gga-miR-455-5p enhanced the expression of type I interferon and the interferon-inducible genes (ISGs) OASL and Mx1 by targeting SOCS3, a negative regulator of type I IFN signaling. Altogether, these findings highlight the crucial role of gga-miR-455-5p in host defense against NDV by targeting the SOCS3 gene to inhibit NDV replication.

1. Introduction

Newcastle disease (ND) is an acute and highly contagious infectious disease in the poultry industry. After first being reported in 1926, ND rapidly spread around the world (Seal et al., 2000). ND is caused by Newcastle disease virus (NDV), which belongs to genus *Avulavirus* of family Paramyxoviridae. NDV has a nonsegmented, negative-sense, single-stranded RNA genome that contains six genes in the order 3′-NP-P-M-F-HN-L-5′ and two nonstructural V and W proteins formed by RNA editing (Steward et al., 1993). Despite the use of vaccines to prevent this disease, ND outbreaks are frequently reported in many countries (Zhu et al., 2016; Dimitrov et al., 2017). Therefore, to prevent an NDV epidemic, there is an urgent need to explore new prevention and treatment strategies to inhibit NDV.

MiRNAs are noncoding small RNAs composed of 20–24 nucleotides that conduct the posttranscriptional regulation of target genes by interaction with their 3′untranslation regions (UTRs), triggering the degradation of mRNA or translation inhibition (He and Hannon, 2004). miRNAs play significant roles in cancer (Qin et al., 2016a; Liu et al., 2016), cell differentiation (Zhou et al., 2007), and cell apoptosis (Guo et al., 2009; Chai et al., 2015). An increasing number of studies have reported that miRNAs play an important role in the process of viral infection (Fu et al., 2017; Wang et al., 2019a). During viral infection, viruses may promote their own replication through utilizing their own encoded miRNAs or host miRNAs (Hu et al., 2015). Moreover, host miRNAs can inhibit viral replication by directly targeting the viral genome or regulating host innate and adaptive immune responses (Fu et al., 2017).

Although NDV is one of the most studied avian viruses, the influence of host miRNAs on NDV infection and their underlying molecular mechanisms have been rarely reported. In our previous study, we found that gga-miR-455-5p was downregulated after NDV infection (Jia et al., 2018). miR-455-5p was reported to be involved in the development and differentiation of cancer (Qin et al., 2016b; Liu et al., 2016; Chai et al., 2015); however, the role of miR-455-5p in the cellular response to viral infection remains unclear. In this study, we demonstrated that gga-miR-455-5p acts as an antagonist of NDV infection *via* suppressing viral replication and upregulating the expression of type I interferon and the interferon-inducible genes (ISGs) OASL and Mx1. We identified suppressors of cytokine signaling 3 (SOCS3), a negative regulator of the JAK-STAT signaling pathway, as targets of gga-miR-455-5p. Ectopic expression of gga-miR-455-5p effectively suppressed NDV replication

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and enhanced IFN-β and ISG expression *via* inhibiting the expression of SOCS3, indicating that gga-miR-455-5p plays a significant role in the host response to NDV infection.

2. Materials and methods

2.1. Ethics statement

All chicken embryos were handled according to the guidelines of the Ethics Committee at Northwest A&F University. Experiments were carried out in accordance with the approved guidelines.

2.2. Cells and virus

Ten-day-old specific pathogen-free (SPF) chicken embryos (Jinan SAIS Poultry Co., Ltd.) were used to make primary chicken embryonic fibroblasts (CEFs). HEK-293 T and BHK-21 cells were maintained in our laboratory and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) in a 5% CO_2 environment in a 37 °C incubator. The NDV virulent strain F48E9 and lentogenic strain LaSota-GFP used in this study were maintained in our laboratory and propagated in 10-day-old SPF chicken embryos. The virus was stored at -80°C until further use.

2.3. Reagents

The psiCHECK2 vector was maintained in our laboratory. The restriction enzymes XhoI and NotI were purchased from TaKaRa (Dalian, China). gga-miR-455-5p mimics and inhibitors were synthesized by GenePharma Company (Shanghai, China), and their sequences are listed in Table 1. Anti-HN monoclonal antibody and anti-SOCS3 polyclonal antibody were developed in our laboratory. Anti-β-tubulin mouse monoclonal antibody was obtained from Sungene Biotech (China). HRP-conjugated goat anti-mouse/rabbit IgG was obtained from Abcam (USA).

2.4. Transfection of miRNA mimics or inhibitors

A total of 5×10^5 CEFs/well were cultured in a 12-well plate overnight and then transfected with gga-miR-455-5p mimics or inhibitors (20 pmoi) using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA). Twenty-four hours after transfection, the cells were infected with NDV (multiplicity of infection (MOI) of 0.1). Cells and the cellular supernatant were collected for further study twenty-four hours after infection.

2.5. RNA isolation and quantitative real-time PCR (qRT-PCR) analysis

Total RNA was extracted from CEFs by using TRIzol reagent (TaKaRa, China) according to the manufacturer's protocol. Total RNA was reverse transcribed using a First-Strand cDNA Synthesis Kit (GeneStar, Beijing, China). Quantitative real-time PCR was carried out with a real-time thermocycler (four-channel, Tianlong, China) using RealStar Green Fast Mixture (GeneStar, Beijing, China) according to the manufacturer's instructions. The gga-miR-455-5p expression level was normalized to that of U6, and other expression levels were normalized to the 28S gene expression level. Relative expression of target mRNAs was calculated using the $2^{-\Delta\Delta CT}$ method as previously described (Livak and Schmittgen, 2001). The primers used in this study are listed in Table 1.

2.6. Plaque assay

Viral titers were measured with a plaque assay as previously described (Chu et al., 2018). In brief, BHK-21 cells grown to a density of 85% in 12-well plates were incubated with the cellular supernatants of different groups after NDV infection for 1 h. The supernatants were replaced with DMEM containing 2% FBS and 1% methylcellulose (Solarbio, Beijing, China). After 3–5 days, cells were fixed with 4% formaldehyde dissolved in phosphate-buffered saline (PBS) and stained with 1% crystal violet.

2.7. Western blot analysis

CEFs from different groups were lysed with 1 × SDS sample buffer, boiled for 10 min and separated by 12% SDS-PAGE, followed by transfer to a nitrocellulose membrane (Millipore, Billerica, MA, USA). After blocking for one night at 4 °C with 10% skim milk, the membranes were incubated with diluted primary and secondary antibodies for 2 h and 1 h, respectively, at room temperature. The result was detected with Clarity Western ECL Substrate (Bio-Rad, Hercules, CA, USA).

2.8. Luciferase reporter assay

The 3′UTR of the SOCS3 gene, which contains gga-miR-455-5p-binding sites, was amplified and cloned into the psiCHECK2vector. We also constructed the luciferase reporter plasmid psiCHECK2-SOCS3 3′UTR-MUT, which contained a putative gga-miR-455-5p-binding site with a mutated region in the 3′UTR. The luciferase reporter assay was performed as previously described (Jia et al., 2018). Briefly, 10^5 HEK-293 T cells/well were cultured in a 24-well plate overnight and then cotransfected with 500 ng of psiCHECK2 containing the target sequence with or without 500 ng of gga-miR-455-5p mimics or miR-NC using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Cells were lysed

Table 1
Primers and miRNA mimics sequences used in this study.

