Molecular Cloning and Identification of the 2′–5′ Oligoadenylate Synthetase 2 Gene in Chinese Domestic Pigs Through Bioinformatics Analysis, and Determination of Its Antiviral Activity Against Porcine Reproductive and Respiratory Syndrome Virus Infection

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Abstract An interferon-mediated antiviral protein, 2′–5′ oligoadenylate synthetase 2, plays an important role in the antiviral response of interferons. In this study, 2′–5′ oligoadenylate synthetase 2 genes were cloned from Chinese domestic pigs. Bioinformatics analysis revealed that the 2024-bp long open reading frame encodes 707 amino acids. There are two conserved regions in this protein: the nucleotidyltransferase domain, and the 2′–5′ oligoadenylate synthetase domain (OAS). Genetic evolution analysis showed that the 2′–5′ oligoadenylate synthetase 2 gene in domestic pigs is closely related to that of cattle. There are multiple antigenic sites, no signal peptide, and no trans-membrane region in the gene, which is predicted to be a hydrophilic protein. Secondary structures were found to be mainly alpha helix-based; its tertiary structure is close to that of humans and cattle, but not that of mice. Tissue distribution results indicated that this protein is distributed in multiple organs, with high distribution in the liver; it is

mainly localized in the cytoplasm. PRRSV infection, interferon-beta, and Poly(I: C) treatment all promoted 2′–5′ oligoadenylate synthetase 2 gene expression. Overexpression and RNA silencing of porcine OAS2 inhibited and promoted PRRSV replication in cells, respectively. The inhibitory effect of porcine OAS2 was mainly dependent on RNase L, similar to what was predicted. This study has laid the foundation for future antiviral studies in pig, and provided a new way of preventing and treating PRRSV in the future.

Keywords 2′–5′ Oligoadenylate synthetase · PRRSV · RNase L · Bioinformatics · Replication

Introduction

Interferon is the first line of defense against viruses and other foreign pathogens. Following the recognition of pathogen-associated molecular pattern (PAMP) by pathogen recognition receptors (PRRS), expressions of interferon cytokines are activated, which further promote expressions of interferon-promoting molecules such as 2′–5′ oligoadenylate synthetase (OAS), myxovirus resistance A (MxA), and protein kinase (PKR) to modulate natural immunity [1, 2].

The 2′–5′-oligoadenylate synthetase is an interferon-induced, dsRNA-activated antiviral enzyme, which plays a critical role in the cellular innate antiviral response. In addition, it may also play a role in other cellular processes such as apoptosis, cell growth, differentiation, and gene regulation [3, 4]. The 2′–5′ oligoadenylate synthetase was first discovered in 1974 [5], and was eventually identified in humans, monkeys, mice, and other tissue cells [6–13]. The human OAS family includes OAS1, OAS2, OAS3, and

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OASL [8, 13, 14], and the pig OAS family includes OAS 1a, OAS1b, OAS2, and OASL [15, 16].

Upon detection of exogenous dsRNA, the OAS enzyme activates the second messenger 2'–5' linked oligoadenylate, which interact with ribonuclease L (RNase L); RNase L then degrades viral and cellular RNA [14]. OAS can inhibit viruses via either the RNase L-dependent pathway or the RNase L-independent pathway. Human OAS displays an antiviral effect against vesicular stomatitis virus (VSV), herpes simplex virus type 2 (HSV-2), and encephalomyocarditis virus (EMCV). It also stimulates alternative antiviral pathways independent of RNase L [17, 18].

Some studies have shown that the OAS system inhibits protein synthesis and induces apoptosis in virally infected cells, which limits viral infection [19]. Alternative splicing generates multiple isoforms of human OAS1, including p41 and the canonical p46. Polymorphisms in the corresponding OAS1 gene have been examined for possible association with increased susceptibility to type 1 diabetes mellitus, multiple sclerosis, and infection by viral pathogens [20–25].

