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RESEARCH ARTICLE

Matrine Inhibits Replication of Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) by Influencing the Activation of Nsp9

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ABSTRACT

Matrine is the major active compound found in the root of Chinese herbal medicine *Sophora flavescens* and has a wide range of pharmacological efficacies. Our previous studies showed that Matrine could inhibit the replication of porcine reproductive and respiratory syndrome virus (PRRSV) directly. However, the underlying inhibition mechanism remains unknown. The main aim of this study was to explore the inhibitory mechanism of Matrine in PRRSV replication *in vitro*. The mRNA expression of PRRSV N gene was determined using RT-qPCR. The expression of pERK1/2 protein was evaluated using Western blot and the effect of Matrine on PRRSV Nsp9 activity was studied using dual luciferase reporter gene assay. The results showed that Matrine suppress the mRNA transcription of PRRSV N gene in Marc-145 cells at 3 h and 24 h post PRRSV infection and had no inhibitory effect on the ERK1/2 signal pathway activated by PRRSV. The results of dual luciferase reporter gene assay showed that Matrine suppressed the interaction of Nsp9 with promoter p107. Taken into account the results above, our study demonstrated that Matrine inhibit the activity of Nsp9 to suppress the virus replication.

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INTRODUCTION

All over the world, significant economic losses have been occured to the swine industry due to porcine reproductive and respiratory syndrome virus (PRRSV) (Nieuwenhuis *et al*., 2012). PRRSV is a single stranded enveloped virus, which belongs to the Arteriviridae Nidovirales (Gorbalenya *et al*., 2006). PRRSV genome size is almost 15 kb and its RNA consist of 10 open reading frames (ORFs), a 5' and a 3'-UTR (untranslated region) (Yun and Lee, 2013; Kappes and Faaberg, 2015). ORF1a and ORF1b makes up about 80% of the entire genome of PRRSV, encoding the nonstructural proteins (Nsps) of the virus, most of which assemble into a membrance-associated replication and transcription complex. The function of this membrane complex is to mediate genomic replication as well as to controls the nested set of subgenomic mRNAs synthesis, who's role is to express the structural proteins (Dokland, 2010; Yun and Lee, 2013; Kappes and Faaberg, 2015). ORF7 encodes the nucleocapsid (N) protein which is highly abundant and immunogenic protein of PRRSV (Dokland, 2010). The Nsp9 of PRRSV, containing the viral replicase RNA-dependent RNA polymerases (RdRp), plays a critical role in the replication of virus (Fang and Snijder, 2010; Lehmann *et al*., 2016). The SDD (Serine-Aspartic acid-Aspartic acid) motif, which located at residues 3050-3052 of Nsp9, has been recognized to be vital for its polymerase activity and virus replication (Zhou *et al*., 2011). Recent studies have found that a number of host proteins interact with Nsp9 and then affect the replication of the virus (Dong *et al*., 2014; Li *et al*., 2014; Zhao *et al*., 2015). PRRSV could be inhibited by specific siRNA targeting Nsp9 gene (Xie *et al*., 2014). Dong (2015) used the dual luciferase reporter assay to detect the interaction between Nsp9 and the E2F reaction promoter p107 and

the results indicated that Nsp9 could activate the promoter p107, consequently promote the cell to enter the S phase. It is conducive to the virus replication.

Chinese traditional herbal medicines could not only directly inactivate the virus, but also enhance the anti-virus ability of immune system. Therefore, increasingly attention was gained on Chinese traditional medicines or their extractive antiviral function such as *Cryptoporus volvatus*, *Kumazasa*, Baicalin, Tea seed saponins and Sodium tanshinone IIA sulfonate etc (Iwata *et al*., 2010; Gao *et al*., 2013; Li *et al*., 2015; Zhu *et al*., 2015; Wang *et al*., 2016). Matrine, an alkaloid that is extracted from *Sophora flavescens Ait*, has a lot of biological activities (Sun *et al*., 2014; Yong *et al*., 2015; Wu *et al*., 2016). Our previous study showed that Matrine has a direct role in the inactivation of PRRSV and causes the inhibition of the virus replication in Marc-145 cells (Sun *et al*., 2014). However, the molecular mechanism of this specific inhibition remains unclear.