Name	Forward primer (5'-3')	Reverse primers (5'-3')
IRF3 (qRT-PCR)	GCTCTCTGACTCTTTCAACCTCTT	AATGCTGCTCTTTTCTCCTCTG
IFN-β (qRT-PCR)	GCTCACCTCAGCATCAACAA	GGGTGTTGAGACGTTTGGAT
OASL (qRT-PCR)	AGATGTTGAAGCCGAAGTACCC	CTGAAGTCCTCCCTGCCTGT
MX1 (qRT-PCR)	AAGCCTGAGCATGAGCAGAA	TCTCAGGCTGTCAACAAGATCAA
28s (qRT-PCR)	GGTATGGGCCCGACGCT	CCGATGCCGACGCTCAT
LNP (FNP) (clone)	CCGCTCGAG ATGTCCTCCGTATTT GATGAG	ATAAGAATGCGGCCGCTCAATACCCCCAGTCGGTGTC
SOCS3 wt (3'UTR)	CCGCTCGAGGAGGCTGGCAGAGGGAAATCAC	ATAAGAATGCGGCCGCGGGTTTATTAAAAAATAGTGC
SOCS3 mut (3'UTR)	CCGCTCGAGCAGATGAAACTTTCGTGTATTT	ATAAGAATGCGGCCGCGGGTTTATTAAAAAATAGTGC
miR-455-5p Reverse Primer		CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAG CGATGTAG
miR-455-5p (qRT-PCR)	ACACTCCAGCTGGGTATGTGCCCTTGGA	AACTGGTGTCGTGGAGTCG
gga-miR-455-5p mimics	UAUGUGCCCUUGGACUACAUCG	AUGUAGUCCAAGGGCACAUAUU
mimics control	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT
gga-miR-455-5p inhibitor	CGAUGUAGUCCAAGGGCACAUA	
Inhibitor Control	CAGUACUUUUGUGUAGUACAA	

36 h after transfection, and luciferase activity was measured using a dual-luciferase reporter assay kit (Beyotime, Shanghai, China) according to the manufacturer's instructions. The primers used in this study are listed in Table 1.

2.9. Statistical analysis

All data presented are from at least three independent experiments. Student's *t*-test was performed for statistical analysis using GraphPad Prism 5 software (San Diego, CA, USA). Statistically significant differences are presented as follows: *P < 0.05, **P < 0.01.

3. Results

3.1. gga-miR-455-5p expression is downregulated during NDV infection in vivo

To identify the miRNAs involved in the host response to NDV infection, we performed a high-throughput sequencing assay to obtain the miRNA profiles of chicken embryos infected with F48E9 or LaSota (Jia et al., 2018). Ninety-eight miRNAs were shown to be involved in the host response to NDV infection. Among these miRNAs, 31 and 25 miRNAs were downregulated in the F48E9-infected (Table S1) and LaSota-GFP-infected (Table S2) groups, respectively. In addition, 17 miRNAs were downregulated in both groups (Fig. 1A, B and Table S3). In a previous study, the miRNAs involved in the response of CEFs to H9N2 infection were also identified (Peng et al., 2015). The gga-miR-455-5p and gga-miR-155 miRNAs were both shown to be downregulated in the two datasets. gga-miR-155 has been well reported to participate in IBDV infection (Wang et al., 2018). We focused on the role of gga-miR-455-5p in the cellular response to NDV infection because the role of this miRNA during NDV infection remains unclear.

3.2. gga-miR-455-5p expression is downregulated in CEFs after NDV infection

To investigate the expression of gga-miR-455-5p *in vitro* during NDV infection, CEFs were infected with F48E9 or LaSota-GFP at an MOI of 0.1 for 6, 12, and 24 h, and qRT-PCR was performed to determine gga-miR-455-5p expression in the NDV-infected cells. As shown in Fig. 2A and B, NDV infection significantly reduced the expression of gga-miR-455-5p in a time-dependent manner, and F48E9 inhibited the expression of gga-miR-455-5p more effectively than LaSota-GFP. Furthermore, the effects of infection with F48E9 or LaSota-GFP at different MOIs on gga-miR-455-5p expression were also determined by qRT-PCR. As shown in Fig. 2C and D, gga-miR-455-5p expression was decreased in CEFs infected with NDV at different MOIs in a dose-dependent manner. These results suggest that gga-miR-455-5p expression is downregulated during NDV infection.

3.3. gga-miR-455-5p inhibits NDV replication

To determine the effects of gga-miR-455-5p overexpression on NDV replication, CEFs were transiently transfected with gga-miR-455-5p mimics or mimics NC, and qRT-PCR showed that gga-miR-455-5p mimics transfection for 24 h significantly increased the expression of gga-miR-455-5p in CEFs (Fig. 3A). Furthermore, at 24 h after transfection with mimic NC or gga-miR-455-5p mimics, CEFs were infected with LaSota-GFP or F48E9 at an MOI of 0.1 for 24 h. As shown in Fig. 3B and C, the rate of LaSota-GFP infection was significantly reduced in CEFs overexpressing gga-miR-455-5p mimics, and the viral titer was also significantly decreased when compared with that of the mimic NC group. Furthermore, we examined expression of the F48E9 HN viral protein. Consistently, overexpression of gga-miR-455-5p also decreased HN protein expression in CEFs compared with that in the mimics NC group (Fig. 3D), and a plaque assay showed that F48E9 replication was significantly decreased in infected CEFs (Fig. 3E). These results suggest that gga-miR-455-5p overexpression decreased NDV replication in CEFs.

3.4. Inhibition of endogenous gga-miR-455-5p enhances NDV infection

To further examine the effects of gga-miR-455-5p on NDV replication, we knocked down the expression of endogenous gga-miR-455-5p in CEFs using inhibitors. At 24 h after transfection, the expression of gga-miR-455-5p was markedly suppressed in CEFs transfected with gga-miR-455-5p inhibitors (miR-455-5p In) compared with its expression in the inhibitors NC (miR-NC In) group (Fig. 4A). At 24 h after transfection

