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LGP2 plays a critical role in MDA5-mediated antiviral activity against duck enteritis virus

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ABSTRACT

Duck viral enteritis (DEV) is a DNA virus that leads to heavy economic losses in the commercial duck industry. As a key cytoplasmic sensor, melanoma differentiation-associated gene 5 (MDA5) can recognize viral RNA and enhance the antiviral immune response. Retinoic acid-inducible gene-I (RIG-I) and MDA5 both belong to the RIG-I-like receptors family, and RIG-I is known to be involved in the anti-DEV signaling pathway. However, the role of MDA5 in DEV infection remains unclear. In this study, we used overexpression and knockdown methods to determine if MDA5 affected DEV infection in ducks. We confirmed that DEV infection was significantly suppressed in MDA5-overexpressing DEF cells, while knockdown of MDA5 by siRNA markedly enhanced DEV growth. We demonstrated that overexpression of duck MDA5 significantly upregulated expression of interferon (IFN)-stimulated genes, including myxovirus resistance protein (Mx), IFN-induced oligodenylate synthetase-like (OASL), IFN-induced transmembrane protein 1 (IFITM1) and IFN-β. In addition, the transcriptional level of MDA5 was upregulated both *in vivo* and *in vitro* upon DEV infection. We also showed that there was an association between MDA5 and laboratory of genetics and physiology 2 (LGP2) in antiviral signaling. LGP2 functioned as a concentration-dependent switch between MDA5-specific enhancement and interference. Overall, these findings indicated that MDA5 restricted DEV replication and LGP2 plays a critical role in MDA5-mediated antiviral activity against DEV.

1. Introduction

Duck viral enteritis, also known as duck plague, is an acute and contagious infection of ducks, geese and swans of all age and species (Davison et al., 1993). Duck viral enteritis is characterized by vascular damage, eruptions on the mucosal surface of the gastrointestinal tract, lesions of the lymphoid organs, and degenerative changes in parenchymal organs (Dhama et al., 2017). The disease is caused by duck enteritis virus (DEV), which is a member of the subfamily Alphaherpesvirinae of the family Herpesviridae (Montali et al., 1976). DEV leads to heavy economic losses in the commercial duck industry due to its high mortality rate and decreased egg production (Burgess and Yuill, 1982; Dhama et al., 2017).

The innate immune response provides the first line of defense against viral infection. Pattern recognition receptors (PRRs) directly sense the presence of pathogen-associated molecular patterns (PAMPs) (Goubau et al., 2013). RIG-I like receptors (RLRs) are a family of cytoplasmic PRRs that sense viral PAMPs in the cytosol (Loo and Gale, 2011). Three members have been identified in this family: RIG-I, MDA5, and LGP2. RIG-I and MDA5 have three domains: two tandem N-terminal caspase recruitment domains (CARDs), a DExD/H-box helicase, and a C-terminal repressor domain (RD). LGP2 has DExD/H-box helicase and RD domains, but lacks the CARD(Loo and Gale, 2011). RIG-I and MDA5 recognize distinct subsets of PAMPs. RIG-I detects the 5'-triphosphate group (5'ppp) and blunt end of short dsRNAs (< 300 base pairs), MDA5 detects long-duplex RNAs (Loo et al., 2008).

While the mechanisms of RIG-I regulation are already well understood, less is known about MDA5 and LGP2 (Barral et al., 2009). As an important RNA sensor implicated in the detection of viral infection, MDA5 is involved in the recognition of many viruses, including norovirus, encephalomyocarditis virus (EMCV), hepatitis C virus (HCV) and highly pathogenic avian influenza virus (HPAIV) (Cao et al., 2015;

Abbreviations: MDA5, melanoma differentiation-associated gene 5; Mx, myxovirus resistance protein; OASL, interferon-induced oligodenylate synthetase-like; IFITM1, interferon induced transmembrane protein 1; LGP2, laboratory of genetics and physiology 2; RIG-I, retinoic acid inducible gene I; DEF, duck embryo fibroblast; DEV, duck enteritis virus; gB, glycoprotein B; siRNA, small interfering RNA

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Dang et al., 2018; Li et al., 2019; Wei et al., 2014). However, few studies have examined the function of MDA5 in DNA virus infections (Lu and Liao, 2013). It is reported that MDA5 senses hepatitis B virus and activates innate immune signaling to suppress virus replication (Lu and Liao, 2013), others also demonstrated that MDA5 was the primary mediator of HSV recognition(Melchjorsen et al., 2010), but the role of duck MDA5 in DEV infection has not been clarified.

