Porcine 2′,5′-oligoadenylate synthetase-like protein inhibits replication of porcine reproductive and respiratory syndrome virus

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Abstract

Background: Porcine reproductive and respiratory syndrome virus (PRRSV) is a serious pathogen that causes $664 million losses per year to the swine industry. There are few useful vaccines that can provide protection against PRRSV infection. 2′, 5′-oligoadenylate synthetase-like protein (OASL) has antiviral activity, this has not been shown for PRRSV and the mechanism is unknown.

Methods: Expression of OASL in porcine alveolar macrophages induced by interferon (IFN)-β stimulation and PRRSV infection was examined by real-time polymerase chain reaction. Exogenous expression and knockdown of OASL were used to determine the role of OASL in the PRRSV replication cycle. The type I IFN signaling pathway was evaluated after OASL overexpression.

Results: In this study, we found that the expression of OASL in porcine alveolar macrophages was significantly increased by IFN-β stimulation and PRRSV infection. Porcine-OASL-specific small interfering RNA (siRNA) promoted PRRSV replication, whereas exogenous expression of porcine OASL inhibited replication of the virus. The anti-PRRSV activity of porcine OASL was lost after knockdown of retinoic acid-inducible gene I (DDX58, also known as RIG-I).

Conclusions: Porcine OASL suppresses PRRSV replication.

1 Background

Porcine reproductive and respiratory syndrome (PRRS) has been the most aggressive disease affecting the swine industry worldwide, resulting in economic losses of $664 million per year in the US [1, 2]. In 2006, the swine industry in China was greatly impacted by the disease, which resulted in substantial economic losses
Porcine reproductive and respiratory syndrome virus (PRRSV), the causative agent of PRRS, belongs to the Arteriviridae family. The entire PRRSV genome contains 10 open reading frames (ORFs), namely, ORF1a, ORF1b, ORF2a, ORF2b, ORF3, ORF4, ORF5, ORF5a, ORF6, and ORF7. ORF1a and ORF1b make up four-fifths of the genome, encoding the main nonstructural proteins involved in viral replication and transcription, whereas the viral structural proteins GP2–GP5, M, N, E, and GP5a are encoded by ORF2–5, ORF6, ORF7, ORF2b, and ORF5a, respectively [4–6]. PRRSV strains are mainly subdivided into types 1 and 2 according to their antigenicity. The majority of China’s epidemic strains are type 2 [7]. Current vaccines hardly provide protection against this virus, finding new ways to control this virus is in urgent need. The host intrinsic restriction factors usually inhibit virus infection by direct interaction with viral proteins, and they are more promising because they are less likely to mutant under drug-mediated selective pressure.

The genes coding for 2’, 5’-oligoadenylate synthetase (OAS) proteins are interferon (IFN)-inducible and play an important role in innate immunity as a host intrinsic restriction factor [8–11]. Upon induction by IFN, the expressed OAS proteins upregulate ribonuclease L (RNase L) [12], leading to the degradation of viral and host RNAs [13] and inhibiting viral replication [14–16]. The OAS family of proteins comprises OAS1, OAS2, OAS3, and OASL. OAS1 has one functional OAS unit, OAS2 has two, and OAS3 has three, whereas OASL has one tandem ubiquitin unit in its C-terminal domain and no oligoadenylate synthetase activity [14, 17–21]. Although OAS1 and OASL are distributed widely, OAS2 and OAS3 are found only in mammals [22]. All these OAS subtypes, except for OAS3, are found in swine [23]. OASL has been shown to inhibit the replication of some viruses. For example, Newcastle disease virus replication in goose embryo fibroblasts is reduced significantly by
overexpression of the goose OASL protein [24]. Human OASL has been shown to inhibit some specific DNA and RNA viruses, such as respiratory syncytial virus (RSV), vesicular stomatitis virus (VSV), dengue virus, and herpes simplex virus-1 (HSV-1) [25, 26]. Nevertheless, human OASL does not protect against encephalomyocarditis virus (EMCV) [27]. Murine OASL2 strongly inhibits RSV replication [25], whereas murine OASL1 fails to do so [25]. Instead, murine OASL1 inhibits the production of type I IFN, and OASL1−/− mice are more resistant to infection with EMCV and HSV-1 [28]. Chicken OASL was shown to inhibit West Nile virus infection [29]. Porcine OASL has been reported to inhibit the Japanese encephalitis virus (JEV) in PK15 cells, but this function is not dependent on the OAS-RNase L pathway [30].

