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Molecular cloning, functional characterization and antiviral activity of porcine DDX3X

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ABSTRACT

Human DDX3X is a newly discovered DEAD-box RNA helicase. In addition to involvement of eukaryotic gene expression regulation, human DDX3X has recently been demonstrated to be a critical molecule in innate immune signaling pathways and to contribute to type I interferon (IFN) induction. In the present study, porcine DDX3X was cloned by RT-PCR from PK-15 cells and its function in regulating IFN-β was characterized. The putative porcine DDX3X ORF encodes 662 amino acids possessing several conserved motifs. Sequence alignments indicated that porcine DDX3X has high identity at the amino acid level to those of horse (96.7%), mouse (97.6%), cattle (98.5%), dog (98.6%) and human (98.9%). Ectopic expression of porcine DDX3X significantly activated IFN-β expression, whereas knockdown of porcine DDX3X inhibited dsRNA- or Sendai virus (SeV)-induced IFN-β. Furthermore, porcine DDX3X co-localized with IPS-1, TBK1 and IKKε, and enhanced IFN-β promoter activation induced by these molecules. We also investigated the role of porcine DDX3X during porcine reproductive and respiratory syndrome virus (PRRSV) infection and found that overexpression of DDX3X significantly inhibited PRRSV replication, indicating that DDX3X is a potential antiviral agent.

1. Introduction

DDX3 (or DBX) is a member of the DEAD-box family of RNA helicases, named for a conserved D-E-A-D sequence. It was first identified in 1997 in a study of the non-recombining region of the Y-chromosome [1]. DDX3 has two homologs, DDX3X and DDX3Y. Differently, DDX3X is ubiquitously expressed in a wide range of tissues and resided in both nucleus and cytoplasm [2], while DDX3Y protein expression is restricted to the male germ line. Like many other DEAD-box helicases, DDX3X is involved in a variety of cellular processes involving RNA, such as transcriptional regulation, splicing, mRNA export, ribosome biogenesis and translational regulation [3]. Interestingly, several studies showed that DDX3X is required for the replication of many viruses, such as Hepatitis C virus (HCV) and Human immunodeficiency virus (HIV) [4–6]. It has therefore been suggested as a potential target for the treatment of viral infection [7].

Recently, DDX3X has received intensive attention because several studies demonstrated that it participates in the regulation of innate immune responses [8,9]. As the first line of defense against invading pathogens, innate immunity has been demonstrated to be regulated by members of helicase family, such as RIG-I (retinoic-acid-inducibel protein I) and MDA5 (melanoma-differentiation-associated gene 5). Both of them recognize viral RNA and signal through a mitochondria-associated adaptor called IPS-1 (IFN-β promoter stimulator 1) [10]. Subsequently, IPS-1 activates inhibitors of IKKε (κB kinase ε) and TBK1 (TANK-binding kinase 1) to phosphorylate various transcription factors, including interferon regulatory factor-3 (IRF3) and IRF7. These transcription factors directly activate type I interferon (IFN) promoters and downstream inflammatory cytokines. Belonging to the same family as RIG-I and MDA5, DDX3X also has a positive role in IFN induction. Somewhat distinct, instead to exert an effect as a pattern recognition receptors (PRRs), DDX3X functions in the downstream of signaling cascade to regulate the expression of IFN [8,9]. Early studies suggested that DDX3X activates IRF3/7 through interacting with IKKε or TBK1 and consequently IFN-β induction [8,9]. Further studies revealed that DDX3X can bind to IFN promoter and act as a transcription factor [9]. Another report suggested that DDX3X interacts with IPS-1 to synergistically stimulate IFN-β promoter [11]. However, in the process of evolution, viruses have developed sophisticated mechanisms to evade or subvert the host antiviral response. For instance, Vaccinia virus (VACV) protein K7 interacts with DDX3X and inhibits the IFN induction [12], Hepatitis B virus (HBV) polymerase inhibits IFN induction by disrupting the interaction between IKKε and DDX3X [13].