Signaling pathways has a crucial role in the diverse biological processes regulation. Virus could utilize these complicated mechanisms to escape by suppressing both the host adaptive and innate immune systems (Zhou and Zhang, 2012). Previous studies have shown that viruses require active ERK1/2 to enter the host cells. ERK1/2 has a function in gene transcription as well as in protein expression of some viruses, or some viruses use ERK1/2 to increase their progeny (Zhou and Zhang, 2012; Kim and Lee, 2015). Previous study had reported that PRRSV could activate ERK1/2 signal pathway at the early stage of PRRSV infection in porcine alveolar macrophages (PAM) cells (Lee and Lee, 2010). Therefore, ERK1/2 signaling pathway can be used as a potent target for the antiviral study.

In this study, the effects of Matrine on the mRNA expression of PRRSV N gene, the protein expression of pERK1/2 and the PRRSV Nsp9 activity were also evaluated to explore the Matrine molecular mechanism against PRRSV *in vitro*.

MATERIALS AND METHODS

Cells, virus, drug and reagents: Marc-145 cells were grown in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin at 37ºC in an atmosphere with 5% CO_2. The PRRSV strain was kindly provided by Jiangsu Academy of Agricultural Sciences. In this study, the final concentration of Matrine is 0.75mg/mL (Sun *et al*., 2014).

U0126-EtOH (U0126) as an inhibitor of MEK1/2 was purchased from Selleck. MTT assays suggested that 30 μM of U0126 was safe and thus was used for this experiment (Date not shown).

Western blot analysis of pERK1/2 protein: Marc-145 cells were seeded in 6-well plates. When the cells were confluent in monolayer, medium was discarded and cells were infected with 2 mL 100$TCID_{50}$ PRRSV for 2 h. Then, 2 mL fresh medium was added to virus control group, 0.75 mg/mL Matrine to Matrine treatment group and 30 μM U0126 to U0126 treatment group, respectively. The cell control group and Matrine alone control group were also prepared. The groups were shown in Table 1. There were three repeats in each group. Cell samples were collected following PRRSV infection at 3 h, 6 h, 12 h and 24 h. Cell lysates were prepared and protein concentration was determined using the bicinchoninic acid protein assay reagent (Beyotime, China). Levels of pERK1/2 (1:1000; Cell Signaling Technology) and GAPDH (1:2000; Proteintech) were measured by Western blot analysis.

Detection of PRRSV N gene using RT-qPCR: RT-qPCR was used to detect the mRNA expression of PRRSV N gene in collected samples of PRRSV control group and Matrine treatment group. Total RNAs were extracted using Trizol Reagent (Invitrogen, USA) and transcribed reversely to cDNA with PrimeScript™ RT reagent Kit with gDNA Eraser (Takara, China). To examine N gene expression, RT-qPCR was performed using SYBR Green qPCR Master Mix (Biotool, China). The N gene primers were listed in Table 2.

Plasmid construction: The p107 promoter DNA or Nsp9 gene was amplified by polymerase chain reaction (PCR) using primers listed in Table 2. PCR products were isolated by Agarose gel electrophoresis and inserted into the expression vector pGL4.10[luc2] (Promega, USA) or pcDNA3.1(+) at the appropriate restriction site. Following selection by colony PCR, all of the plasmid sequences were confirmed by DNA sequencing (Beijing Genomics Institute, China). The yielding plasmid was named as pGL4.10-p107 and pcDNA3.1-Nsp9. pGL4.74 [hRluc/TK] (pGL4.74) vector (Promega, USA) was transfected into the cells as the internal reference to detect the expression of firefly's luciferase gene. All plasmids were extracted according to the manufacturer's instructions of QIAGEN Plasmid Mini Kit.

Table 1: The groups for studying the effect of Matrine on the expression of pERK1/2 protein

Reagents	Cell control group	PRRSV control group	Matrine alone control group	Matrine treatment group	U0126 treatment group
PRRSV	-	+	-	+	+
Matrine	-	-	+	+	-
U0126	-	-	-	-	+

Table 2: PCR Primers used in this study and the PCR product size

Gene	Primer Sequences (5'—3')	PCR product size
N	F: AGAAGCCCCATTTCCCTCTA R: CGGATCAGACGCACAGTATG	196 bp
Nsp9	F:*CGGGATCC*ACCATGCTAGCCGCCAGCGGCTTGAC R:*GCTCTAGA*AACTCATGATTGGACCTGAGTT	1929 bp
p107	F : *CGAGCTC*GGAAGATCTTTTTTTTGTCCCGCCGGCTC R:*GCTCTAGA*CCCAAGCTTTTCAGGCCCCGCGGGCGGCG	268 bp