For LGP2, the absence of CARDs results in failure of autonomous transducer signaling, but it potentially cooperates with other PRRs to regulate IFN signaling (Komuro and Horvath, 2006). However, the exact roles of LGP2 in regulating IFN signaling remain controversial. The antithetical roles of LGP2 as a negative or positive regulator in antiviral responses have been reported (Bruns and Horvath, 2015; Rodriguez et al., 2014). In this study, we examined the relationship between duck MDA5 and DEV infection, and between MDA5 and LGP2. The results of this study help to clarify the roles of MDA5 and LGP2 in the regulation of antiviral signaling.

2. Materials and methods

2.1. Cells and viruses

Duck embryo fibroblasts (DEFs), derived from the 11-day-old duck embryo, were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, China) supplemented with 5% fetal bovine serum (FBS; Clark, USA) at 37°C in 5% CO_2. DEV CSC strain was obtained from the China Institute of Veterinary Drug Control (Beijing, China). Mouse monoclonal antibody against glycoprotein B (gB) was maintained in our laboratory, and produced by Genscript biotechnology limited company.

2.2. Plasmid construction and transfection

The duck MDA5 and LGP2 genes were amplified from DEF cDNA using the primers listed in Table 1 and then cloned into a pCAGGS-MCS-C-Flag and pCAGGS-MCS-C-Myc vector, respectively. DEF cells (10^6) were seeded overnight in 6-well plates, and transfected with 2 μg pFlag-MDA5 or pMyc-LGP2 using Lipofectamine 3000 (Invitrogen). The cells were used for further analysis at 24 h post-transfection.

2.3. Viral $TCID_{50}$ determination

Viral titers were determined by an endpoint dilution assay and expressed as the $TCID_{50}$/ml using the Reed-Münch method. DEF cells were seeded in 96-well plates and inoculated with serial 10-fold dilutions of virus, with eight replicates per dilution. The supernatants was removed after adsorption for 2 h at 37°C, and the cells were washed three times with PBS, then DMEM with 2% FBS was added to each well. The plates were incubated for 96 h, and the viral titers were calculated based on the cytopathic effect.

2.4. Real-time quantitative polymerase chain reaction

Viral DNA was extracted from DEF cells using a Nucleic Acid Extraction Kit (Axygen, USA) and quantified by TaqMan real-time quantitative polymerase chain reaction (RT-qPCR). The primers and probes are listed in Table 1. RT-qPCR was performed with the following cycling conditions: 95 °C for 30 s for activation, followed by 40 cycles at 95 °C for 5 s and 60 °C for 50 s. Total RNA was isolated from DEF cells using an RNeasy Mini Kit (Qiagen, Germany) and reverse transcribed into cDNA with aPrimeScript™ RT Reagent Kit with gDNA Eraser (Takara, Japan). The cDNA generated was analyzed by RT-qPCR with 2 × SYBR Green qPCR Master Mix (S-2014-T) (US Everbright Inc, Suzhou, China). The specific primers used to amplify duck MDA5, IFN-β, Mx, OASL, IFITM1 and β-actin cDNAs have been described previously (Barber et al., 2010; Chen et al., 2017, 2018). qPCR was conducted under the following cycling conditions: 95 °C for 30 s for activation, followed by 40 cycles at 95 °C for 5 s and 60 °C for 34 s. The relative expression levels of duck RIG-I, IFN-β, Mx, OASL and IFITM1 were determined using the comparative Ct ($2^{-\Delta\Delta t}$) method with the β-actin gene as a control.

2.5. Western blotting

The cell lysates were resolved on 10% SDS-PAGE, and the separated proteins were electroblotted onto nitrocellulose membranes (Millipore, USA). The membranes were blocked with 5% skimmed milk and incubated with rabbit-anti-MDA5 (Sigma, Canada), mouse anti-β-actin (Sigma, Canada), or mouse anti-gB antibody. IRDye-800-CW-COnjugated goat anti-mouse IgG antibody (LI-COR Biosciences, USA) or IRDye-800CW-COnjugated goat anti-rabbit IgG antibody (LI-COR Biosciences, USA) was used as the secondary antibody at a dilution of 1:5000. Signal detection was performed using an Odyssey Infrared Fluorescence Scanning Imaging System (LI-COR Biosciences, USA).

2.6. RNAi assay

siRNA sequence targeting duck MDA5 was prepared. siMDA5 and negative control siRNA were synthesized by GenePharma (Suzhou, China). The siMDA5 sequence were 5′-GGAUGUCGCUACAGAAGA UTT-3′ (sense) and 5′-AUCUUCUGUAGCGACAUCCTT-3′ (antisense),

Table 1
Primers used in this study.