Recently, as it is potential to be a more economic model for the study of human immune system, there has been an increasing

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interest in swine immune system [14]. However, there is no available information for porcine DDX3X. In this study, porcine DDX3X was cloned from PK-15 cells and its function in regulating type I IFN signaling was investigated. We demonstrated that porcine DDX3X is a potent activator of type I IFN and exhibited significant antiviral activity against porcine reproductive and respiratory syndrome virus (PRRSV).

2. Materials and methods

2.1. Cells and virus

Porcine kidney (PK-15) cells and MARC-145 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 0.25 μg/ml fungizone, 100 U/ml penicillin, 10 μg/ml streptomycin sulfate, and 5 μg/ml gentamicin in a humidified 37 °C, 5% CO_2 incubator. PRRSV strain WUH3 [15], originally isolated from the brains of pigs with "high fever syndrome" in China in 2006 and identified as a highly pathogenic North American type PRRSV, was used in this study.

2.2. Plasmid constructions

Human DDX3X (GenBank accession number AB451220.1) coding sequence were used to search the pig Expressed Sequence Tag (EST) database by BLAST. Two pig EST sequence (GenBank accession number BW976898.1 and CJ009128.1) were found. The primers were designed using these EST sequence. The cDNA fragment of potential porcine DDX3X was amplified by reverse transcriptase-polymerase chain reaction (RT-PCR) from total RNA extracted from PK-15 cells. The PCR product was purified and cloned into the pEGFP-C2 and pCAGGS-HA. The DNA fragments encoding the full-length porcine TBK1, IKKε, and IPS-1 were amplified by PCR using the primers described in Table S1 and subcloned into pDsRed-C1, resulting in the RFP-fusion expression constructs pDsRed-TBK1, pDsRed-IKKε and pDsRed-IPS-1, respectively. The luciferase reporter plasmid IFN-β-luc was described previously [16].

2.3. Sequence alignment and phylogenetic analysis

Amino acid sequences were aligned, and phylogenetic and molecular evolutionary analyses were conducted using the Clustal X 1.83 program. To predict the presence and location of helicase domains in porcine DDX3X, the putative amino acid sequences were analyzed using the PROSITE tools (http://prosite.expasy.org/), and compared to different species by Clustal.

2.4. Transfection and confocal imaging

PK-15 cells were seeded in 24-well plates and incubated until the cells reached approximately 70–80% confluence. The pEGFP-DDX3X was co-transfected with pDsRed-TBK1, pDsRed-IKKε or pDsRed-IPS1. At 24 h post-transfection, cells were fixed by 4% paraformaldehyde for 10 min followed by permeabilization with 0.1% Triton X-100 for 10 min at room temperature (RT). After three washes with PBS, cells were incubated with DAPI for 5 min. After washing with PBS, fluorescent images were performed using confocal laser scanning microscope (LSM 510 Meta, Carl Zeiss).

2.5. Reporter assay

PK-15 cells cultured in 24-well plates were transfected with the expression vectors for TBK1, IKKε, IPS-1, DDX3X or empty vector, together with the reporter plasmid and internal control vector pRL-TK (Promega) using Lipofectamine 2000 reagent (Invitrogen) as specified by the manufacturer. Luciferase assays were performed at 24 h after transfections. Luciferase activity in these cultures was quantified using the Dual-Luciferase Assay Kit (Promega) according to the manufacture's instructions.

2.6. RNA exaction and Real-time RT-PCR

Total cellular RNA was extracted from the cells by using TRIzol reagent (Invitrogen). RNAs (1 μg) were reverse transcribed to cDNA using reverse transcriptase from TOYOBO, and 1 μl of the total cDNA volume was used in a SYBR green PCR assay (Applied Biosystems). Each sample was performed in triplicate and normalized to porcine GAPDH (glyceraldehyde-3-phosphate dehydrogenase) mRNA level. Primers were designed with Primer Express software v.3.0 (Applied Biosystems) and listed in Table S1.

2.7. Quantitative real time RT-PCR (qPCR) for quantitation of PRRSV

Quantitation of PRRSV cDNA was performed by qPCR as previously described [17,18]. Viral RNAs were extracted from samples using TRIzol reagent. Total RANs (1 μg) were reverse transcribed into cDNA using reverse transcriptase form TOYOBO, and 1 μl of total cDNA was used in a qPCR assay (Applied Biosystems). The primers and probe were designed from the ORF7 gene with the Primer Express™ software. The sequences are listed in Table S1. The thermal cycler program consisted of 60 °C for 10 min and 95 °C for 15 s, and then 40 cycles at 90 °C for 15 s and 60 °C for 60 s. For each assay, a standard curve was generated using serially diluted plasmid standards at 10^8–10^{14} copies/μl.