Previous transcriptomics showed that after PRRSV infection, OAS expression increased [31, 32]. The anti-PRRSV effects of porcine OAS1 and OAS2 have been proven [33, 34], but those of porcine OASL and the relationship between OASL and IFN are not clear. Moreover, OASL has a different sequence at its C terminus (relative to other OAS proteins), it remains to be determined whether this feature has a different inhibitory effect on viral replication as compared with the other three OAS subtypes. Therefore, in this study, we evaluated the effect of porcine OASL on PRRSV replication in vitro and attempted to elucidate the mechanisms underlying its antiviral activity.

2. Materials and methods

2.1. Cell lines and the virus

Porcine alveolar macrophages (PAMs), isolated from lung lavage samples of 7-week-old pigs which were brought from Henan academy of agricultural sciences, free of
PRRSV, pseudorabies virus, porcine circovirus type 2, and classical swine fever virus (CSFV), were cultured in the Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum (FBS; Life Technologies, Carlsbad, CA, USA) at 37 °C, in a humidified atmosphere containing 5% CO₂.

CRL-2843-CD163, a stable porcine macrophage cell line that can be infected by PRRSV, was kindly provided by Prof. Enmin Zhou (Northwest A&F University, Lingyang, China) [35]. This cell line was grown in RPMI 1640 medium supplemented with 6% of FBS (Sijiqing, ZhejiangTianhang Biotechnology Co. Ltd., China) at 37 °C, in a humidified atmosphere containing 5% CO₂.

Marc-145 cells (a monkey kidney cell line, ATCC catalogue numbers CRL-12231) were grown in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% FBS (Sijiqing, ZhejiangTianhang Biotechnology Co. Ltd., China) at 37 °C, in a humidified atmosphere containing 5% CO₂.

The type 2 PRRSV BJ-4 strain (GenBank accession No. AF331831) was kindly provided by Prof. Hanchun Yang (China Agricultural University, Beijing, China).

2.2. Expression of OASL during PRRSV infection of PAMs

PAMs were infected with PRRSV strain BJ-4 at a multiplicity of infection (MOI) of 1.0 for different periods (0, 6, 12, 24, 36, or 48 h). The cells were then processed for quantitative reverse-transcription polymerase chain reaction (qRT-PCR) analysis of OASL mRNA expression.

2.3. Expression of OASL after stimulation of PAMs with IFN

PAMs were stimulated with human IFN-β (PeproTech) which was diluted with PBS at a concentration of 1000 IU/mL for different periods (0, 6, 12, 24, 36, or 48 h). The cells were then subjected to treatment for qRT-PCR determination of OASL mRNA
expression.

2.4. Molecular cloning

Porcine OASL (GenBank accession No. NM_001031790.1) was cloned from the complementary DNA (cDNA) extracted from PAMs, using the following primer sequences: 5′-CCGGAATTCTGGAGCTATTTTACACCCAGC-3′ (OASL-For) and 5′-AAGGAAAAAGCGCCGCTCAGTCACAGCCTTTGGCTGAGA-3′ (OASL-Rev). After double digestion, the purified product was ligated with the p3xFLAG-CMV™-7.1 vector (Sigma, USA) to generate the pCMV-3xFLAG-7.1-OASL expression plasmid with the Mix & Go! E. coli Transformation Kit and ZymoPURE II Plasmid Midiprep Kit (ZYMO RESEARCH, USA).