Primer	Sequence (5'-3')	Use
Flag-dMDA5-F	TATTAGCATGCTCATGTCGACGGAGTGC	Amplification of dMDA5
Flag-dMDA5-R	CGGCGCTAGCTTAGTCTTCATCACT	Amplification of dMDA5
Myc-dLGP2-F	ATAGAATTCGAATGGAGCTGCGCGGGTAC	Amplification of dLGP2
Myc-dLGP2-R	AGCTCGAGCGAAGGACTCGTCCTC	Amplification of dLGP2
Q-dIFN-β-F	AGATGGCTCCCAGCTCTACA	QRT-PCR for detection of dIFN-β
Q-dIFN-β-R	AGTGGTTGAGCTGGTTGAGG	QRT-PCR for detection of dIFN-β
Q-dMDA5-F	GCTACAGAAGATAGAAGTGTCA	QRT-PCR for detection of dMDA5
Q-dMDA5-R	CAGGATCAGATCTGGTTCAG	QRT-PCR for detection of dMDA5
Q-dDEV-F	TGGGAAGGCTTTCGGTCGC	QRT-PCR for detection of DEV
Q-dDEV-R	CATTCGCGCCTTTGCTAAATTCTCT	QRT-PCR for detection of DEV
Q-dMx-F	TGCTGTCCTTCATGACTTCG	QRT-PCR for detection of dMx
Q-dMx-R	GCTTTGCTGAGCCGATTAAC	QRT-PCR for detection of dMx
Q-dOASL-F	TCTTCCTCAGCTGCTTCTCC	QRT-PCR for detection of dOASL
Q-dOASL-R	ACTTCGATGGACTCGCTGTT	QRT-PCR for detection of dOASL
Q-dIFITM1-F	AACCCTACGGCAGGAATG	QRT-PCR for detection of dIFITM1
Q-dIFITM1-R	GAAGACAAGAGCGAGGAAGC	QRT-PCR for detection of dIFITM1
Q-dβ-actin-F	GATCACAGCCCTGGCACC	QRT-PCR for detection of dβ-actin
Q-dβ-actin-R	CGGATTCATCATACTCCTGCTT	QRT-PCR for detection of dβ-actin

and the non-targeting control siRNA (siNC) sequences were 5′-UUCUC CGAACGUGUCACGUTT-3′ (sense) and 5′-ACGUGACACGUUCGGAGA ATT-3′ (antisense). DEFs were transfected with siRNAs with Lipofectamine RNAiMAX Transfection Reagent (Thermo Fisher, USA) according to the manufacturer's instructions. The DEF cells were then infected with DEV at an MOI of 0.1 at 36 h post-transfection. The cell medium was replaced with DMEM containing 2% FBS after 2 h, and the cells were then incubated at 37℃. For 24, 36, and 48 h, respectively, after which the supernatants were collected for analysis. DEF cells were collected for further analysis at 48 h post-transfection.

2.7. Animal infection experiments

Eighteen 21-day-old specific-pathogen-free ducklings were obtained from the Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences. The ducklings were divided randomly into two groups: an infection group that received an intramuscular injection of 0.2 ml DEV per duck ($TCID_{50} = 10^5$/ml) and a control group that received an intramuscular injection of 0.2 ml DMEM. Three ducklings from each group were killed at 1, 3, and 5 days post-infection (dpi), respectively, and tissue samples were collected. MDA5 mRNA expression levels were evaluated by qPCR as described above.

3. Results

3.1. MDA5 inhibits DEV infection

To explore the antiviral activity of MDA5 against DEV, we transfected DEF cells with pFlag-MDA5 and then challenged them with DEV at MOI of 0.1 and 1. The extracellular titers of progeny virus (Fig. 1A), the number of viral genomic copies (Fig. 1B), and the intracellular viral genome copy numbers in infected cells (Fig. 1C) were significantly reduced in MDA5-transfected DEFs compared with vector-treated control DEF cells at 24, 36, and 48 h post-transfection. The intracellular expression level of the gB protein were reduced in MDA5-transfected cells (Fig. 1D). These data indicated that MDA5 exerts antiviral effects against DEV infection.

3.2. Inhibition of MDA5 facilitates DEV infection

To further examine the antiviral effects of MDA5 on DEV, siRNA was used to silence the expression of endogenous MDA5 in DEFs, resulting in efficient reduction of MDA5 expression (Fig. 2A and B). Subsequently, the replication efficiency of DEV was assessed upon silencing MDA5. The viral titer and genome copy number were higher in siMDA5-transfected compared with siNC-transfected DEF cells (Fig. 2C and D). The expression of gB protein were increased in siMDA5-transfected DEF cells (Fig. 2E). The results demonstrated that inhibition of MDA5 expression facilitated DEV infection

3.3. Overexpression of MDA5 induces IFN-β and ISG expression in DEF cells

We further examined the ability of MDA5 to induce IFN-β and ISGs by analyzing the expression levels of IFN-β, Mx, OASL and IFITM1 by qPCR. Overexpression of MDA5 in infected or uninfected DEF cells