2.8. $TCID_{50}$ assay for PRRSV

PRRSV titers were determined by an endpoint dilution assay and the titers were expressed as the tissue culture infectious dose 50 ($TCID_{50}$) per milliliter using the Reed–Muench method [19]. Briefly, MARC-145 cells were seeded in 96-well plates, following, infected with serial 10-fold dilutions (100 μl) of the original PRRSV samples in eight replicates. Allow virus to adsorb to cells at 37 °C for 1 h, then, the virus inoculum was removed, and DMEM containing 5% FBS was added. Plates were incubated for an additional 72–96 h. Virus titers were calculated by determining the dilution giving 50% of wells containing cells that display cytopathic effects.

2.9. Statistical analysis

All experiments were performed at least three times with reproducible results. Data are presented as means ± SD. Statistical values of $^{*}P < 0.05$ were considered significant and $^{**}P < 0.01$ highly significant.

3. Results and discussion

3.1. Cloning and sequence analysis of porcine DDX3X

The primers were designed based on the porcine EST sequence. RT-PCR was performed to amplify the potential porcine DDX3X from total RNA of PK-15 cells. As shown in Fig. 1A, the full-length cDNA of porcine DDX3X contains 1986 bp and encodes 662 amino acid residues (GenBank accession number HQ266638). Comparison of putative amino acid sequences of porcine DDX3X to the identified or predicted DDX3X of other species showed that the porcine DDX3X had sequence similarity at the aa level to the DDX3X of horse (96.7%), mouse (97.6%), cattle (98.5%), dog (98.6%) and human (98.9%). Phylogenetic analysis indicated that porcine DDX3X

(A)

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Porcine DDX3X   MSHVAVENALGLDQQFAGLDLNSSDNQSGGSTASKGRYIPPHLRNREATKGFYDKDSSRW 60
Porcine DDX3X   SSNKDKDAYSSFGSRNDSRGKSSFFSDRGSGSRGRFDDRGRSDYDGIGSRGDRGGFGKYE 120
Porcine DDX3X   RGGHSRWCDKSDEDDWSKPLPPSERLEQELFSGGNTGINFEKYDDIPVEATGNNCPPHIE 180
                                Q Motif                 Motif I
Porcine DDX3X   SFSDVEMGEIIMGNIELTRYTRPTPVQKHAIPIIKEKRDLMACAQTGSGKTAAFLLPILS 240
                                        Motif Ia
Porcine DDX3X   QIYSDGPGEALRAMKENGRYGRRKQYPISLVLAPTRELAVQIYEEARKFSYRSRVRPCVV 300
                                Motif Ib                Motif II
Porcine DDX3X   YGGADIGQQIRDLERGCHLLVATPGRLVDMMERGKIGLDFCKYLVLDEADRMLDMGFEPQ 360
                                Motif III
Porcine DDX3X   IRRIVEQDTMPPKGVRHTMMFSATFPKEIQMLARDFLDEYIFLAVGRVGSTSENITQKVV 420
                                Motif IV
Porcine DDX3X   WVEESDKRSFLLDLLNATGKDSLTLVFVETKKGADSLEDFLYHEGYACTSIHGDRSQRDR 480
                                Motif V                 Motif VI
Porcine DDX3X   EEALHQFRSGKSPILVATAVAARGLDISNVKHVINFDLPSDIEEYVHRIGRTGRVGNLGL 540
Porcine DDX3X   ATSFFNERNANITKDLLDLLVEAKQEVPSWLENMAYEHHYKGSSRGRSKSRFSGGFGARD 600
Porcine DDX3X   YRQSSGASSSSFSSSRASSSRSGGGGHGSSRGFGGGGYGGFYNSDGYGGNYNSQGVDWWG 660
Porcine DDX3X   NZ 662
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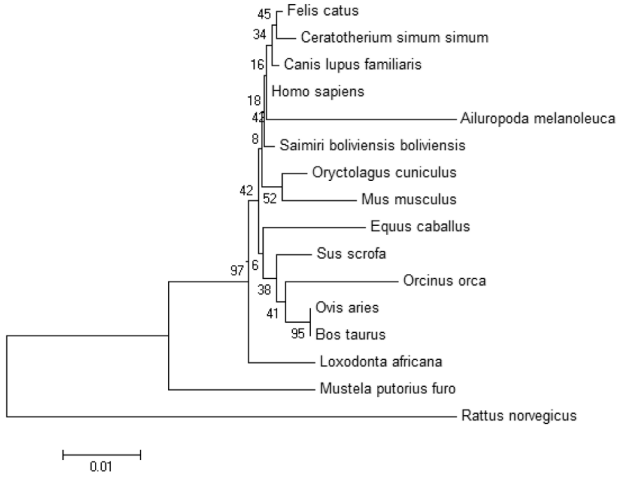
(B)