Table 3: The groups for dual luciferase reporter gene assay

Plasmids	Vector control group I	Vector control group II	Reference group	Treatment group
pGL4.10-p107	+	+	+	+
pGL4.74 [hRluc/TK]	+	+	+	+
pcDNA3.1(+)	-	+	-	-
pcDNA3.1-Nsp9	-	-	+	+
0.75 mg/mL Matrine	-	-	-	+

Dual luciferase reporter gene detection: Marc-145 cells were seeded in 24-well plate. At around 60% confluence, the plasmids were transfected using 0.75 μL Lipofectamine® 3000 (Thermo Fisher Scientific, USA). The experiment included four groups as shown in Table 3. At 6 h following the transfection, 0.5 mL DMEM containing 2% FBS was added in vector control group and reference group, and 0.5 mL of 0.75mg/mL Matrine to treatment group. All experiments were performed in triplicates. Transfected cells were collected at 24 h and 48 h. The cells were lysed and the supernatants were collected. The luciferase intensity was detected according to the instructions of Dual luciferase reporter gene detection kit (Beyotime, China) by Glomax 96 Microplate luminometer (Promega).

Statistical analyses: Image J software was used to analyze the gray scale of Western blot images. Statistical analyzes were performed using one-way ANOVA and Student's *t* test by Graphad Prism 5 software.

RESULTS

The effect of Matrine on phosphorylation of ERK1/2: In order to investigate the effect of Matrine on PRRSV-induced ERK1/2 signaling pathway, the expression of pERK1/2 was examined by Western blot. As shown in Fig.1A and B, the expression of pERK1/2 protein was reached the highest point at 6 h after PRRSV infected Marc-145 cells (P<0.05). The protein expression at 6 h post infection was exhibited in Fig.1C and D. Compared with the cell control group, the expression of pERK1/2 was significantly increased in the PRRSV group, Matrine alone control group and the Matrine treatment group (P<0.05). Compared with the PRRSV group, the expression of pERK1/2 was significantly increased in the Matrine alone control group and the Matrine treatment group (P<0.05). The pERK1/2 expression of Matrine alone control group is more significant than the Matrine treatment group (P<0.05).

Matrine's inhibitory effect on the replication of PRRSV: As shown in Fig. 2, at 24 h post infection by PRRSV, there is almost no significant morphological change in the cells. At 48 h, Marc-145 cells shows disintegration and death but Matrine treatment group shows no cytopathic effect, comparing with normal cells: the cell has a complete shape with clear edges and has uniform individual cell size. In order to check the effect of Matrine on the early stage PRRSV replication, the mRNA expression of N gene was detected by RT-qPCR. The mRNA expression of N gene in infected cells within Matrine treatment was significantly lower than that of virus infection group at 3 h, 6 h, 12 h and 24 h post infection (P<0.05) (Fig. 3).

Matrine suppressed the activity of PRRSV Nsp9: To find the effect of Matrine on the virus replication enzyme, we used dual luciferase reporter gene assay to assess the effect of Matrine on Nsp9 activity. The results showed that at 24 h (Fig. 4A) and 48 h (Fig. 4B) following the transfection, compared with vector control group, the relative luciferase activity level is increased significantly in the reference group (P<0.05). That means the increase in the luciferase activity is specifically related to the Nsp9 not for any other element produced by the plasmid sequence. Compared with the reference group, the level of the relative luciferase activity was significantly reduced in treatment group (P<0.05).