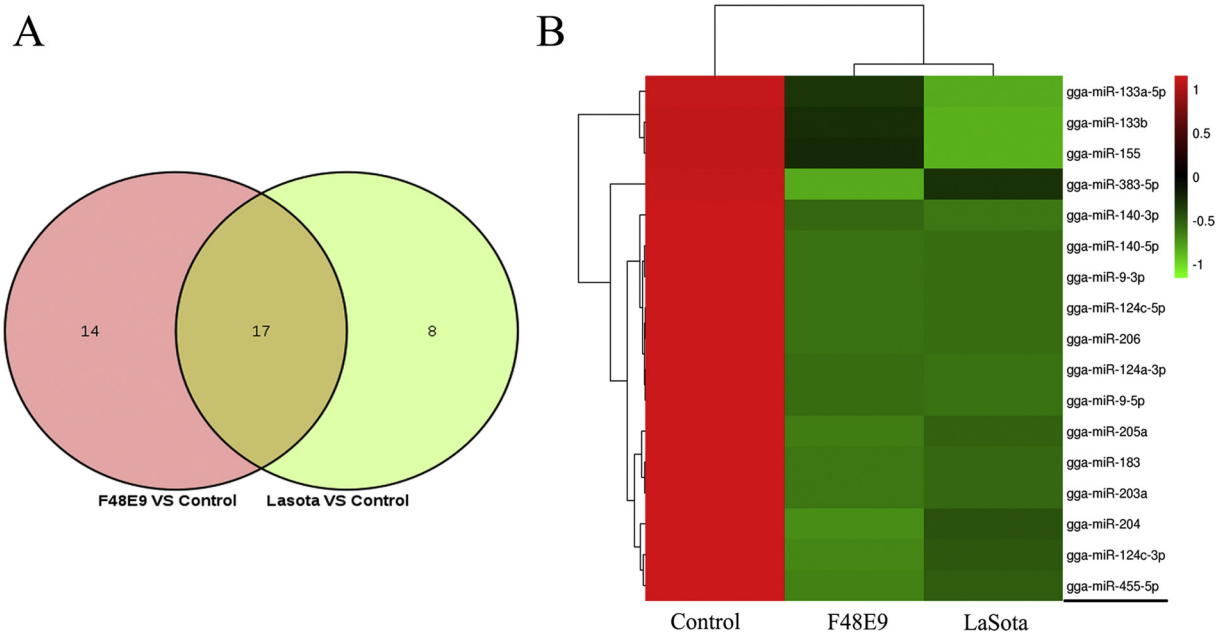

Fig. 1. Downregulated miRNAs indicated by transcriptome data from both F48E9-infected chicken embryos and LaSota-infected chicken embryos. (A) Venn diagram displaying the number of downregulated miRNAs in different groups. (B) Heat map analysis was used to classify gene expression patterns.

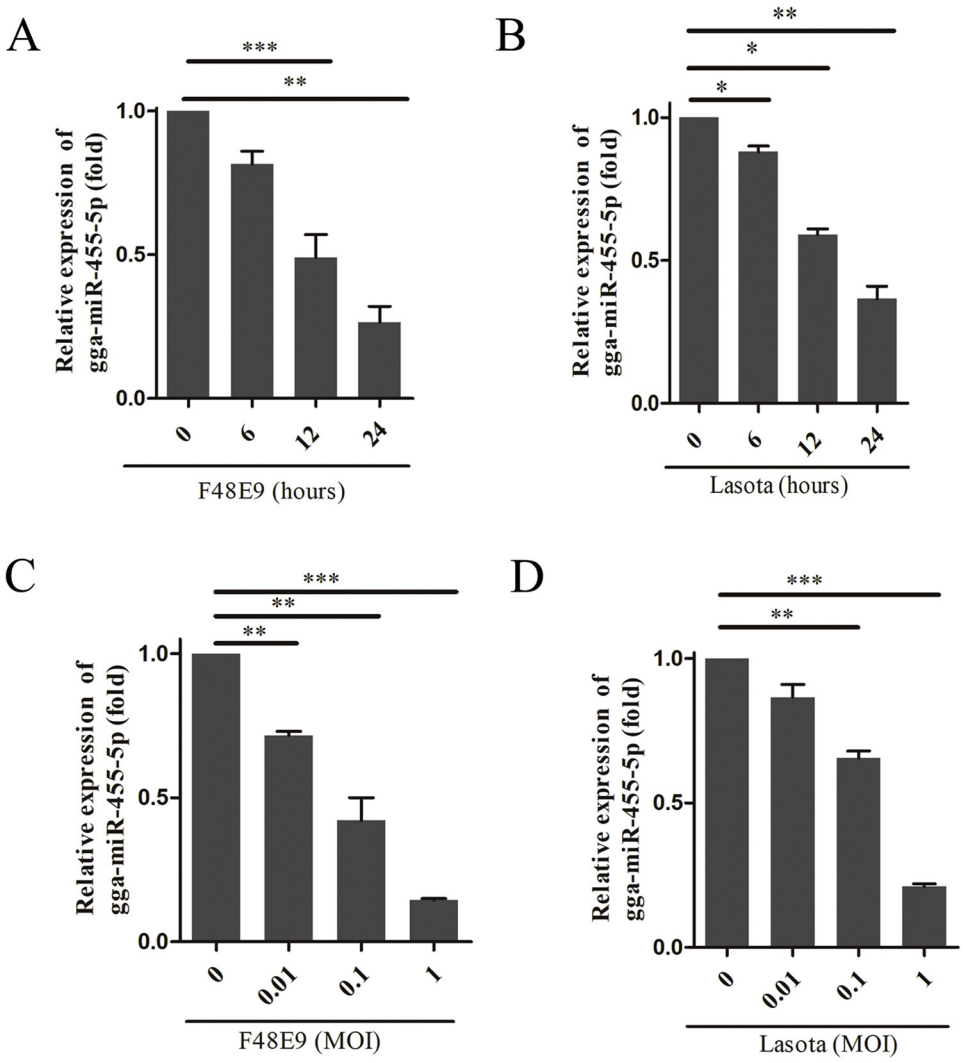

Fig. 2. Effects of F48E9 and Lasota-GFP infection on gga-miR-455-5p expression in CEFs. (A, B) The relative expression of gga-miR-455-5p in CEFs after infection with F48E9 or LaSota-GFP at an MOI of 0.1 for 6, 12, and 24 h was determined by qRT-PCR. (C, D) The relative expression of gga-miR-455-5p in CEFs after infection with F48E9 or LaSota-GFP at an MOI of 0.01, 0.1, and 1 for 24 h was determined by qRT-PCR. Values are the means ± SDs of three independent experiments. Significant differences between groups are indicated by $*p < 0.05$, $**p < 0.01$.

with inhibitors NC or miR-455-5p In, CEFs were infected with LaSota-GFP or F48E9 at an MOI of 0.1 for 24 h. As shown in Fig. 4B and C, the rate of LaSota-GFP infection was significantly increased in CEFs over-expressing miR-455-5p In, and the viral titer was also significantly higher than that in the mimics NC group. Furthermore, we examined expression of the F48E9 HN viral protein. Consistently, the inhibition of endogenous gga-miR-455-5p increased HN protein expression in CEFs compared with its expression in the mimics NC group (Fig. 4D), and a plaque assay showed that F48E9 replication was significantly increased in infected CEFs (Fig. 4E). These results clearly establish the role of gga-miR-455-5p as an antiviral factor in the cellular response to NDV infection.