2.5. Small interfering RNA (siRNA) synthesis

siRNAs were employed to identify the genes or proteins involved in the antiviral mechanism of OASL. The control siRNA (si-Ctrl, a scrambled control siRNA), OASL siRNA (si-OASL), RIG-I siRNA (si-RIG-I), RNase L siRNA (si-RNase L), and melanoma differentiation-associated protein 5 (MDA5) siRNA (si-MDA5) were all ordered from GenePharma Co., Ltd. (Suzhou, China). The siRNA sequences can be found in Table 1.
Table 1
Primers used in the research

| primer      | sequences                                      |
|-------------|------------------------------------------------|
| OASL- For   | CCGGAATTCTGGAGCTATTTTACACCACCCAGC             |
| OASL- Rev   | AAGGAAAAAGCGGCGCTCAGTCAAGCGACTTTG            |
| qOASL-F     | CTTGGTTGACATTCTGTTCGT                        |
| qOASL-R     | AAGATGGTGAAGCGGATACG                        |
| qGAPDH-F    | CTGCCGCTGGAGAAACCT                           |
| qGAPDH-R    | GCTGTACACAAATTTCACTCTTGCG                    |
| qIRF3-F     | AAGGTTGTCATCGTGGCCATCTGCC                    |
| qIRF3-R     | GGAATGTCAGTCAGTCCACCGTGT                    |
| qIRF7-F     | TCCGCCGAGATGCTATAAGT                        |
| qIRF7-R     | GTCCAAGTTCTGGCCGATGT                        |
| qNF-F       | AAACCCAGTCCAGGCAAGGAAG                      |
| qNF-R       | GCAAAACTAAACTTCCACAGTGTA                    |
| qIFN-beta-F | CTAGCACTGCTGGGAATAGGACG                    |
| qIFN-beta-R | GGCCCTCAGTAAATGCAGAAATC                     |
| qTNF-alpha-F| CACACGCTCTTGCTCCCTA                       |
| qTNF-alpha-R| ACGGGCTTATCGAGTGTGAG                     |
| qIL-8-F     | GGCAGTTTTCTCCTGTCT                           |
| qIL-8-R     | CAGTGGGTCTCCACTCTCA                       |
| qRIG-I-F    | CAGAGCAGCGGCGGAGAA                      |
| qRIG-I-R    | ACTCAAGGTTGCCAT                          |
| qTLR7-F     | GAACTGTCTTCTCACA                           |
| qTLR7-R     | AGACTGTCAATTCAGTCA                       |
| qTLR3-F     | TACTGTACACAACTTCTAC                       |
| qTLR3-R     | TTTAATCCTCCATCCAAGG                       |
| qNF-KB-F    | CCCAGCCTGCCATCCCTAC                        |
| qNF-KB-R    | ACATCAACCCAAAGACAC                      |
| qMDA5-F     | CGAATTAACAGGACCCAGAT                      |
| qMDA5-R     | CTCAGAGCTTTGAGCTGTCT                      |
| qMyD88-F    | CTCGGAGCCGAGCTCCCTC                       |
| qMyD88-R    | GCCAGCCAGTCCAGTCC                       |
| qTBK1-F     | CAGTAGGATGTCCAAAT                       |
| qTBK1-R     | CTCCACATGGACAAAT                        |
| Si-OASL     | GCCACAUUGAGCGGUUUCCAATT                     |
| Si-RIG-I     | GCAGGUAUUCUGGACUUUTT                     |
| Si-MDA-5    | CUCAGAUUUGGACUAAATT                     |
| Si-RNase L  | UGGAGAGAUGAAUGCAUATT                     |

2.6. Transfection and infection

CRL-2843-CD163 cells were transfected with 800 ng of the pCMV-3xFLAG-7.1-OASL expression plasmid or control expression vector (pCMV-3xFLAG-7.1) by means of Lipofectamine 2000 (Life Technologies).

After 24 h of incubation, the cells were infected with PRRSV (at MOI of 0.1 or 1.0) for 24 h. qRT-PCR was then carried out to determine the mRNA expression of various factors, such as PRRSV nucleoprotein (N, ORF7), OASL, RIG-I, and MDA5, as well as the cytokines IFN-β, IFN-α, and pro-interleukin 1 beta (pro-IL-1β).

Total IRF3 and phosphorylated IRF3 (p-IRF3) proteins were quantified by western
For the siRNA experiments, PAMs were transfected with 60 nM si-OASL, si-RNase L, si-RIG-I, si-MDA5, or si-Ctrl via the Lipofectamine RNAiMAX Transfection Reagent (Invitrogen). At 24 h post-transfection, the cells were infected with PRRSV (MOI 1.0) for another 24 h, mRNA expression of the respective genes was determined by qRT-PCR, and the viral titer was tested by TCID\textsubscript{50}.