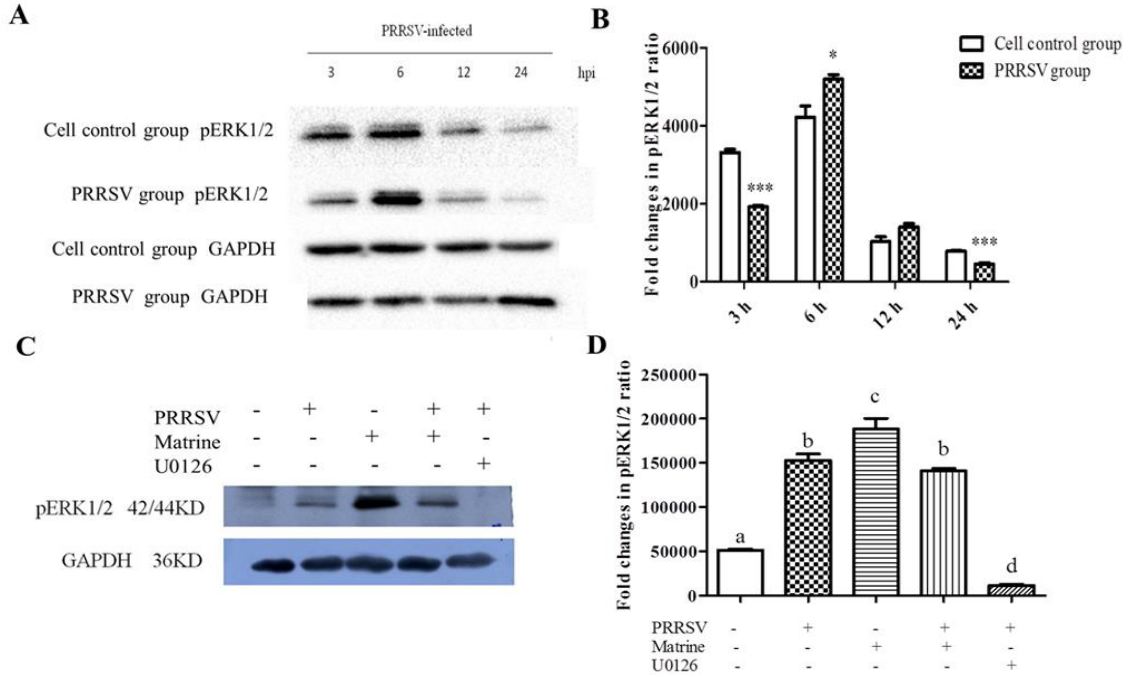

Fig. 1: The expression of pERK1/2 protein detected by Western blot. A. The expression of pERK1/2 at different time points following PRRSV infection. At 3 h, 6 h, 12 h and 24 h following the infection, the cell samples were collected and the expression level of pERK1/2 were analyzed by Western blot. The results showed that PRRSV could significantly increase the expression of pERK1/2 at 6 h following the infection. (*represent P<0.05, ***represent P<0.001). C. The effect of Matrine on the expression of pERK1/2 at 6 h following the infection. The results revealed that Matrine inhibited PRRSV replication was independent of ERK signaling pathway. B and D. Quantification of pERK1/2 was normalized to GAPDH using Image J software. Data with different letters (a, b, c, d) indicate significant differences between groups (P<0.05). The blot was reacted with mouse MAb against GAPDH to verify the amount of protein loaded.

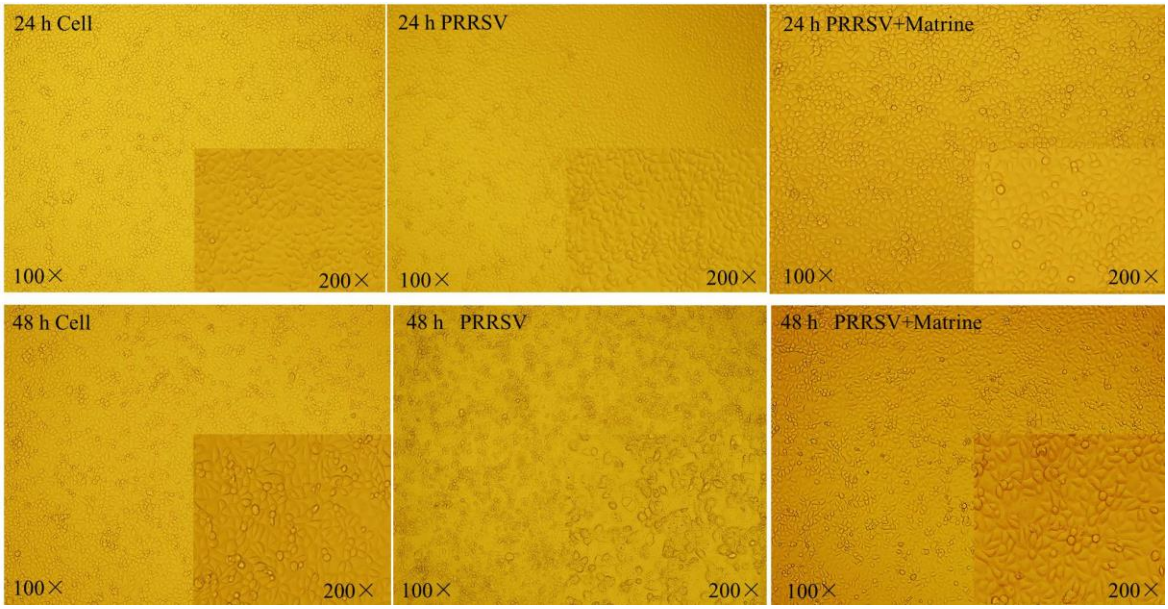

Fig. 2: The state of Marc-145 cells infected with PRRSV at 24 h and 48 h. Post 2 h PRRSV infection, discard the supernatant and add 2 mL 0.75 mg/mL Matrine to Matrine treatment group and add 2 mL 2% FBS medium to PRRSV group. The figures showed that the cells begin to disintegrate and die post 48 h infection while Matrine inhibit PRRSV from cell destruction.