3.5. gga-miR-455-5p enhances antiviral immune responses by targeting the 3′UTR of SOCS3

Next, we investigated the molecular mechanism underlying gga-miR-455-5p-mediated suppression of NDV. Using TargetScan prediction software, we identified SOCS3, which can utilize a feedback loop to inhibit the type I interferon-dependent antiviral signaling pathway (Shuai and Liu, 2003), as a putative target gene of gga-miR-455-5p in host cells. A region of the SOCS3 3′UTR contains a gga-miR-455-5p target site (Fig. 5A). The expression of SOCS3 was significantly upregulated in the miRNA profiles of chicken embryos infected with F48E9 or LaSota-GFP (Fig. 5B). Thus, we constructed a firefly luciferase reporter gene plasmid containing the predicted target site in SOCS3 (psiCheck2-SOCS3-WT) and another construct with mutations in the seed region (psiCheck2-SOCS3-Mut) (Fig. 5A) and transfected CEFs with these reporter gene plasmids and miRNA mimics. gga-miR-455-5p significantly inhibited the luciferase activities of psiCheck2-SOCS3-WT-transfected CEFs. In contrast, transfection with psiCheck2-SOCS3-Mut abolished gga-miR-455-5p-mediated inhibition of the gga-miR-455-5p target sequence (Fig. 5C). Furthermore, overexpression of gga-miR-455-5p reduced the cellular expression of SOCS3 at both the mRNA and protein levels, and overexpression of miR-455-5p In enhanced the cellular expression of SOCS3 at both the mRNA and protein levels (Fig. 5D–G). These data demonstrate that gga-miR-455-5p directly inhibits NDV replication *via* targeting SOCS3.

3.6. gga-miR-455-5p enhances NDV-induced expression of type I interferon and ISGs

SOCS3 can utilize a feedback loop to inhibit the type I interferon-dependent antiviral signaling pathway (Shuai and Liu, 2003). To

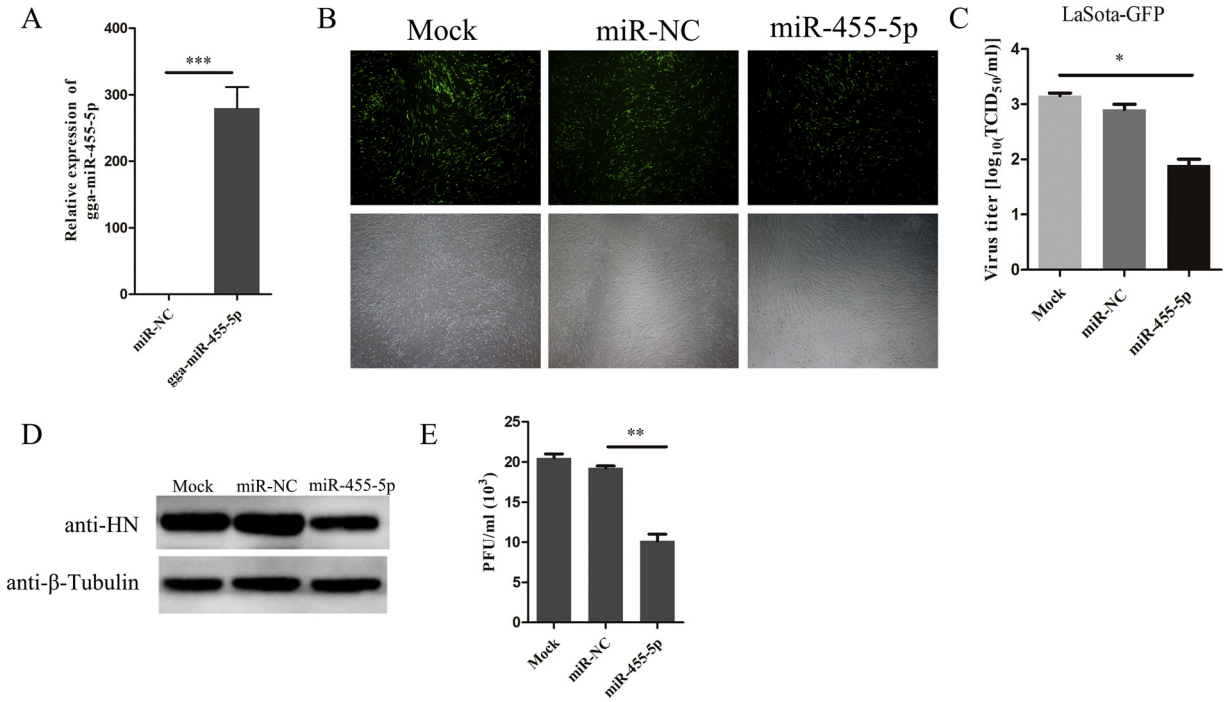

Fig. 3. Overexpression of gga-miR-455-5p decreases NDV replication. (A) The relative expression of gga-miR-455-5p in CEFs was determined by qRT-PCR. CEFs were transfected with mimics NC or gga-miR-455-5p mimics for 24 h. Then, the cells were infected with LaSota-GFP or F48E9 at an MOI of 0.1 for 24 h. (B) The LaSota-GFP infection rate was determined by fluorescence microscopy. (C) Viral titers in culture supernatants of the LaSota-GFP-infected group were measured as the TCID50. (D) The protein expression of HN in the F48E9-infected group was determined by WB. (E) Viral titers in culture supernatants of the F48E9-infected group were measured by plaques assay. Values are the means ± SDs of three independent experiments. Significant differences between groups are indicated by $*p < 0.05$, $**p < 0.01$.