For the siRNA and plasmid cotransfection experiments, CRL-2843-CD163 cells were transfected with 60 nM si-RNase L, si-RIG-I, si-MDA5, or si-Ctrl and 800 ng plasmid via the Lipofectamine 2000 Transfection Reagent (Invitrogen). At 24 h post-transfection, the cells were infected with PRRSV (MOI 1.0) for another 24 h, mRNA expression of the respective genes was determined by qRT-PCR, and the viral titer was tested by TCID\textsubscript{50}.

2.7. qRT-PCR

Total-RNA samples from PAMs and CRL-2843-CD163 cells were extracted with the TRIzol Reagent (Life Technologies) and then subjected to reverse-transcriptase treatment by means of the First Strand cDNA Synthesis Kit (Takara, Dalian, China) and qPCR. qPCR was carried out on a 7500 Fast Real-time PCR system (Applied Biosystems, Foster City, CA, USA) with the primers listed in Table 1. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was analyzed as an internal control, and relative changes in the expression of the target genes were calculated by the $2^{-\Delta\Delta Ct}$ method [36].

2.8. Cell survival experiments

The toxicity of porcine OASL and various siRNAs toward PAMs and CRL-2843-CD163 cells was tested with the Enhanced Cell Counting Kit-8 (Solarbio, Beijing, China).
2.9. Western blot analysis

Western blotting was carried out as described previously [37–39]. The primary antibodies were as follows: an anti-FLAG monoclonal antibody (1:200; Abnova, Taipei, Taiwan), anti-GAPDH antibody (1:500; Beijing Biosynthesis Biotech Co., Ltd., Beijing, China), anti-IRF3 antibody (1:1000, Proteintech Group, China), anti-p-IRF3 antibody (1:500; Cell Signaling Technology, Danvers, MA, USA), anti-OASL antibody (1:1000; Abcam, USA), anti-RNase L antibody (1:200; Santa Cruz, USA), anti-RIG-I antibody (1:1000; Cell Signaling Technology, Danvers, MA, USA), and anti-MDA-5 antibody (1:1000; Cell Signaling Technology, Danvers, MA, USA). Secondary antibodies were a horseradish peroxide-conjugated goat anti-rabbit IgG antibody (1:10000, Zhongshan Golden Bridge Biotechnology, China), a horseradish peroxide-conjugated goat anti-mouse IgG antibody (1:10000, Jackson ImmunoResearch, West Grove, PA, USA), or a horseradish peroxide-conjugated goat anti-chicken IgY antibody (1:10000, Abbkine, USA).

2.10. Luciferase reporter assay

CRL-2843-CD163 cells were transfected with 100 ng of pIFN-beta-Luc, 20 ng of pRL-TK, and 400 ng of the expression vector or control vector by means of Lipofectamine 3000 (Life Technologies). After 12 h, 1.5 μg of poly (I: C) (InvivoGen, Paris, France) was transfected into the cells for 9 h. The cells were then subjected to a luciferase reporter assay (Promega, Madison, WI, USA) to determine the IFN-β promoter reporter activity. In another experiment, CRL-2843-CD163 cells transfected as described above were infected with PRRSV (MOI 1.0) for 24 h, and the luciferase reporter assay was then conducted.

2.11. Virus titers
Marc-145 cells were used to determine the PRRSV titers in the supernatants of cultures treated with different vectors and siRNAs. The tissue culture infectious dose 50 (TCID<sub>50</sub>) was determined.

2.12. Statistical analysis

All experiments were biologically repeated three times, data represent means ± standard deviations of three independent experiments, and all assays were conducted in triplicate. The results were analyzed by the Student’s t-test. Data with a P value < 0.05 were considered statistically significant.

3. Results

3.1. Porcine OASL mRNA expression was increased by IFN-β and PRRSV infection

After 6 h of stimulation with 1000 IU/mL IFN-β, porcine OASL mRNA expression in the PAMs increased quickly to a peak of 125 times than that in the untreated control cells (Fig. 1A). The OASL mRNA expression peak occurred at 12 h post-stimulation. The OASL mRNA expression level in the PRRSV-infected PAMs peaked at 36 h post-infection (Fig. 1B), showing a 15-fold increase compared to the untreated cells.