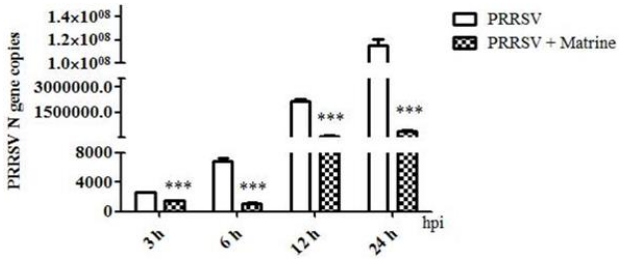

Fig. 3: The effect of Matrine on the mRNA expression of PRRSV N gene following Marc-145 cells infected with PRRSV at 3 h, 6 h, 12 h and 24 h. The results showed that Matrine inhibit the expression of PRRSV N gene at all time points following the virus infection. (* the mRNA expression of N gene was significantly different with that in the virus only group ($P<0.05$).

DISCUSSION

PRRSV has a great economic loss to the swine industry throughout the glob as the virus has an epidemic outbreak in most of the swine producing countries. At present, vaccines have been widely used but it cannot provide complete protection for vaccinated pigs. Therefore, the development of novel antivirals is urgently needed.

Chinese traditional herbal medicines have become a research hotspot due to low drug resistance, hypotoxicity and antiviral effect with broad spectrum. Propolis flavone and *Epimedium* polysaccharide synergistically inhibit the cellular infectivity of Newcastle disease virus (NDV) and improved the treatment of Newcastle Disease in chicken (Fan *et al*., 2011). *Kumazasa* extract had the anti-pseudorabies virus activity (Iwata *et al*., 2010). *Cryptoporus volvatus* extract inhibited PRRSV *in vitro* and *in vivo* (Gao *et al*., 2013). Matrine has an antiviral activity in PAM co-infected by PRRSV and porcine circovirus type 2 (PCV2) (Sun *et al*., 2016). Our previous studies revealed that Tea seed saponins, Dipotassium Glycyrrhizinate, Sodium tanshinone IIA sulfonate, Ginsenoside Rb1, chlorogenic acid and scutellarin had the antiviral activity. Matrine could directly inactivate and significantly inhibit the replication of PRRSV at 48 h post-infection (Sun *et al*., 2014). However, previous data did not show the antiviral activity of Matrine at the early stage of PRRSV infection, as well as the antiviral mechanism.

Viruses possess the ability to stimulate host signaling pathways including the Mitogen-activated protein kinase (MAPK) cascades and JAK-STAT signal pathway, which regulate a wide range of cellular functions and facilitate virus replication (Lee and Lee, 2012). ERK1/2 signal pathway is involved in a wide range of cellular functions, mediating and regulating cell growth and differentiation and it is closely related to the invasion, gene transcription, protein expression and the release of virus particles in the host cell (Zhou and Zhang, 2012; Kappes and Faaberg, 2015). Recent studies had shown that porcine epidemic diarrhea virus, influenza virus and PCV2 have an influence on the ERK signaling pathway for the regulation of both gene expression and viral replication, to produce the high level of virus and the optimal viral infection (Kim and Lee, 2015). A recent study had shown that the ERK signaling pathway of PAM is activated by PRRSV to increase virus infectivity and propagation (Lee and Lee, 2010). Moreover, recent researches have shown that the nuclear translocation of STAT1 and STAT2 are blocked by PRRSV that will cause the inhibition of IFN signaling pathway via the Nsp1β (Patel *et al*., 2010; Wang *et al*., 2013), PRRSV Nsp5 has an antagonistic effect on JAK/STAT3 signaling pathway due to the degradation of STAT3 (Yang *et al*., 2017). This suggests that the JAK/STAT antagonism could be one of the reasons to interfere the development of protective immune response by PRRSV (Yang and Zhang, 2017). JAK-STAT signaling is also interconnected with the MAPK/ERK pathways; MAPK help the STATs to increase the transcription process (Rawlings *et al*., 2004). However, one study shows that MAPK activation could reduce STAT3 activity (Jain *et al*., 1998). Therefore, we suspected