Porcine reproductive and respiratory syndrome (PRRS) is a viral disease caused by the porcine reproductive and respiratory syndrome virus (PRRSV), and contributes to significant economic losses to the pig industry worldwide [26–31]. The disease was first discovered in 1975, which was then spread worldwide. In 1996, China reported the first case of this disease. It broke out in 2006 [32], and was mutated into a new subtype in 2010 [33]. Currently, there are no effective drugs and vaccines available.

The full virus genome is 15,000 bp, and is divided into 10 open reading frames (ORF), of which ORF7 encodes the nucleocapsid (N) protein of the virus [29]. The main natural target cells of PRRSV are porcine alveolar macrophages (PAMs); it can also replicate in several cell lines, such as MARC-145 cells [34].

Previous results demonstrated that PRRSV infection in PAMs leads to elevated protein levels of OAS1 and OAS2 [35]. However, the mechanism of action and immunological characteristics of porcine OAS2 (pOAS2) are still unknown. In this study, we cloned the OAS2 gene of Chinese domestic pigs, performed bioinformatics analysis on the gene, and carried out associated experiments to verify the bioinformatics results. This study aims to lay the foundation for determining important functions of the OAS2 gene.

Material and Method

Animal, Cell, Virus

Domestic pigs were obtained from remote mountains, and polymerase chain reactions (PCR) indicated that they were not infected by any pathogens. Marc-145 cells and the

Type 2 PRRSV XH-GD strain (GenBank No. EU624117) were preserved in the laboratory.

RNA Extraction and cDNA Preparation

RNA Extraction was performed using TRIzol reagent (Life Technology, USA) and the Mini BEST Viral RNA/DNA Extraction Kit (Takara, Dalian, China). First strand cDNA synthesis was accomplished using the Prime Script First Strand cDNA Synthesis Kit (Takara, Dalian, China) according to manufacturer's instructions.

OAS2 Cloning

Following cDNA synthesis, PCR products were amplified using OAS2-F and OAS2-R primers. After purification and digestion, the product was ligated to the pcDNA3.1-flag vector, and was verified by sequencing. The primer sequences used are shown in Table 1.

PCR

PCR was performed using the LA Taq DNA polymerase (Takara, Dalian, China), according to manufacturer's protocol.

Bioinformatics Analysis

After obtaining the complete ORF, isoelectric point, aliphatic index, and basic physicochemical characteristics were predicted with ProtParam at the following website: <http://web.expasy.org/protparam/>. Alignment analyses with other species were analyzed using the ClustalX software. A phylogenetic tree was constructed using the MEGA version 4.0 (Tamura, Peterson, Stecher, Nei, and Kumar 2011) program, as previously discussed [36]. Antigenicity was

Table 1 Primers used in the research

Primer	Sequence
OAS2-F	TATGAATTCAATGGGAACTGGGGGTCCC
OAS2-R	CGCGACGACTCAGTCCATGAATCTCC
qOAS2-F	ATGGTGTGAACGCAAACCTGA
qOAS2-R	AACCTTCAGCCGTGTCAAAG
qGAPDH-F	CTGCCGCCTGGAGAAACCT
qGAPDH-R	GCTGTAGCCAAATTCATTGTCTG
Si-OAS2	CCACCAAACUGAAGGAUUU AAAUCCUUCAGUUUGGUGG
Si-RNase L	UGGAAGAGAUGAAUGCAUA UAUGCAUUAUCUCUCCA
qN-F	AAACCACTCCAGAGGCAAGG
qN-R	GCAAACATAAATCCACAGTGTAA