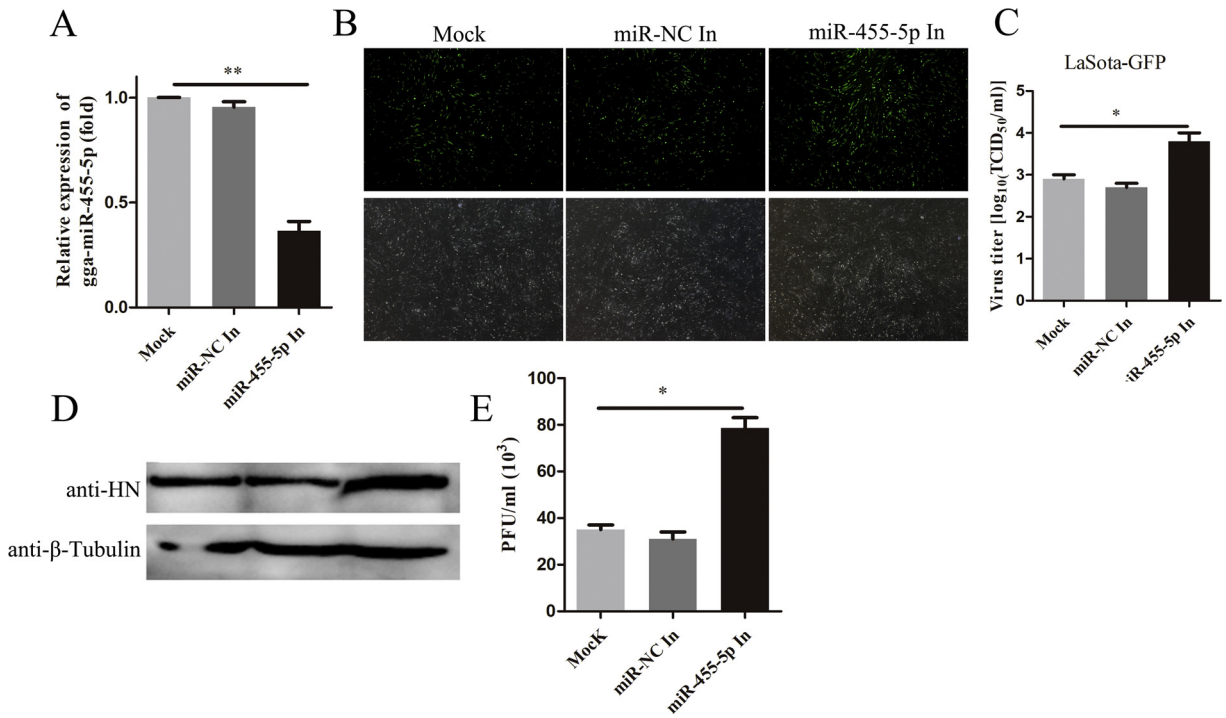

Fig. 4. Inhibition of endogenous gga-miR-455-5p enhances NDV replication. (A) The relative expression of gga-miR-455-5p in CEFs was determined by qRT-PCR. CEFs were transfected with inhibitors NC or gga-miR-455-5p inhibitors for 24 h. Then, the cells were infected w LaSota-GFP or F48E9 at an MOI of 0.1 for 24 h. (B) The LaSota-GFP infection rate was determined by fluorescence microscopy. (C) Viral titers in culture supernatants of the LaSota-GFP-infected group were measured as the TCID50. (D) The protein expression of HN in the F48E9-infected group was determined by WB. (E) Viral titers in culture supernatants of the F48E9-infected group were measured by plaque assay. Values are the means ± SDs of three independent experiments. Significant differences between groups are indicated by $*p < 0.05$, $**p < 0.01$.

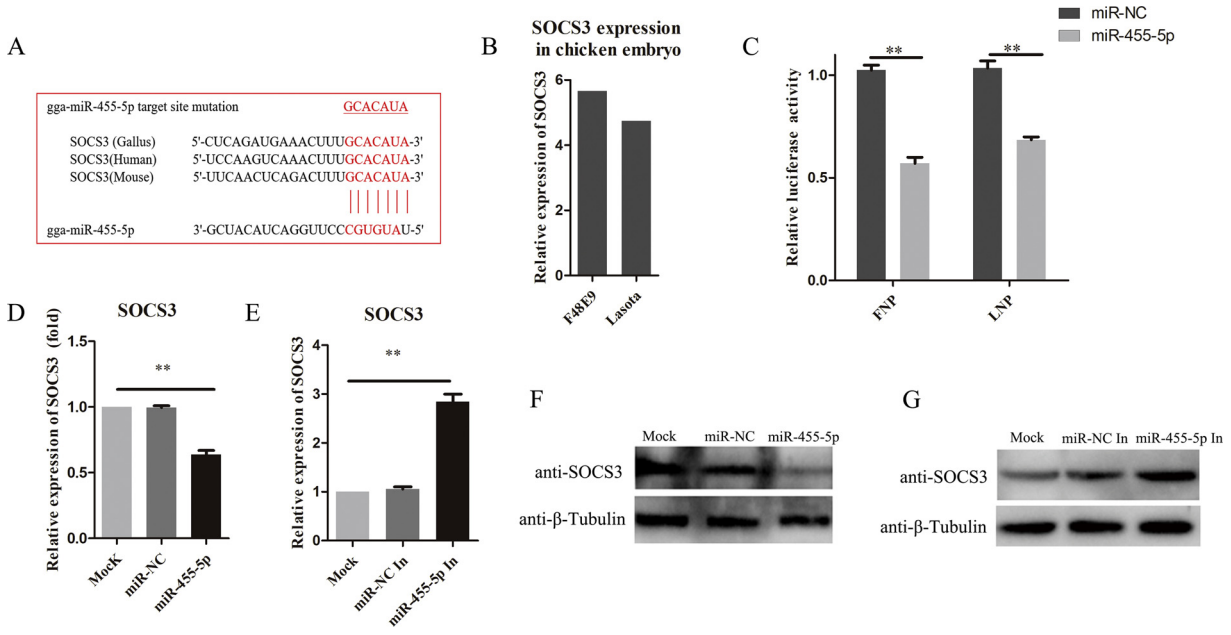

Fig. 5. SOCS3 is a direct target of gga-miR-455-5p. (A) Diagram of the predicted gga-miR-455-5p target sites in SOCS3. gga-miR-455-5p seed regions and mutated gga-miR_455-5p are shown in red. (B) SOCS3 expression indicated by the miRNA profiles of chicken embryos infected with F48E9 or LaSota. (C) Transfection with gga-miR-455-5p reduced the expression of SOCS3 but not mutant SOCS3. CEFs were cotransfected with gga-miR-455-5p mimics or a mimics control and WT or mutant SOCS3 luciferase reporter vectors. Thirty-six hours after transfection, the cells were lysed, and luciferase reporter gene assays were performed to measure SOCS3 expression. (E, F) gga-miR-455-5p inhibits the mRNA and protein expression of SOCS3. CEFs were transfected with gga-miR-455-5p mimics or a mimics control. Twenty-four hours after transfection, the cells were infected with NDV at an MOI of 0.1. Twenty-four hours after infection, qRT-PCR (D) and WB (F) were performed to detect the expression of SOCS3. (E, G) gga-miR-455-5p inhibitors promote the mRNA and protein expression of SOCS3. CEFs were transfected with gga-miR-455-5p inhibitors or an inhibitors control. Twenty-four hours after transfection, cells were infected with NDV at an MOI of 0.1. Twenty-four hours after infection, qRT-PCR (E) and WB (G) were performed to detect the expression of SOCS3. Values are the means ± SDs of three independent experiments. Significant differences between groups are indicated by *$p < 0.05$, **$p < 0.01$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

explore the effect of miRNAs on antiviral signaling pathways, we transfected CEFs with gga-miR-455-5p and then examined the expression of IFN-β and ISGs in response to NDV infection. The overexpression of gga-miR-455-5p significantly enhanced NDV-induced expression of IRF3, IFN-β, Mx1, and OASL (Fig. 6A–D). Furthermore, knockdown of endogenous gga-miR-455-5p with an inhibitor markedly suppressed NDV-induced expression of IRF3, IFN-β, Mx1, and OASL (Fig. 6E–H). These data suggest that gga-miR-455-5p enhances the expression of IFN-β and ISGs in response to NDV infection.