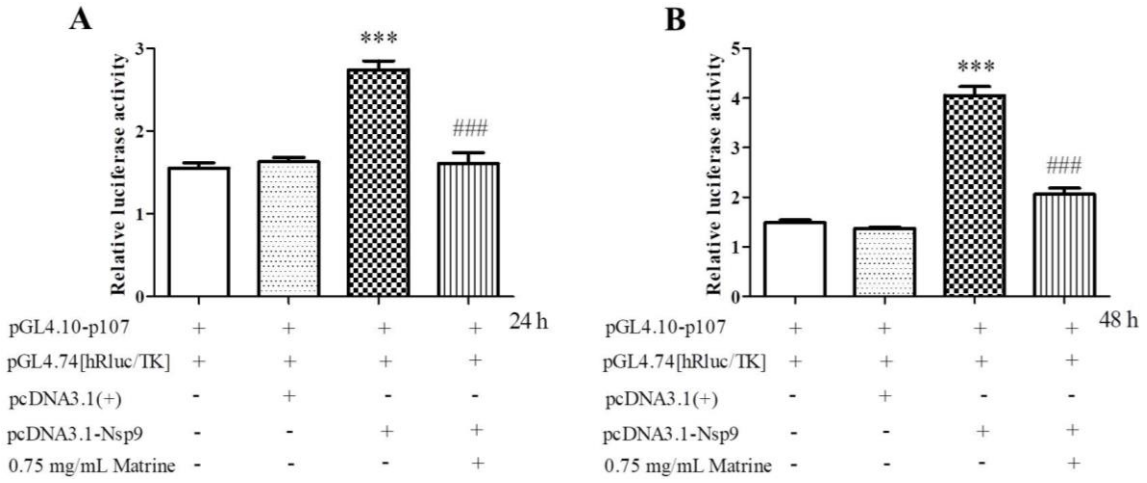

Fig. 4: The results of dual luciferase reporter gene assay. Marc-145 cells were inoculated in the 24 wells plate, at around 60% confluence, transfected with plasmids pGL4.10-p107, pGL4.74[hRluc/TK], pcDNA3.1(+) and pcDNA3.1-Nsp9. The experiment were consisted of four groups, 1) vector control group I: transfected pGL4.10-p107 and pGL4.74[hRluc/TK] vector; 2) vector control group II: transfected pGL4.10-p107, pGL4.74[hRluc/TK] vector and pcDNA3.1(+) vector; 3) reference group: transfected pGL4.10-p107, pcDNA3.1-Nsp9 and pGL4.74[hRluc/TK] vector without treatment with Matrine; 4) treatment group: the same as the reference group but with treatment of Matrine after 6 hours of transfection. At 24 h and 48 h post transfection, collected cell samples and detected the relative luciferase activity. These results showed that Nsp9 activate the promoter p107 and Matrine inhibit the activity of Nsp9 (* the relative luciferase activity was significantly different from the vector group (P<0.001) and # the relative luciferase activity was significantly different from the reference group (P<0.001)).

Martine might as an ERK1/2 inhibitor to inhibit the replicate of PRRSV. In present study, we found that the protein expression of pERK1/2 in Marc-145 cells was increased at 6 h post PRRSV infection, which was similar with the previous report (Lee and Lee, 2010). In PRRSV infected Marc-145 cells, the level of pERK1/2 in Matrine treated group was less than that in Matrine alone group. Moreover, we examined the Matrine's effect on the transcription of PRRSV related receptors including CD163, Heparan sulfate, Vimentin and CD151, but the results had no obvious discipline (Data not shown). However, in PRRSV infected Marc-145 cells, Matrine suppressed the mRNA expression of N gene from 3 h to 24 h post infection. This indicated that PRRSV replication has been inhibited by Matrine. The results suggested that Matrine could affect the replication of PRRSV, and this mechanism was independent of the entry process of PRRSV, as well as ERK1/2 signal pathway.