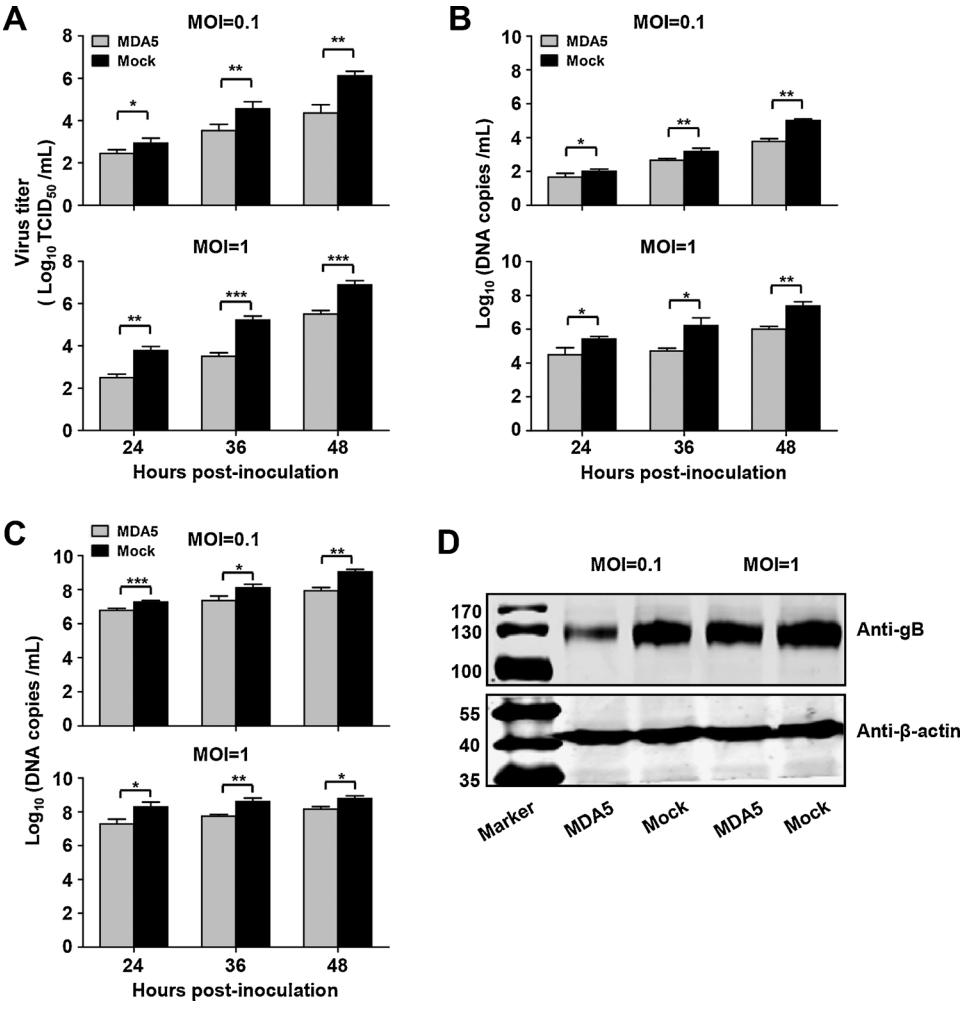

Fig. 1. MDA5 inhibits DEV infection. DEF cells were transfected with pFlag-MDA5 or mock vector, and were infected with DEV at MOI 0.1 or 1 for 24, 36 and 48 h, respectively. (A) Viral titers in the supernatants were detected by endpoint dilution assay and are presented as $TCID_{50}\,ml^{-1}$. (B) Copies of DEV in the supernatants and (C) Intracellular genomic copy numbers of DEV were quantified by RT-qPCR. (D) Expression of gB in cell lysates was analyzed by western blotting, with β-actin protein as the control. Each sample was run in triplicate. Error bars represent standard deviations. Significant differences in virus titer and DNA copies between MDA5-transfected and mock-transfected DEF cells were assessed using the Student's *t*-test, *P < 0.05, **P < 0.01, ***P < 0.001.