analyzed using the DNASTAR package version 7.0 (DNASTAR, Madison, WI, USA), and the transmembrane region was analyzed by the TMHMM Server v. 2.0 at the following web site: <http://www.cbs.dtu.dk/services/TMHMM/>. Conservative regions were analyzed by NCBI Blast at the following website: <https://blast.ncbi.nlm.nih.gov/Blast.cgi>. Signal peptide analysis was performed by the SignalP 4.1 Server at <http://www.cbs.dtu.dk/services/SignalP/>. Analysis of hydrophilicity was carried out by ProtScale at <http://web.expasy.org/protscale/>. Glycosylation sites were analyzed by the NetNGlyc 1.0 Server at <http://www.cbs.dtu.dk/services/NetNGlyc/>. Analysis of tertiary structure was taken carried out by Swiss-mode at <https://swissmodel.expasy.org/>. Secondary structure was analyzed at the following website: https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html. Phosphorylation sites were predicted by NetPhos-3.1 at <http://www.cbs.dtu.dk/services/NetPhos-3.1/>. Subcellular localization was predicted by PSORT at <https://psort.hgc.jp/form2.html>. Protein function was predicted by the ProtFun 2.2 server at <http://www.cbs.dtu.dk/services/ProtFun/>. Proteins that interact with porcine OAS2 were predicted by the String program, at http://string-db.org/cgi/input.pl?UserId=8AG3IJsO1vO1&sessionId=CqLMDWa9qxEm&input_page_active_form=single_identifier.

Tissue Distribution

RNA was extracted from different tissues in Chinese domestic pigs. RNA samples were reverse transcribed, and were amplified via quantitative polymerase chain reaction (qPCR) to determine the distribution of OAS2 in different tissues.

PRRSV Infected Cells

Marc-145 cells were infected with an MOI of 0.1; cell samples were collected at different time points (0, 6, 12, 24, 36, 48 h), and OAS2 mRNA expression in the cells was measured by qPCR.

Interferons Stimulates Cells

Marc-145 cells were stimulated at a dose of 1000 IU/mL; cell samples were collected at different time points (0, 6, 12, 24 h), and were subjected to qPCR to determine OAS2 mRNA expression.

Poly(I: C) Stimulates Cells

Marc-145 cells were stimulated at a dose of 1.5 µg/mL; cell samples were collected at different time points (0, 4, 6 h), and OAS2 mRNA expression was measured by qPCR.

Transfection

Transfection was performed using the lipofectamine 2000 reagent (Invitrogen, USA), according to manufacturer's instructions.

Western Blotting

Western blotting was carried out as previously discussed [37], where the primary antibody was FLAG monoclonal antibody (1:200, Abnova, Taipei, Taiwan). GAPDH (1:1000, Beijing Biosynthesis Biotech Co., Ltd, China) was used as the control.

Indirect Immunofluorescence Assay (IFA)

IFA was performed as previously outlined [38], with FLAG monoclonal antibody (Abbkine, USA) as the primary antibody.

TCID₅₀

PRRSV titer was tested by TCID₅₀ on Marc-145 cells.

qPCR

SYBR Ex Taq™ (Takara, Dalian, China) was used in qPCR; primers are listed in Table 1.

Cytotoxicity Test

Enhanced Cell Counting Kit-8 assay (Beyotime Biotech, China) was used for determination of cytotoxicity, as per manufacturer's instructions.

siRNAs

The siRNA against OAS2 was synthesized by GenePharma Co., Ltd (Suzhou, China); primer sequences are shown in Table 1.

Data Analysis

Each experiment was performed in triplicates, with three technical replicates. Figures were made using the Graph-Pad Prism 5 software. **P* < 0.05, ***P* < 0.01.

Results

Sequence Analysis

Sequence analysis showed that the whole ORF spans a length of 2021 bp, and encodes 708 amino acids. There are