3.2. Porcine OASL restricted PRRSV replication

Western blot results showed that porcine OASL was well-expressed in the CRL-2843-CD163 cells transfected with p3xFLAG-CMV™-7.1-OASLfor 48 h (Fig. 2A). Furthermore, OASL exerted no cytotoxic activity toward this cell line after transfection for 48 h (Fig. 2B).

After OASL transfection for 24 h, PRRSV infected the cells, and 24 post-transfection, the PRRSV N mRNA level and viral titer were tested. In comparison with empty
vector-transfected cells, the PRRSV N mRNA level decreased significantly in the CRL-2843-CD163 cells transfected with p3xFLAG-CMV™-7.1-OASL (Fig. 2C). PRRSV TCID50 for the p3xFLAG-CMV™-7.1-OASL group was also lower (Fig. 2D).

After OASL transfection for 24 h, in the cells transfected with p3xFLAG-CMV™-7.1-OASL, mRNA levels of the IFN-α and IFN-β were higher by 1.9- and 1.6-fold, respectively, relative to the levels in the empty vector-transfected cells. The mRNA expression levels of IRF3, TLR-3, RIG-I, NF-κB, MDA5, MyD88, and TBK1 were higher by 1.8-, 4.0-, 3.9-, 2.1-, 2.0-, 5.5-, and 1.6-fold, respectively. On the other hand, those of TNF-α, IL-8, pro-IL-1β, and IRF7 did not differ significantly from empty vector-transfected cells (Fig. 3A and 3B).

The phosphorylation level of IRF3 was higher in the OASL-overexpressing cells than in the empty vector-transfected cells (Fig. 3C). After poly (I:C) treatment for 6 h and PRRSV infection for 24 h, IFN-β promoter activity was tested, and the results show that OASL also increased the IFN-β promoter activity (Fig. 3D).

3.3. OASL siRNA increased PRRSV replication

si-OASL (60 nM) was transfected into PAMs for 48 h, resulting in efficient reduction of OASL expression (Fig. 4A and 4B). si-OASL itself was not cytotoxic to the cells (Fig. 4C). After 60 nM si-OASL was transfected into PAMs, PRRSV infected the cells for 48 h, and the results showed that in the presence of si-OASL, the PRRSV N mRNA level was higher than that in cells treated with si-Ctrl (Fig. 4D). The TCID50 results were in agreement with the mRNA results (Fig. 4E). After 60 nM si-OASL was transfected into PAMs, the cells were subjected to Poly(I:C) treatment for 6 h; the results showed that IFN-β and IFN-α mRNA levels in the OASL knockdown group were also significantly lower (Fig. 4F and 4G).
3.4. Anti-PRRSV activity was dependent on RIG-I but not RNase L and MDA5

si-RNase L (60 nM) was transfected into CRL-2843-CD163 for 48 h, resulting in efficient reduction of RNase L expression (Fig. 5A and 5B). OASL (800 ng) and 60 nM si-RNase L were cotransfected into CRL-2843-CD163 cells, 24 h later, and 1.0 MOI PRRSV-infected CRL-2843-CD163 cells for 48 h. In the 800 ng OASL and 60 nM si-RNase L cotransfected and PRRSV (MOI 1.0)-infected CRL-2843-CD163, there were significant decreases in both the PRRSV N mRNA level and virus titers relative to the control group levels (Fig. 5C and 5D).

si-RIG-I (60 nM) was transfected into CRL-2843-CD163 for 48 h, resulting in efficient reduction of RIG-I expression (Fig. 5A and 5B). By contrast, in the 800 ng OASL and 60 nM si-RIG-I cotransfected and PRRSV (MOI 1.0)-infected CRL-2843-CD163 cells, there were no decreases in either the PRRSV N mRNA expression level or virus titers relative to the levels in the control group (Fig. 6C and 6D).

In the case of MDA5, 60 nM si-MDA5 was transfected into CRL-2843-CD163 cells for 48 h. mRNA results showed that MDA5 mRNA expression in CRL-2843-CD163 cells was decreased by approximately 72% (Fig. 6E); the western blot confirmed this result (Fig. 6F). In the CRL-2843-CD163 cells cotransfected with 800 ng OASL and 60 nM si-MDA5 and then infected with PRRSV (MOI 1.0), there were significant decreases in both the PRRSV N mRNA expression level and virus titers relative to the control levels (Fig. 6G and 6H).