RdRp is indispensable for the replication and transcription of all the positive strand RNA viral genome (Fang and Snijder, 2010; Lehmann *et al.*, 2016). Recent study had demonstrated that host proteins such as Annexin A2 and DEAD-box RNA helicase 5 and retinoblastoma protein, affect the replication of PRRSV by interacting with Nsp9 (Dong *et al.*, 2014; Li *et al.*, 2014; Zhao *et al.*, 2015). Wu *et al.* (2010) discovered that anti-viral mechanism of barramundi Mx against betanodavirus involves the viral RNA synthesis inhibition by interfering the RdRp and Liu *et al.* (2015) also demonstrated that PRRSV replication could be blocked fully or is strongly inhibited by nanobody 6, when expressed in the cytoplasm of Marc-145 cells. Gao *et al.* (2013) confirmed that the aqueous extract from the fruiting body of *Cryptoporus volvatus* had the potential to inhibit PRRSV infection and the extract directly inhibited PRRSV RdRp activity through filter-binding assays. Lee *et al.* (2010) developed a Renilla luciferase reporter system to measure NS5B polymerase activity of Hepatitis C virus (HCV) and an imidazole derivative compound was evaluated as a candidate HCV RdRp inhibitor. Peng *et al.* (2013) demonstrated that 2-(4-nitroanilino)-6-methylenzothiazole inhibited HCV RdRp activity and HCV RNA replication in a dose-dependent manner using the same method. Dong (2015) was reported that PRRSV Nsp9 had the binding site of promoter p107. Therefore, we constructed an eukaryotic expression plasmid with Nsp9 and promoter p107, and co-transfected into Marc-145 cells with the dual luciferase reporter genes to study the effect of Matrine on the activity of Nsp9. Our results showed that PRRSV Nsp9 was able to activate the promoter p107 and Matrine inhibit the interaction between Nsp9 and promoter p107.

Conclusions: Our findings revealed that the Matrine inhibits the replication of PRRSV in Marc-145 cells by inhibiting the activity of Nsp9, resulting in impeding the synthesis of PRRSV RNA and protein in cells. Nsp9, one of the important polymerase proteins in PRRSV, can be used as a potential anti-viral target.

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Authors contribution: PS, NS, ZW and HL conceived and designed the experiments; PS and NS performed the experiments and analyzed the data; PS, ZW and JG provided manuscript editing. YH guided the RT-qPCR and Western blot assay. All authors read and approved the final manuscript.

REFERENCES

Dokland T, 2010. The structural biology of PRRSV. Virus Res 154:86-97.

Dong J, Zhang N, Ge X, *et al.,* 2014. The interaction of nonstructural protein 9 with retinoblastoma protein benefits the replication of genotype 2 porcine reproductive and respiratory syndrome virus in vitro. Virology 464-5: 432-40.

Dong J, 2015. Molecular mechanism of the interaction between porcine reproductive and respiratory syndrome virus nsp9 and host cellular protein pRb. China Agriculture University. PhD. Dissertation (in Chinese) pp:50-61.

Fan Y, Liu J, Wang D, *et al.,* 2011. Epimedium polysaccharide and propolis flavone can synergistically inhibit the cellular infectivity of NDV and improve the curative effect of ND in chicken. Int J Biol Macromol 48:439-44.

Fang Y and Snijder E, 2010. The PRRSV replicase: exploring the multifunctionality of an intriguing set of nonstructural proteins. Virus Res 154:61-76.

Gao L, Zhang W, Sun Y, *et al.,* 2013. *Cryptoporus volvatus* extract inhibits porcine reproductive and respiretory syndrome virus (PRRSV) *in vitro* and *in vivo*. PLoS One 8:e63767.

Gorbalenya A, Enjuanes L, Ziebuhr J, *et al.,* 2006. Nidovirales: evolving the largest RNA virus genome. Virus Res 117:17-37.

Iwata K, Naito E, Yamashita K, *et al.,* 2010. Anti-pseudorabies virus activity of kumazasa extract. Biocontrol Sci 15:123-8.

Jain N, Zhang T, Fong SL, *et al*., 1998. Repression of Stat3 activity by activation of mitogen-activated protein kinase (MAPK). Oncogene 17:3157-67.

Kappes M and Faaberg K, 2015. PRRSV structure, replication and recombination: Origin of phenotype and genotype diversity. Virology 479-80: 475-86.

Kim Y and Lee C, 2015. Extracellular signal-regulated kinase (ERK) activation is required for porcine epidemic diarrhea virus replication. Virology 484: 181-93.

Lee J, Tseng C, Chen K, *et al.,* 2010. A cell-based reporter assay for inhibitor screening of hepatitis C virus RNA-dependent RNA polymerase. Anal Biochem 403:52-62.

Lee Y and Lee C, 2010. Porcine reproductive and respiratory syndrome virus replication is suppressed by inhibition of the extracellular signal-regulated kinase (ERK) signaling pathway. Virus Res 152:50-8.

Lee Y and Lee C, 2012. Stress-activated protein kinases are involved in porcine reproductive and respiratory syndrome virus infection and modulate virus-induced cytokine production. Virology 427:80-9.

Lehmann K, Gorbalenya A, Snijder E, *et al.,* 2016. Arterivirus RNA-dependent RNA polymerase: Vital enzymatic activity remains elusive. Virology 487:68-74.