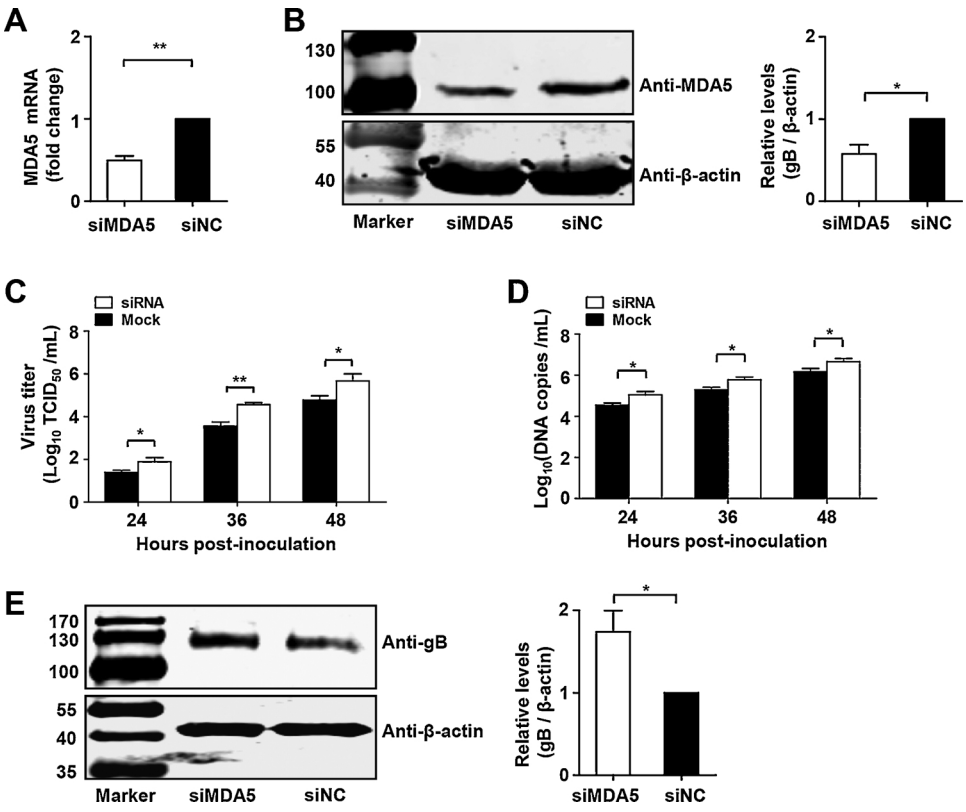

Fig. 2. Knockdown of duck MDA5 promotes DEV infection. (A) DEF cells transfected with siMDA5 or siNC were harvested at 36 h after transfection and the efficiency of RIG-I knockdown was confirmed by RT-qPCR. (B) DEF cells transfected with siMDA5 or siNC were harvested at 36 h after transfection. Expression of MDA5 in the cell lysates was analyzed by western blotting, with β-actin protein as the control. (C–E) DEF cells were transfected with siMDA5 or siNC mRNA for 36 h, and then infected with DEV at MOI 0.1 or 1 for 24, 36 or 48 h, respectively. (C) Viral titers in the supernatants were detected by endpoint dilution assay and are presented as $TCID_{50}\,ml^{-1}$. (D) Copies of DEV in the supernatants were assessed by RT-qPCR. (E) Expression of gB in the cell lysates was analyzed by western blotting, with β-actin protein as the control.Each sample was run in triplicate. Error bars represent standard deviations. Statistical significance was assessed using the Student's *t*-test, *P < 0.05, **P < 0.01, ***P < 0.001.