Fig. 1 a Sequence alignment of porcine OAS2 with mouse, cattle, and human OAS2. Pig (GenBank No. MG799561), mouse (GenBank No. NP_035984), cattle (GenBank No. NP_001019728), and human OAS2 (GenBank No. NM_016817) were analyzed by Clustal X alignment. **b** phylogenetic tree analysis of OAS2 in different species. OAS2 from various species were subjected to phylogenetic analysis: pig (GenBank No. MG799561), mouse (GenBank No. NP_035984), cattle (GenBank No. NP_001019728), human (GenBank No. NM_016817), sheep (GenBank No. XP_009180025), dog (GenBank No. XP_005636083), horse (GenBank No. XP_005612605), monkey (GenBank No.), rat (GenBank No. NP_001009682), cat (GenBank No. M3VUJ0), rat (GenBank No. NP_001009715.1), *Callithrix jacchus* (GenBank No. GL283483), *Ictidomys tridecemlineatus* (GenBank No. JH393478), *Pan troglodytes* (GenBank No. XP_001151534), *Macaca mulatta* (GenBank No. KQ730758), *Ailuropoda melanoleuca* (GenBank No. GL193642), *Otolemur garnettii* (GenBank No. GL873557), *Chlorocebus sabaeus* (GenBank No. XP_008002987.1), *Papio anubis* (GenBank No. XP_009180025.1), and *Gorilla gorilla* (GenBank No. G3QYM7). The unrooted phylogenetic tree was generated by the distance-based neighbor-joining method using MEGA version 4.0 (Tamura, Peterson, Stecher, Nei, and Kumar 2011). Bootstrap values were calculated on 1000 replicates in the alignment

multiple conserved regions in the sequence, including the nucleotidyltransferase (NTase) and 2'-5' oligoadenylate synthase domains (Fig. 1a); no mutation was found in the D-box domain. Previous studies indicated that two OAS domains are present in the sequence with no UBL domain. The phylogenetic tree showed that the porcine OAS2 is on the same branch of the tree as that of the cattle (Fig. 1b).

Bioinformatics Analysis

Using the ProtParam software, the fat index aliphatic index was found to be 87.84; the average hydrophilic coefficient was -0.285; the isoelectric point was 8.31; the molecular formula was $C_{3661}H_{5690}N_{964}O_{1045}S_{27}$; the molecular weight was 80,794.90. The total number of negatively (Asp + Glu) and positively (Arg + Lys) charged residues were 80 and 86, respectively; the structural pattern is shown in Fig. 2a. Amino acid homology analysis indicated that pig and cattle OAS2 had the greatest similarity (74%, Fig. 2b). Antigenicity analysis indicated that multiple antigenic sites are present within the protein (Fig. 2c). Transmembrane prediction results showed no transmembrane region within the sequence (Fig. 2d). Conserved region prediction is shown in Fig. 2e, Two conserved regions, the NTase and OAS domains, were found based on conserved region prediction analysis (Fig. 2e), and were consistent with sequence analysis. Secondary structure prediction indicated that the percentages of alpha helix and random coil were 47.67 and 29.14%, respectively (Fig. 2f).

Based on the signal peptide prediction, no signal peptide is present in this protein (Fig. 3a). Hydrophilicity prediction results showed that many hydrophilic amino acids are