4. Discussion

In this study, we showed that exogenous expression of porcine OASL restricts PRRSV replication, the schematic representation of the signaling pathway is presented in
Reports have revealed that human OASL interacts with RIG-I and exerts an antiviral effect. Even though it has no enzymatic activity, OASL is usually maintained at low expression levels in cells. When viruses infect human cells, human OASL is notably upregulated by the double-stranded RNA, and IFN is induced by IRF3 [27, 40, 41]. In the present study, after PRRSV infection of the cells, porcine OASL was produced, which then induced the phosphorylation of IRF3 to enhance IFN production, whereupon viral replication was inhibited. In stark contrast to our results, Lee demonstrated that murine OASL1 downregulates IFN via IRF7 to impede its expression and therefore aids in viral replication [28]. The discrepancies in the results could be explained as follows: effects of porcine OASL are mediated by IRF3, and different OASL isoforms might have different regulatory mechanisms in the signaling pathway.

Since porcine OASL had a nucleotidyltransferase region (data not shown), we surmised that its antiviral activity is dependent on RNase L. Nevertheless, our results indicated that this was not the case; porcine OASL did not exert its action via the OASL-RNase L pathway, and there may be another critical factor influencing the antiviral effect. Similarly, another study showed that porcine OASL also inhibits JEV replication but not through the OASL-RNase L signaling pathway [30]. Thus, our finding for PRRSV is the same as that for JEV.

Another study showed that porcine OASL inhibits replication of some RNA viruses through the MDA5-dependent pathway [42], but in our study, porcine OASL did not inhibit PRRSV via this pathway. The discrepancy may lie in the difference between the viruses and cells employed for these studies because we used PRRSV and PAMs, and Li et al used CSFV and PK15 cells. PAMs are macrophages, whereas PK15 is an
epithelial cell line, and they may have different immune characteristics. The difference in biological specificity between these two viruses may be related to the antiviral activity of porcine OASL.

In line with other studies that have shown the inhibition of PRRSV replication by porcine OAS1 and OAS2, our study proves that porcine OASL inhibits PRRSV replication as well. Besides, porcine OAS1 and OAS2 inhibit the replication of JEV, whereas porcine OASL inhibits the replication of CSFV, thus confirming the antiviral effects of the OAS protein family.

On the other hand, the inhibition of PRRSV replication by porcine OAS2 is dependent on RNase L, whereas that by OASL is not. This phenomenon may be related to the structures of porcine OAS2 and porcine OASL, which need further experimental verification.

Although porcine OASL does not interact with RIG-I directly [42], its anti-PRRSV activity depends on this protein factor. There may be some intermediary proteins between OASL and RIG-I that drive the antiviral action.

A limitation of this study is that testing of other virulent strains was not done. The NADC-30 strains responsible for the most recent epidemics are presumed to follow the same trends as PRRSV, largely because of their ability to induce IFN. Therefore, future studies should include these viruses.

In conclusion, we demonstrate that pOASL is an anti-PRRSV factor. Upregulation of pOASL activity may boost host immunity to limit PRRSV infection. Knockout of pOASL may increase the PRRSV titer during the virus production. Future investigation of pOASL activity might provide the insight and opportunities needed for the therapeutic development and improved vaccine candidate.
5. Conclusions

Porcine OASL was proven to inhibit PRRSV replication in vitro through a RIG-I-dependent process. These data may point to future directions regarding new ways to target these viruses. Further research regarding the regulation of OASL may provide insight and new antiviral strategies for therapeutic development.

Abbreviations

porcine reproductive and respiratory syndrome (PRRS)
porcine reproductive and respiratory syndrome virus (PRRSV)
open reading frames (ORFs)
interferon (IFN)
ribonuclease L (RNase L)
respiratory syncytial virus (RSV)
vesicular stomatitis virus (VSV)
herpes simplex virus-1 (HSV-1)
encephalomyocarditis virus (EMCV)
glyceraldehyde-3-phosphate dehydrogenase (GAPDH)
indirect immunofluorescence assay (IFA)

Declarations

Ethics approval and consent to participate

This study was approved by the Institutional Animal Care and Use Committee of Henan Academy of Agricultural Sciences. All processes were followed the “Guidelines for the care and use of laboratory animals in biomedical research”.