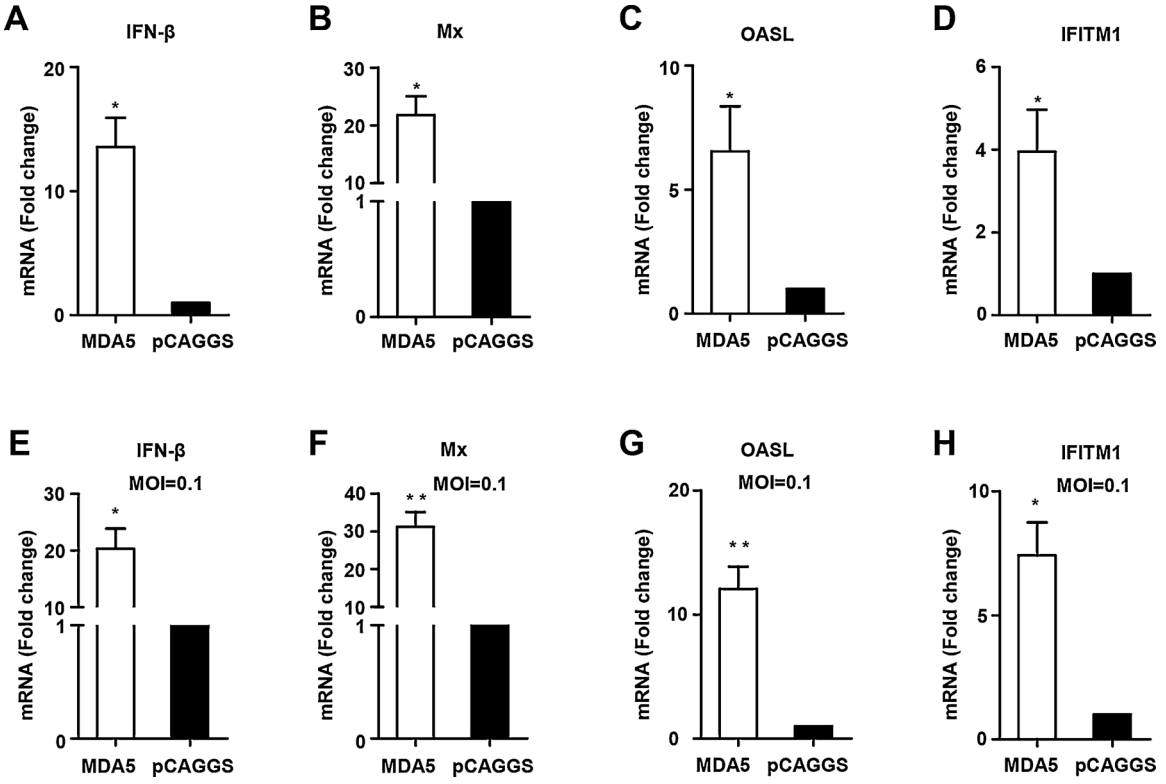

Fig. 3. IFN-β and ISG expression levels in DEF cells. (A–D) DEF cells were transfected with pFlag-MDA5 or mock vector for 48 h and IFN-β, Mx, OASL and IFITM1 gene expression levels were detected by RT-qPCR. (E–H) DEF cells were transfected with pFlag-MDA5 or mock vector for 24 h, and were infected with DEV at MOI 1 for 24 h. IFN-β, Mx, OASL and IFITM1 gene expression levels were detected by RT-qPCR. Significant differences in IFN-β, Mx, OASL and IFITM1 gene expression levels between MDA5-transfected and mock-transfected DEF cells were assessed using the Student's *t*-test, *P < 0.05, **P < 0.01, ***P < 0.001.

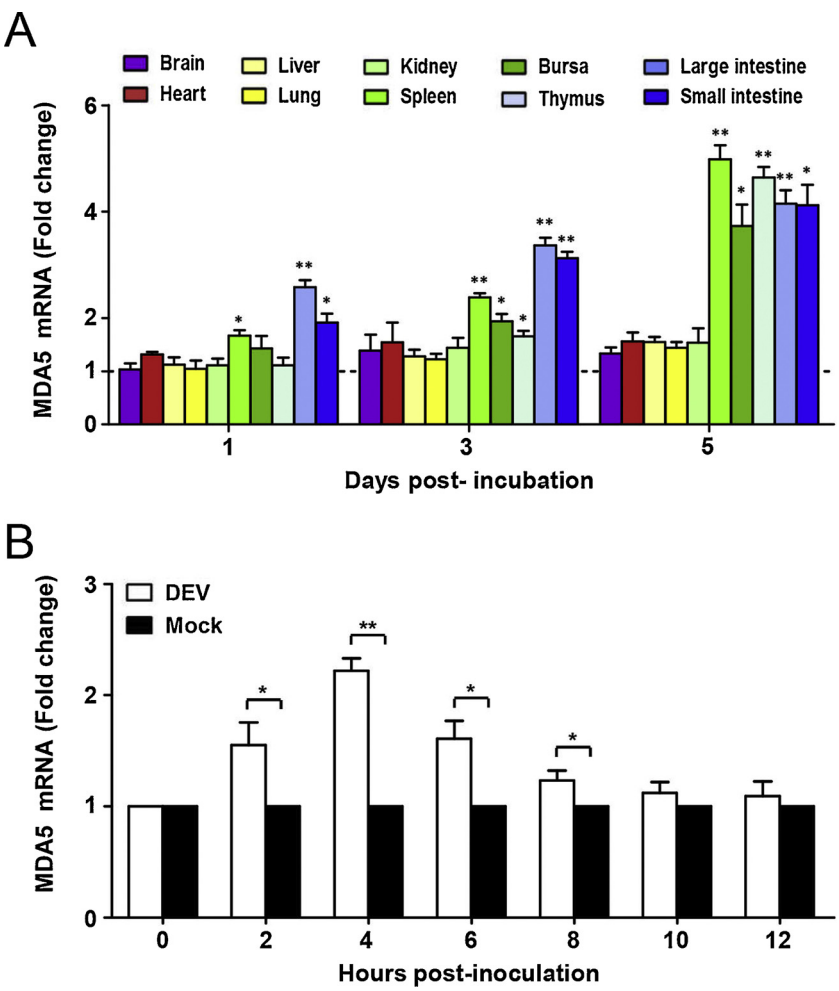

Fig. 4. Effects of DEV on expression of MDA5 *in vivo* and *in vitro*. (A) Eighteen 21-day-old specific-pathogen-free ducklings were divided randomly into an infection group (intramuscular injection of 0.2 ml DEV ($TCID_{50} = 10^5\ ml^{-1}$) and control group (intramuscular injection of 0.2 ml DMEM). Three ducklings from each group were killed and tissue samples were collected at 1, 3, and 5 dpi. MDA5 gene expression levels were measured in various organs by RT-qPCR. (B) DEF cells were infected with DEV at MOI 0.1 for 0, 2, 4, 6, 8, 10 or 12 h and cell samples were collected. MDA5 gene expression levels in DEV-infected DEF cells were examined by RT-qPCR. Each sample was run in triplicate. Error bars represent standard deviations. Significant differences in MDA5 expression levels between DEV-infected and mock-infected groups were assessed using the Student's *t*-test, *P < 0.05, **P < 0.01, ***P < 0.001.