A	p0AS2	MGNWGSHTYDRPPQKLGEFVQNLRPSEDCQKIDQTVDITICKVLQDAEQPLPVISVARG
	m0AS2	MGNWGSHTYDRPPQKLGEFVQNLRPSEDCQKIDQTVDITICKVLQDAEQPLPVISVARG
	c0AS2	MGSRRESHLYEKPSSEKLEEFIQHNLRPSEDCKQIDQSVDTICEVLQEPCPSLTVTGVAKG
	hu0AS2	MNGESQLSSYPAQKLGFITQEYLKPYEECQTLIDEMVNITCDVLQEPEQFPFLVQGVAIG **,**,*
	p0AS2	GSYGRKTVLRGNSDGLSIVIFISDLEKFQDQSKNHSELLSQIWAQLKCCQLTRKLEAKMEI
	m0AS2	GSYGRKTVLRGNSDGLSIVIFISDLEKFQDQSKNHSELLSQIWAQLKCCQLTRKLEAKMEI
	c0AS2	GSYGRRTVLRGNSDGLVVFFGDLEQFQDEKRQYELLSKIWAQMKHCESTWKLAAMKEL
	hu0AS2	GSYGRKTVLRGNSDGLTILVFSDLKQFQDKRSQRDILDKTGDKLKFCFLTWWLNKNFEI *****:***** ***,*,*,*:****,: :*,*: :*,* * *:***:
	p0AS2	QNFN-SGPTTIQLFAKEQSITFKILPAFNALGLEKSPSPWITYRDLKRSLDMMKASPGEFS
	m0AS2	QNFN-SGPTTIQLFAKEQSITFKILPAFNALGLEKSPSPWITYRDLKRSLDMMKASPGEFS
	c0AS2	QNTNRSRVTTILSTRQQSITFNVLPAFNALGLEKSSLYTYRELKRSLDMVKARPGEFS
	hu0AS2	QKSL—DGFTIQVFTKNRQISFEVLAAPNALNDNPSPWITYRELKRSLDKTNASPGEFA *:, **,*:*,*
		YAELELLV
	p0AS2	VCFTELQERFFNNLPRLKLDILLVKYWQQCEKLAVSFQLPVYALELLTVYAWEQGCG
	m0AS2	VCFTELQERFFNNLPRLKLDILLVKYWQQCEKLAVSFQLPVYALELLTVYAWEQGCG
	c0AS2	VCFTELQEKFSNPNYSKLDILLVKHWFQKCQEKLINPSSLPPYALELLTVYAWEQGCG
	hu0AS2	VCFTELQKKFFDNRPGLKLDILLIKHWHQQCQKKIKDLPSPYALELLTVYAWEQGCCR *****;**, * *****:*:,*,*,*,*, *, *****
	p0AS2	AEDFDIAEGLRTVLGLIRKPGELCVYWTNVYNFEDETVRNVLLGQLRARRPVILDPTDPT
	m0AS2	AEDFDIAEGLRTVLGLIRKPGELCVYWTNVYNFEDETVRNVLLGQLRARRPVILDPTDPT
	c0AS2	AEDFDMAEGVRTVLRLIEKQEQLCVYWTNVYNFGDETVINILLSQLQAPRPVILDPTDPT
	hu0AS2	KDNFDIAEGVRTVLELIKQEKELCIYWMVNYNFEDETIRNILLSQLQSARPVILDPDVPT :**;****:*** ***,*:*,* ***** ***,*,*,* **: ; *****,**,

present in the protein, which form hydrophilic regions and proteins (Fig. 3b). Furthermore, multiple glycosylation sites were also predicted, as shown in Fig. 3c. Tertiary structure prediction is shown in Fig. 3d. It was found that porcine OAS2 is homologous to that of humans and monkeys, but is dissimilar to that of mice. As shown in Fig. 3e, proteins that may interact with porcine OAS2 include TRIP12, RABGEF1, ribosomal protein S4 (RPS4), 26S proteasome non-ATPase regulatory subunit (PSMD4), 40S ribosomal S3a (RPS3A), RPS27A, and ubiquitin specific peptidase 43 (USP43).

Fig. 1 continued

Phosphorylation site prediction results showed that there are eight serine sites, two threonine sites, and two tyrosine sites on the protein. Functional prediction indicated that the protein has a 77.3% possibility of being a transport and binding protein, 63.2% possibility of being a nonenzyme protein, and 13.4% possibility of being a transferase enzyme.

Fig. 2 Bioinformatics analysis of pig OAS2. **a** Porcine OAS2 structural model was constructed via the IBS Software. **b** Amino acid homology between different species were analyzed using the DNASTAR softwares. **c** Antigenicity of pOAS2 was analyzed by the

DNASTAR softwares. **d** TMHMM was used to predict the transmembrane domain of pOAS2. **e** NCBI was used to predict conserved regions within pOAS2. **f** Secondary structures of pOAS2 were predicted by PSIPRED

Tissue Distribution

As shown in Fig. 4a, the OAS2 protein is distributed in multiple organs, with especially high expression in the liver.

Subcellular Localization

Prediction results showed that 47.8% of the protein is localized in the cytoplasm, 17.4% is localized in the mitochondria, and 17.4% is localized in nucleus. IFA results showed that the main localization for the protein is the cytoplasm (Fig. 4b).