4. Discussion

Host cells produce type I interferon upon the recognition of viral dsRNA by pattern recognition receptors (PRRs) and then produce a large number of ISGs through the JAK-STAT signaling pathway to combat viral infection (Yoneyama et al., 2004). In turn, NDV has evolved diverse strategies to counter the antiviral activity of IFN-I during infection (Motz et al., 2013; Qiu et al., 2016). Recently, miRNAs, a new player involved in regulating gene expression programs, were shown to play important roles during viral infection. In this study, we defined a novel signaling pathway involved in NDV evasion of the host innate immune response. First, NDV infection was shown to downregulate gga-miR-455-5p expression and upregulate SOCS3 expression. In addition, overexpression of gga-miR-455-5p decreased NDV replication in CEFs, while knockdown of gga-miR-455-5p expression enhanced viral replication. Furthermore, mechanistic research showed that gga-miR-455-5p downregulated SOCS3 protein expression by directly targeting its 3′UTR. Finally, gga-miR-455-5p enhanced the expression of IFN-β and ISGs in response to NDV infection (Fig. 7). Collectively, our results demonstrate for the first time the role of gga-miR-455-5p in NDV replication and that the effects of gga-miR-455-5p may be mediated *via* regulating SOCS3.

The role of miRNAs in host defense against viral infection has been well illustrated. Mounting evidence has revealed that miRNAs can assist in host defense against viral infection (Wang et al., 2018; Fu et al., 2017). The role of miRNAs in NDV infection has been elucidated in several studies. The miRNAs gga-miR-375 (Wang et al., 2019b), miR-324-5p (Kumar et al., 2018), and miR-485 (Ingle et al., 2015) were reported to be involved in regulating NDV replication. Previously, we performed a high-throughput sequencing assay to obtain miRNA profiles of chicken embryos infected with F48E9 or LaSota (Jia et al., 2018) and found that a subset of miRNAs was dysregulated during NDV infection. Among these cellular miRNAs, gga-miR-455-5p attracted our attention because it is also involved in H9N2 infection and its role in viral infection remains unclear (Peng et al., 2015). miR-455-5p has been shown to have an antitumor effect in various cancers (Qin et al., 2016a; Liu et al., 2016; Chai et al., 2015). For instance, miR-455-5p can suppress cell viability and induce cell apoptosis in colorectal cancer and colorectal cancer by targeting RAF1 and RAB18, respectively (Chai et al., 2015; Liu et al., 2016). In this study, we demonstrated that NDV infection decreases the expression of gga-miR-455-5p and that overexpression of gga-miR-455-5p inhibits NDV replication, while knockdown of gga-miR-455-5p promoted NDV replication, indicating that gga-miR-455-5p plays an important role during NDV infection.

Next, we explored the mechanisms underlying the effects of gga-miR-455-5p on NDV replication. Using bioinformatics analysis, we found a region of the SOCS3 3′UTR containing a gga-miR-455-5p target site. SOCS3 belongs to the suppressor of cytokine signaling (SOCS) family of proteins, which have been shown to regulate the JAK/STAT pathway through negative feedback (Qin et al., 2012; Baker et al., 2009). SOCS3 can inhibit the autophosphorylation of JAK1 and JAK2 through binding and inhibiting JAKs through its Src homology 2 (SH2)

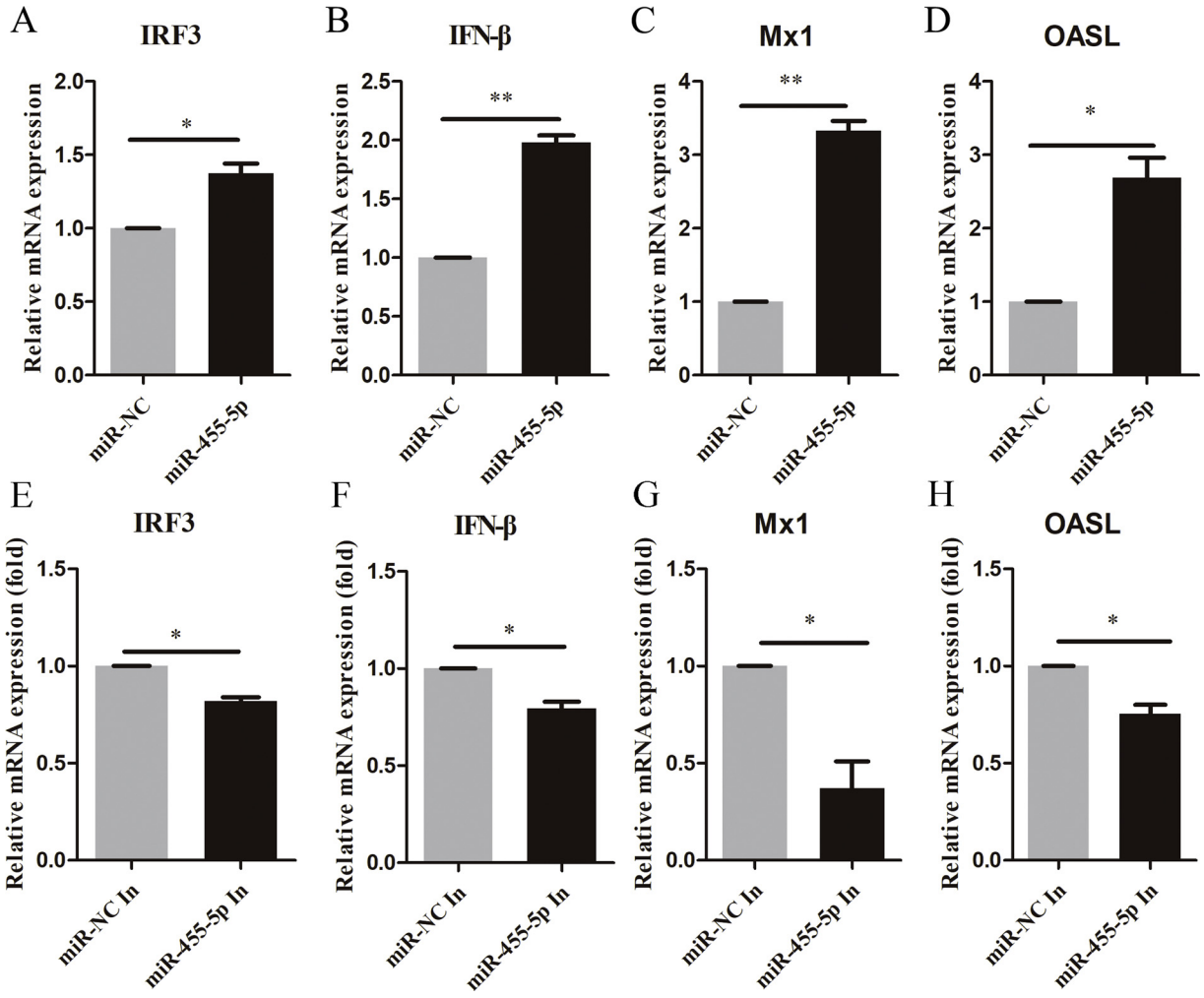

Fig. 6. gga-miR-455-5p enhances NDV-induced expression of IRF3, IFN-β, Mx1, and OASL in CEFs. (A–D) CEFs were transfected with gga-mi-455-5p mimics or a mimics control. Twenty-four hours after transfection, cells were infected with NDV at an MOI of 0.1. Twenty-four hours after infection, cells were harvested, and the expression of IRF3 (A), IFN-β (B), Mx1 (C), and OASL (D) was quantified by qRT-PCR. (E–H) CEFs were transfected with gga-miR-455-5p inhibitors mimics or an inhibitors control. Twenty-four hours after transfection, cells were infected with NDV at an MOI of 0.1. Twenty-four hours after infection, cells were harvested, and the expression of IRF3 (E), IFN-β (F), Mx1 (G), and OASL (H) was quantified by qRT-PCR. Values are the means ± SDs of three independent experiments. Significant differences between groups are indicated by $*p < 0.05$, $**p < 0.01$.