Li E, Sun N, Zhao J, *et al.,* 2015. *In vitro* evaluation of antiviral activity of tea seed saponins against porcine reproductive and respiratory syndrome virus. Antivir Ther 20:743-52.

Li J, Guo D, Huang L, *et al*., 2014. The interaction between host Annexin A2 and viral Nsp9 is beneficial for replication of porcine reproductive and respiratory syndrome virus. Virus Res 189:106-13.

Liu H, Wang Y, Duan H, *et al.,* 2015. An intracellularly expressed Nsp9-specific nanobody in MARC-145 cells inhibits porcine reproductive and respiratory syndrome virus replication. Vet Microbiol 181:252-60.

Nieuwenhuis N, Duinhof T and van Nes, 2012. Economic analysis of outbreaks of porcine reproductive and respiratory syndrome virus in nine sow herds. Vet Rec 170:225.

Patel D, Nan Y, Shen M, *et al*., 2010. Porcine reproductive and respiratory syndrome virus inhibits type I interferon signaling by blocking STAT1/STAT2 nuclear translocation. J Virol 84:11045-55.

Peng H, Chen W, Lin Y, *et al.,* 2013. Anti-hepatitis C virus RdRp activity and replication of novel anilinebenzothiazole derivatives. Antiviral Res 100: 269-75.

Rawlings JS, Rosler KM and Harrison DA, 2004. The JAK/STAT signaling pathway. J Cell Sci 117:1281-3.

Sun N, Sun P, Lv H, *et al.,* 2016. Matrine displayed antiviral activity in porcine alveolar macrophages co-infected by porcine reproductive and respiratory syndrome virus and porcine circovirus type 2. Sci Rep 6:24401.

Sun N, Wang Z, Wu C, *et al.,* 2014. Antiviral activity and underlying molecular mechanisms of Matrine against porcine reproductive and respiratory syndrome virus *in vitro*. Res Vet Sci 96:323-7.

Wang J, Sun P, Feng Y, *et al.,* 2016. Sodium tanshinone IIA sulfonate affects Marek's disease virus replication by inhibiting gB expression. Pharm Biol 54:701-4.

Wang R, Nan Y, Yu Y, *et al*., 2013. Porcine reproductive and respiratory syndrome virus Nsp1β inhibits interferon-activated JAK/STAT signal transduction by inducing karyopherin-α1 degradation. J Virol 87:5219-28.

Wu C, Xu Z, Gai R, *et al.,* 2016. Matrine ameliorates spontaneously developed colitis in interleukin-10-deficient mice. Int Immunopharmacol 36:256-62.

Wu Y, Lu Y and Chi S, 2010. Anti-viral mechanism of barramundi Mx against betanodavirus involves the inhibition of viral RNA synthesis through the interference of RdRp. Fish Shellfish Immunol 28:467-75.

Xie J, Zhou H, Cui J, *et al.,* 2014. Inhibition of porcine reproductive and respiratory syndrome virus by specific siRNA targeting Nsp9 gene. Infect Genet Evol 28:64-70.

Yang L, Wang R, Ma Z, *et al*., 2017. Porcine reproductive and respiratory syndrome virus antagonizes JAK/STAT3 signaling via nsp5, which induces STAT3 degradation. J Virol 91:e02087-16.

Yang L and Zhang YJ, 2017. Antagonizing cytokine-mediated JAK-STAT signaling by porcine reproductive and respiratory syndrome virus. Vet Microbiol 209:57-65.

Yong J, Wu X and Lu C, 2015. Anticancer advances of matrine and its derivatives. Curr Pharm Des 21:3673-80.

Yun S and Lee Y, 2013. Overview: Replication of porcine reproductive and respiratory syndrome virus. J Microbiol 51:711-23.

Zhao S, Ge X, Wang X, *et al.,* 2015. The DEAD-box RNA helicase 5 positively regulates the replication of porcine reproductive and respiratory syndrome virus by interacting with viral Nsp9 in vitro. Virus Res 195: 217-24.

Zhou A and Zhang S, 2012. Regulation of cell signaling and porcine reproductive and respiratory syndrome virus. Cell Signal 24:973-80.

Zhou Y, Zheng H, Gao F, *et al.,* 2011. Mutational analysis of the SDD sequence motif of a PRRSV RNA-dependent RNA polymerase. Sci China Life Sci 54: 870-9.

Zhu H, Han L, Shi X, *et al*., 2015. Baicalin inhibits autophagy induced by influenza A virus H3N2. Antiviral Res 113:62-70.