IFN Increases OAS2 Expression

Following IFN stimulation, OAS2 mRNA expression was significantly increased; the highest level of expression was found at 12 h, with an approximate 100-fold increase in expression (Fig. 4c).

Poly(I: C) Increases OAS2 Expression

Poly(I: C) promoted an approximately 15-fold increase in OAS2 expression, with peak expression reached at 6 h post-treatment (Fig. 4d).

Fig. 2 continued

PRRSV Infection Promotes OAS2 Expression

Elevated OAS2 expression was observed with PRRSV infection, and peaked at 12 h post-infection (14-fold increase) before gradually declining (Fig. 4e).

OAS2 Over Expression Inhibits PRRSV

Western blot results showed that OAS2 protein was abundantly expressed in the cells (Fig. 5a). In addition, increase in OAS2 expression was associated with reduced PRRSV titer and mRNA level (Fig. 5b–e). Cell activity experiments showed that the transfected protein did not exhibit obvious toxicity (Fig. 5f).

Si-OAS2 Promoted PRRSV Replication

Si-OAS2 was transfected into cells, and qPCR result confirmed OAS2 mRNA knockdown (Fig. 6a). A significant increase in viral titers and mRNA levels was found following si-OAS2 transfection (Fig. 6b, c); si-OAS2 did not exhibit any cytotoxicity (Fig. 6d).

Inhibitory Effect of OAS2 is Dependent on RNase L

Si-RNase L was transfected into cells, and qPCR results confirmed effective knock down of RNaseL (Fig. 7a); there is no cytotoxicity was observed for si-RNase L (Fig. 7b).

After the co-transfection of si-OAS2 and si-RNase L, the virus titer and mRNA level did not change significantly as compared with that of the control group (Fig. 7c, d).

Discussion

In the present study, OAS2 was cloned from Chinese domestic pigs; sequence analysis revealed multiple conserved regions within the OAS2 gene. The P-LOOP motif is involved in ATP binding, and the D-box contributes to binding of Mg^{2+} ; no deletion or mutation of P-LOOP and D-box were detected (Fig. 1a). This result suggested that NTase activity is intact in the protein, and that its antiviral activity is dependent on RNase L [39]. Subsequent experimental results further confirm our previous prediction. We did not detect any UBL domains, therefore, function of OAS2 may not be dependent on RIG-I. Sequence analysis

Fig. 3 Bioinformatics analysis of porcine OAS2. **a** Signal4.0 was used to predict the signal peptide of pOAS2. **b** Expasy was used to predict hydrophilicity. **c** Glycosylation sites were predicted by the NetNGlyc 1.0 Server. **d** Swiss-mode was used to predict the tertiary structure of pOAS2. **e** String was used to predict proteins that may interact with pOAS2

Fig. 3 continued

Fig. 4 Tissue distribution and subcellular localization of pOAS2 under different stimuli. **a** Tissue distribution of pOAS2, as analyzed by qPCR. **b** Determination of pOAS2 localization in cells by IFA. **c** pOAS2 mRNA expression in cells during IFN-beta stimulation, as

analyzed by qPCR. **d** pOAS2 mRNA expression in cells during Poly(I: C) stimulation, as analyzed by qPCR. **e** pOAS2 mRNA expression in cells during PRRSV infection, as analyzed by qPCR

suggested that different OAS species are structurally conservative conserved at key sites, and highlighted the important genetic evolution of the protein.