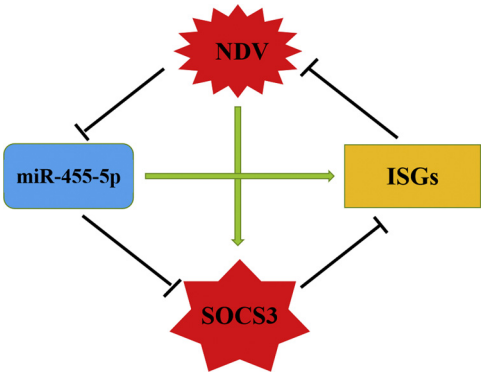

Fig. 7. A model to explain the role of the gga-miR-455-5p/SOCS3 axis in assisting NDV in evading the host innate immune response.

domain and N-terminal kinase inhibitory region (KIR) (Sasaki et al., 1999). The JAK-STAT signaling pathway plays a critical role in the host response to viral infection (O'Shea and Plenge, 2012; Stark and Darnell, 2012). Previous studies have demonstrated that many viruses, such as HIV (Sood et al., 2019), HCV (Collins et al., 2014), DHAV-1 (Xie et al., 2019), and IAV (Pauli et al., 2008; Jia et al., 2010), induce the expression of SOCS3 to escape the IFN-mediated antiviral response. We previously found that overexpression of SOCS3 promotes NDV infection (Wang et al., 2019b). In this study, we found that the targeting of gga-miR-455-5p to SOCS3 was upregulated during NDV infection. Overexpression of gga-miR-455-5p enhanced the expression of INF-β and ISGs (OASL and Mx1). Determining whether gga-miR-455-5p affects the JAK-STAT signaling pathway during NDV infection requires further investigation.

The interactions between miRNAs and viruses are complicated. This study raised several questions that need to be further investigated. For example, how does NDV inhibit the expression of gga-miR-455-5p? Are any other miRNAs besides gga-miR-455-5p involved in the host response to NDV infection? Does gga-miR-455-5p act as a broad-spectrum antiviral agent in the host defense against other viruses? More efforts will be required to elucidate the molecular mechanisms underlying the pathogenesis of NDV infection.

We propose a working model to explain the role of the gga-miR-455-5p/SOCS3 axis in evasion of the innate immune response by NDV. In this model, NDV infection strongly induces SOCS3 expression through inhibiting gga-miR-455-5p expression. The inhibition of gga-miR-455-5p expression inhibits ISGs, promoting NDV replication. In summary,

although more research is needed to understand the delicate regulatory mechanisms by which NDV evades innate immunity, our current findings highlight the function of the gga-miR-455-5p/SOCS3 axis as a critical regulator of NDV-induced immune escape and provide a potential target for the treatment of NDV infection in the future.

Author contributions

Xiangwei Wang and *Zengqi Yang* conceived and designed the experiments, *Xiangwei Wang, Xinglong Wang* and *Haijin Liu* performed the experiments and drafted manuscript, *Xiangwei Wang and Yanqing Jia* analyzed the data, *Juan Ren, Na Huo* and *Sa Xiao* contributed to reagents and materials. All authors read and approved the final manuscript.

Declaration of Competing Interest

All authors declare that there is no conflict of interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2019.108460.

References

Baker, B.J., Akhtar, L.N., Benveniste, E.N., 2009. SOCS1 and SOCS3 in the control of CNS immunity. Trends Immunol. 30, 392–400.

Chai, J., Wang, S., Han, D., Dong, W., Xie, C., Guo, H., 2015. MicroRNA-455 inhibits proliferation and invasion of colorectal cancer by targeting RAF proto-oncogene serine/threonine-protein kinase. Tumor Biol. 36, 1313–1321.

Chu, Z.L., Ma, J.G., Wang, C.Y., Lu, K.J., Li, X.Q., Liu, H.J., Wang, X.L., Xiao, S., Yang, Z.Q., 2018. Newcastle disease virus V protein promotes viral replication in HeLa cells through the activation of MEK/ERK signaling. Viruses 10, E489.

Collins, A.S., Ahmed, S., Napoletano, S., Schroeder, M., Johnston, J.A., Hegarty, J.E., O'Farrelly CStevenson, N.J., 2014. Hepatitis C virus (HCV)-induced suppressor of cytokine signaling (SOCS) 3 regulates proinflammatory TNF-α responses. J. Leukoc. Biol. 96, 255–263.

Dimitrov, K.M., Afonso, C.L., Yu, Q., Miller, P.J., 2017. Newcastle disease vaccines-a solved problem or a continuous challenge? Vet. Microbiol. 206, 126–136.

Fu, M., Wang, B., Chen, X., He, Z., Wang, Y., Li, X., Cao, H., Zheng, S.J., 2017. MicroRNA gga-miR-130b suppresses infectious bursal disease virus replication via targeting of the viral genome and cellular suppressors of cytokine signaling 5. J. Virol. 92, e01646–e01717.

Guo, C.J., Pan, Q., Li, D.G., Sun, H., Liu, B.W., 2009. miR-15b and miR-16 are implicated in activation of the rat hepatic stellate cell: an essential role for apoptosis. J. Hepatol. 50, 766–778.

He, L., Hannon, G.J., 2004. MicroRNAs: small RNAs with a big role in gene regulation. Nat. Rev. Genet. 5, 522–531.

Hu, X., Ye, J., Qin, A., Zou, H., Shao, H., Qian, K., 2015. Both microRNA-155 and virus-encoded miR-155 ortholog regulate TLR3 expression. PLoS One 10, e0126012.

Ingle, H., Kumar, S., Raut, A.A., Mishra, A., Kulkarni, D.D., Kameyama, T., Takaoka, A., Akira, S., Kumar, H., 2015. The microRNA miR-485 targets host and influenza virus transcripts to regulate antiviral immunity and restrict viral replication. Sci. Signal. 8, ra126.