upregulated IFN-β expression 13.56- and 20.33-fold, respectively (P < 0.05; Fig. 3A and E). Mx expression increased by 21.79- and 31.19-fold, respectively (P < 0.01; Fig. 3B and F). OASL expression increased by 6.54- and 12.05-fold, respectively (P < 0.01; Fig. 3C and G). IFITM1expression increased by 3.95-and 7.41-fold, respectively (P < 0.05; Fig. 3D and H).

3.4. Effects of DEV on MDA5 expression in vivo and in vitro

To determine if DEV infection activated the host innate immune response *in vivo*, we collected brain, heart, liver, spleen, lung, kidney, bursa of Fabricius, thymus, large intestine, and small intestine from infected ducklings, and assessed MDA5 expression. MDA5 expression was universally upregulated in response to DEV infection in ducks (Fig. 4A). MDA5 was upregulated in the spleen and intestinal tract at 1 dpi, and MDA5 mRNA levels were increased in the spleen, bursa of Fabricius, thymus, large intestine and small intestine at 3 and 5 dpi. The results demonstrated that MDA5 expression was significantly upregulated in the spleen, large intestine and small intestine at all time points.

To confirm that DEV infection activated the host innate immune response *in vitro*, we infected DEF cells with DEV and determined MDA5 mRNA expression levels from 0 to 12 h. MDA5 mRNA levels were increased at 2 h post-infection (hpi), peaked at 4 hpi, and began to decline from 6 hpi (Fig. 4B). MDA5 mRNA levels increased significantly at 2 hpi and returned to baseline at 12 hpi (Fig. 4B). These data demonstrate that MDA5 expression increased significantly *in vitro* following DEV infection, indicating that they may play important roles in resisting DEV infection.

3.5. Association of MDA5 with LGP2 in antiviral signaling

To determine if LGP2 associated with MDA5 and regulated MDA5-mediated antiviral signaling, pFlag-MDA5 was transfected at a constant 2 μg plasmid/well, while the amount of pMyc-LGP2 was titrated. Titration of pMyc-LGP2 by plasmid transfection revealed that low concentrations of LGP2 (0.5 μg plasmid/well) enhanced the MDA5-mediated antiviral effect, while high concentrations (2 μg plasmid/well) inhibited MDA5 antiviral effect (Fig. 5A). The results also showed that low concentration of LGP2 (0.5 μg plasmid/well) enhanced IFN-β expression, while high concentration of LGP2 (2 μg plasmid/well) downregulated IFN-β expression (Fig. 5B).

4. Discussion

As a key cytosolic sensor of RNA viruses, MDA5 has been shown to play a critical role in H5N1 highly pathogenic avian influenza virus infections (Wei et al., 2014). However, there remains a lack of research into its antiviral activity in other avian species. In this study, we showed that MDA5 inhibited DEV infection, and its knockdown promoted DEV infection. Further analysis revealed that overexpression of MDA5 significantly upregulated expression of ISGs, including Mx, OASL, IFITM1 and IFN-β. Furthermore, we revealed that LGP2 associated with MDA5 and regulated MDA5-mediated antiviral signaling.

MDA5 has been reported to play an important role in regulating host immunity against various viruses (Banos-Lara Mdel et al., 2013; Chauveau et al., 2012; Kim and Myoung, 2018). Since RIG-I are already well understood and we have previously demonstrated that RIG-I restricts DEV infection (Huo et al., 2019), less is known about MDA5. We