Phylogenetic analysis demonstrated high homology between pig and cattle OAS2 (Fig. 1b), similar to results based on amino acid sequence similarity. Phylogenetic tree

Fig. 5 pOAS2 overexpression inhibited PRRSV replication. **a** Western blot of FLAG expression during OAS2 overexpression; GAPDH was used as control. **b** pOAS2 (MOI = 0.1) was transfected into cells for 24 h, and TCID₅₀ was used to determine the virus titer. **c** Following transfection of pOAS2 (MOI = 0.1), viral mRNA levels were

determined by qPCR. **d** Following transfection of pOAS2 (MOI = 1), TCID₅₀ was used to determine the virus titer. **e** pOAS2 were transfected into cells for 24 h (MOI = 1), and qPCR was used to quantify viral mRNA. **f** Enhanced cell counting Kit-8 assay (Beyotime Biotech, China) was used to determination cytotoxicity of pOAS2

Fig. 6 si-OAS2 promotes virus replication. **a** si-OAS2 was transfected into cells, and OAS2 mRNA expression levels were determined by qPCR. **b** Enhanced cell counting Kit-8 assay (Beyotime Biotech, China) was used to determine cytotoxicity of si-OAS2. **c** Cells were transfected with si-OAS2 (MOI = 0.1), after which viral mRNA level was determined by qPCR. **d** Viral titers were determined via TCID₅₀

Fig. 7 anti-PRRSV activity was dependent on RNase L. **a** si-RNase L was transfected into cells, and RNase L mRNA expression levels were determined by qPCR. **b** Enhanced cell counting kit-8 assay (Beyotime Biotech, China) was used to determine cytotoxicity of RNase L siRNA. **c** Cells were infected with RNase siRNA (MOI = 0.1), and TCID₅₀ was used to determine the virus titer. **d** Cells were infected with RNase siRNA (MOI = 0.1), and viral mRNA was determined by qPCR

analysis indicated that the OAS2 sequence is conserved in mammals.

Sequence analysis showed that there are multiple antigenic sites within this protein, suggesting that it can be used as an immunogen to prepare antibodies. No transmembrane regions or signal peptides were found, which suggested that OAS2 gene encodes a hydrophilic, secretory protein. The tertiary structure of this protein showed high similarity between humans and cows; the secondary structures present were mainly alpha helices.

OAS2 was found to be mainly distributed in the liver and the stomach, as well as organs that are rich in immune cells, which suggested that it can be used to target specific organs. pOAS2 was also found in other immune tissues, which further confirmed its role in immune function. This

was similar to the tissue distribution of human OAS, and suggested that pOAS2 may have important immune functions.

It was predicted that the protein resides in the cytoplasm. Consistent with our prediction, IFA results indicated that pOAS2 is largely distributed in the cytoplasm. Interestingly, the major PRRSV proteins replicate within the cytoplasm, which suggested that OAS may interact with PRRSV proteins.

Expression of OAS was activated during various stimuli; interferon promoted the strongest OAS expression, followed by Poly(I:C). In addition to previous studies have shown that OAS2 in PAMs protein levels, the study is at the mRNA level. On the previous conclusion consistent. This experiment further confirmed the inductive effect of

interferon on OAS2 expression. Other types of stimuli may also promote OAS expression, and will be verified in subsequent experiments.

Our overexpression results indicated that the protein is efficiently expressed, and plays a role in inhibition of viral replication. This inhibitory effect on PRRSV is dependent on RNase L, similar to its role in JEV inhibition [40]. Further studies are required to determine its effect on other RNA or DNA viruses. It has previously been reported that viruses may evade innate immunity by inhibiting OAS expression through self-encoded structural or non-structural proteins [41–45]. Whether such mechanism exists in PRRSV, and which protein is specific to this virus, remain to be determined.

We found that pOAS2 interacts with proteins such as USP43. However, whether pOAS2 is upstream or downstream of these proteins is yet unknown. Future experiments will aim to explore the immunological role of pOAS2 in various pathways.

In this study, highly pathogenic viral strains were used, whereas previous studies have focused on low pathogenic species. Highly pathogenic strains have stronger ability to induce interferons, which may enhance OAS2-induced inhibition.

In summary, this study predicted novel functions of pOAS2, and laid the foundation for further studies on the molecular immunological characteristics of OAS2. pOAS2 is an interferon-inducible protein located in the cytoplasm, and inhibits PRRSV replication.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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