Jia, D., Rahbar, R., Chan, R.W., Lee, S.M., Chan, M.C., Wang, B.X., Baker, D.P., Sun, B., Peiris, J.S., Nicholls, J.M., Fish, E.N., 2010. Influenza virus non-structural protein 1 (NS1) disrupts interferon signaling. PLoS One 5, e13927.

Jia, Y.Q., Wang, X.L., Wang, X.W., Yan, C.Q., Lv, C.J., Li, X.Q., Chu, Z.L., Adam, F.E.A., Xiao, S., Zhang, S.X., Yang, Z.Q., 2018. Common microRNA-mRNA interactions in different newcastle disease virus-infected chicken embryonic visceral tissues. Int. J. Mol. Sci. 19, E1291.

Kumar, A., Kumar, A., Ingle, H., Kumar, S., Mishra, R., Verma, M.K., Biswas, D., Kumar, N.S., Mishra, A., Raut, A.A., Takaoka, A., Kumar, H., 2018. MicroRNA hsa-miR-324-5p suppresses H5N1 virus replication by targeting the viral PB1 and host CUEDC2. J. Virol. 92, e01057–e01118.

Liu, J., Zhang, J., Li, Y., Wang, L., Sui, B., Dai, D., 2016. MiR-455-5p acts as a novel tumor suppressor in gastric cancer by down-regulating RAB18. Gene 592, 308–315.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2 (-DeltaDeltaC(T)) Method. Methods 25, 402–408.

Motz, C., Schuhmann, K.M., Kirchhofer, A., Moldt, M., Witte, G., Conzelmann, K.K., Hopfner, K.P., 2013. Paramyxovirus V proteins disrupt the fold of the RNA sensor MDA5 to inhibit antiviral signaling. Science 339, 690–693.

O'Shea, J.J., Plenge, R., 2012. JAK and STAT signaling molecules in immunoregulation and immune-mediated disease. Immunity 36, 542–550.

Pauli, E.K., Schmolke, M., Wolff, T., Viemann, D., Roth, J., Bode, J.G., Ludwig, S., 2008. Influenza A virus inhibits type I IFN signaling via NF-kappaB-dependent induction of SOCS-3 expression. PLoS Pathog. 4, e1000196.

Peng, X., Gao, Q.S., Zhou, L., Chen, Z.H., Lu, S., Huang, H.J., Zhan, C.Y., Xiang, M., 2015. MicroRNAs in avian influenza virus H9N2-infected and non-infected chicken embryo fibroblasts. Genet. Mol. Res. 14, 9081–9091.

Qin, H., Holdbrooks, A.T., Liu, Y., Reynolds, S.L., Yanagisawa, L.L., Benveniste, E.N., 2016a. SOCS3 deficiency promotes M1 macrophage polarization and inflammation. J. Immunol. 189, 3439–3448.

Qin, L., Zhang, Y., Lin, J., Shentu, Y., Xie, X., 2016b. MicroRNA-455 regulates migration and invasion of human hepatocellular carcinoma by targeting Runx2. Oncol. Rep. 36, 3325–3332.

Qiu, X.S., Fu, Q., Meng, C.C., Yu, S.Q., Zhan, Y., Dong, L.N., Song, C.P., Sun, Y.J., Tan, L., Hu, S.L., Wang, X.Q., Liu, X.W., Peng, D.X., Liu, X.F., Ding, C., 2016. Newcastle disease virus V protein targets phosphorylated STAT1 to block IFN-I signaling. PLoS One 11, e0148560.

Sasaki, A., Yasukawa, H., Suzuki, A., Kamizono, S., Syoda, T., Kinjyo, I., Sasaki, M., Johnston, J.A., Yoshimura, A., 1999. Cytokine-inducible SH2 protein-3 (CIS3/SOCS3) inhibits Janus tyrosine kinase by binding through the N-terminal kinase inhibitory region as well as SH2 domain. Genes Cells 4, 339–351.

Seal, B.S., King, D.J., Sellers, H.S., 2000. The avian response to Newcastle disease virus. Dev. Comp. Immunol. 24, 257–268.

Shuai, K., Liu, B., 2003. Regulation of JAK-STAT signalling in the immune system. Nat. Rev. Immunol. 3, 900–911.

Sood, V., Lata, S., Ramachandran, V.G., Banerjea, A.C., 2019. Suppressor of cytokine signaling 3 (SOCS3) degrades p65 and regulate HIV-1 replication. Front. Microbiol. 10, 114.

Stark, G.R., Darnell, J.E.Jr., 2012. The JAK-STAT pathway at twenty. Immunity 36, 503–514.

Steward, M., Vipond, I.B., Millar, N.S., Emmerson, P.T., 1993. RNA editing in Newcastle disease virus. J. Gen. Virol. 74, 2539–2547.

Wang, B., Fu, M., Liu, Y., Wang, Y., Li, X., Cao, H., Zheng, S.J., 2018. gga-miR-155 enhances type I interferon expression and suppresses infectious burse disease virus replication via targeting SOCS1 and TANK. Front. Cell. Infect. Microbiol. 8, 55.

Wang, X.L., Jia, Y.Q., Wang, X.W., Wang, C.Y., Lv, C.J., Li, X.Q., Chu, Z.L., Han, Q.S., Xiao, S., Zhang, S.X., Yang, Z.Q., 2019a. MiR-375 has contrasting effects on newcastle disease virus growth depending on the target gene. Int. J. Biol. Sci. 15, 44–57.

Wang, X.W., Jia, Y.Q., Ren, J., Huo, N., Liu, H.J., Xiao, S., Wang, X.L., Yang, Z.Q., 2019b. Newcastle disease virus nonstructural V protein upregulates SOCS3 expression to facilitate viral replication depending on the MEK/ERK pathway. Front. Cell. Infect. Microbiol. 9, 317.

Xie, J.Y., Wang, M.S., Cheng, A.C., Zhao, X.X., Liu, M.F., Zhu, D.K., Chen, S., Jia, R.Y., Yang, Q., Wu, Y., Zhang, S.Q., Liu, Y., Yu, Y.Y., Zhang, L., Chen, X.Y., 2019. DHAV-1 inhibits type I interferon signaling to assist viral adaption by increasing the expression of SOCS3. Front. Immunol. 10, 731.

Yoneyama, M., Kikuchi, M., Natsukawa, T., Shinobu, N., Imaizumi, T., Miyagishi, M., Taira, K., Akira, S., Fujita, T., 2004. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. Nat. Immunol. 5, 730–737.

Zhou, B., Wang, S., Mayr, C., Bartel, D.P., Lodish, H.F., 2007. miR-150, a microRNA expressed in mature B and T cells, blocks early B cell development when expressed prematurely. Proc. Natl. Acad. Sci. U. S. A. 104, 7080–7085.

Zhu, J., Hu, S.L., Xu, H.X., Liu, J.J., Zhao, Z.Z., Wang, X.Q., Liu, X.F., 2016. Characterization of virulent Newcastle disease viruses from vaccinated chicken flocks in Eastern China. BMC Vet. Res. 12, 113.