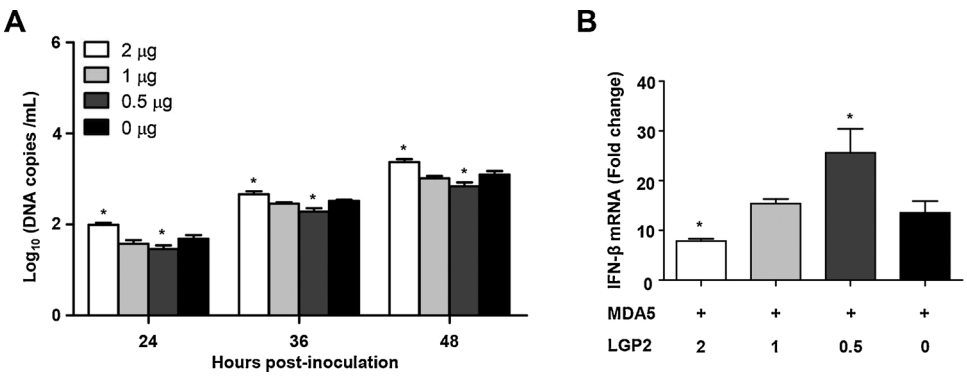

Fig. 5. Association of MDA5 with LGP2 in antiviral signaling. pFlag-MDA5 was transfected at a constant 2 μg plasmid/well, while the amount of pMyc-LGP2 was titrated. (A) DEFs were challenged with DEV at MOI 0.1 at 24 h after transfection. The numbers of viral genome copies in the supernatant of DEFs at 24, 36 and 48 hpi were quantified by RT-qPCR. (B) At 48 h after transfection, IFN-β expression levels were detected by RT-qPCR. Significant differences in IFN-β expression levels between LGP2-transfected and mock-transfected DEF cells were assessed using the Student's *t*-test, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

therefore speculated that MDA5 plays an important role in DNA viral infection in ducks. We explored this using MDA5 overexpression and knockdown methods, and showed that MDA5 significantly suppressed DEV infection in DEF cells, while MDA5 was knocked down by siRNA promoted DEV infection in DEF cells. These data suggested that MDA5 acted as a host antiviral factor during DEV infection in ducks.

Upon activation of MDA5 by virus, the CARDs interact with MAVS, ultimately leading to the transcription of the genes encoding IFNs. IFN stimulation subsequently induces transcription of ISGs, such as Mx, OASL and IFITM1. ISGs encode specific proteins that have antiviral properties, interfering with virus at different stages of the replication cycle (Hartmann, 2017). In the context of ISGs as antiviral molecules, we explored the mechanism of MDA5-mediated antiviral activity. IFN-β, Mx, OASL and IFITM1 expression levels were upregulated when MDA5 was overexpressed in DEV-infected or mock-infected DEF cells. This indicated that MDA5 played an important role in regulating expression of IFN-β and ISGs in ducks, and the antiviral effect of MDA5 may be due to subsequent ISGs expression.

In the event of viral infection, the host innate immune system is the first defense mechanism invoked to eliminate the virus. IFNs are a group of antiviral cytokines involved in antiviral immunity, and binding of IFNs to their cognate receptors induces expression of IFNs and ISGs, thus eliciting an antiviral response (Katze et al., 2002). Our previous study has shown that viral loads continued to increase in various organs in ducklings infected with DEV throughout the period of infection, with the highest viral load in the intestinal tract, followed by the spleen. These data indicated that DEV primarily replicates in the mucosa of the digestive tract and then spreads to the immune organs (Huo et al., 2019). In this study, MDA5 expression was upregulated by DEV infection *in vivo*; notably, in the intestinal tract and immune organs. These results indicate that a MDA5-dependent pathway might be involved in the anti-DEV immune response in ducks. MDA5 mRNA levels increased significantly at 2 hpi and returned to baseline at 12 hpi *in vitro*. We speculated that DEV infection induces a host type I IFN response including MDA5 up-regulation during early infection but functionality of this response is subsequently attenuated by viral proteins, so the mRNA level of MDA5 returned to baseline.

Many lines of evidence support the concept of synergy between the cytosolic RNA sensor LGP2 and MDA5. Combining information from a variety of sources has suggested a theoretical foundation for understanding the mechanisms of LGP2 as both a positive and negative regulator of RLR signaling (Bruns et al., 2014, 2013; Childs et al., 2013). We demonstrated that there is an association of MDA5 with LGP2 in antiviral signaling, and that LGP2 functions as a concentration-dependent switch between MDA5-specific enhancement and interference. Titration of LGP2 yielded a biphasic effect on MDA5 signaling. The primary effect of LGP2 was to enhance MDA5-mediated IFN-β production, and further titration of LGP2 expression ultimately reached a concentration that inhibits MDA5 signaling activity, driving IFN-β expression back toward baseline. These data are consistent with other results (Bruns et al., 2013; Pippig et al., 2009), indicating that MDA5 and LGP2 were associated as accomplices and antagonists of anti-DEV signal transduction.

In conclusion, this study demonstrated that duck MDA5 inhibited DEV infection and MDA5 played an essential role in the induction of IFN-β and ISG expression. Furthermore, we demonstrated that DEV infection induced expression of MDA5 in various organs in ducks and in DEF cells. Our data also showed that titration of LGP2 yields a biphasic effect on MDA5 signaling. The finding will be helpful for understanding the molecular mechanism of MDA5 against DEV infection and may provide important information for the future development of new antiviral drugs. However, further studies are required to clarify the precise mechanism underlying the antiviral activity of MDA5 in ducks.

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Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

Ethical statement

This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and approved by the Harbin Veterinary Research Institute. The animal Ethics Committee approval number is SYXK (Hei) 2017-009.

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