Consent for publication
Not applicable.

**Author Contributions**
MZ, RW conceived and designed the experiments. MZ, RW, YK, HL, WW performed the experiments and wrote the manuscript. MZ analysed the data; all authors read and approved the final manuscript.

**Availability of data and materials**
All datasets are available in the main manuscript.

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**Competing interests**
The authors declare that they have no competing interests.

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Figures
FIGURE 1

IFN-β stimulation and PRRSV infection induce OASL expression. (A) Porcine alveolar macrophages (PAMs) were treated with IFN-β (1000 IU/ml) for designated times (6, 12, 24, 36, or 48 h), mRNA was extracted, and then qRT-PCR was carried out. PAMs were seeded into 24-well plates at a density of 2 × 10⁴ cells per well. After one day, PAMs were treated with IFN-β for the designated times. Cells were harvested and subjected to mRNA extraction and real-time PCR analysis. Data represent means ± standard deviations of three independent experiments. **P < 0.01; ***P < 0.001.
Ectopic expression of porcine OASL inhibits PRRSV replication. (A) CRL-2843-CD1#
OASL enhances RIG-I signaling. (A) qRT-PCR quantitation of the TNF-α, IL-1β, IFN-α, IL-8, and IFN-β mRNA levels in CRL-2843-CD163 cells transfected with 800 ng of pCMV-3xFLAG-7.1 or pCMV-3xFLAG-7.1-OASL for 2 days. Data represent means ± standard deviations of three independent experiments, *P < 0.05.

(B) qRT-PCR quantitation of the TBK1, RIG-I, IRF3, IRF7, NF-κB, TLR-3, MyD88, TLR-7, and MDA5 mRNA levels in CRL-2843-CD163 cells transfected with 800 ng of pCMV-3xFLAG-7.1 or pCMV-3xFLAG-7.1-OASL for 2 days. Data represent means ± standard deviations of three independent experiments, *P < 0.05.

(C) Western blot analysis of pIRF3 and IRF3 levels with anti-IRF3 and anti-p-IRF3 antibodies, respectively.

(D) Porcine OASL enhances IFN-β promoter luciferase activity. On day 0, CRL-2843-CD163 cells were seeded in 24-well plates; on day 1, 200 µM of IFN-α and the IFN-β promoter luciferase reporter was transfected into the cells. On day 2, the cells were either treated with 1.5 µg of poly(I:C) and the IFN-β promoter activity was tested 9 h later, or they were infected with PRRSV (MOI 1.0) and the IFN-β promoter luciferase reporter assay was performed 24 h later. All experiments were biologically repeated three times. Data represent means ± standard deviations of three independent experiments. *P < 0.05.
The porcine OASL knockdown increases virus replication in macrophages. (A) qRT-PCR quantitation of the OASL mRNA levels in the siRNA-transfected and PRRSV-infected PAMs. Data represent means ± standard deviations of three independent experiments. *P < 0.05. (F) qRT-PCR quantitation of the IFN-β mRNA levels in the siRNA-transfected and PRRSV-infected PAMs. Data represent means ± standard deviations of three independent experiments. **P < 0.01. (G) qRT-PCR quantitation of the IFN-α mRNA levels in the siRNA-transfected and PRRSV-infected PAMs. Data represent means ± standard deviations of three independent experiments. **P < 0.01.

Figure 5

OASL inhibition of PRRSV replication is not dependent on RNase L. (A) qRT-PCR quantitation of RNase L mRNA levels in the siRNA-transfected and PRRSV-infected PAMs. Data represent means ± standard deviations of three independent experiments. **P < 0.01.
OASL inhibition of PRRSV is dependent on RIG-I but not on MDA5. (A) qRT-PCR quantitation of the RIG-I mRNA relative to the negative control. Data represent means ± standard deviations of three independent experiments. **P < 0.01.

Figure 6

Schematic representation of the signaling pathways involved in OASL-induced IFN expression.