(C)

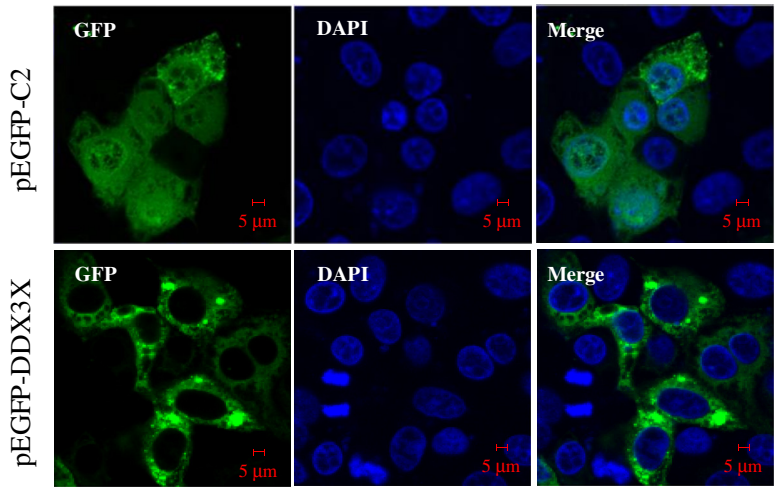


Fig. 1. The conserved motifs, phylogenetic tree and subcellular localization analysis of porcine DDX3X. (A) The conserved motifs of porcine DDX3X. The numbers indicate the amino acid position. Nine conserved motifs are highlighted. (B) A phylogenetic tree of the amino acid sequences of sixteen identified or predicted DDX3X from different species. The uprooted tree was built using the neighbor-joining method based on the alignment of DDX3X amino acid sequences. The scale bar is 0.1. (C) PK-15 cells were transfected with pEGFP-C2 or pEGFP-DDX3X. Cells were fixed and permeabilized at 24 h post-transfection. Cellular nuclei were stained with DAPI. The porcine DDX3X localization was observed under an LSM-510 Meta confocal fluorescence microscope. Scale bar: 5 μm.

belonged to a new branch of the CLUSTAL dendrogram (Fig. 1B), and was more phylogenetically close to human DDX3X compared with mouse, supporting the concept that swine may be a better model animal for the study of human immune system [14].

Based on sequence alignments, nine conserved motifs have been identified in the helicase core of DEAD-box proteins [20,21]. The simultaneous presence of these motifs is a criterion for inclusion of a protein into the family. As shown in Fig. 1A, the nine putative motifs could be in porcine DDX3X, indicating that these motifs are highly conserved in porcine DDX3X.

3.2. Porcine DDX3X locates in the cytoplasm

Is has been reported that human DDX3X shuttles between the cytoplasm and the nucleus [6]. Further, Martina et al. demonstrated that DDX3X was mostly in the cytoplasmic fraction under normal condition [8]. To investigate the location of porcine DDX3X, the recombinant plasmid that expresses the fusion protein of EGFP and full-length porcine DDX3X (pEGFP-DDX3X) was transfected into PK-15 cells, and the cells transfected with pEGFP-C2 plasmid as control. At 24 h post-transfection, cells were examined under confocal laser scanning microscope. As shown in Fig. 1C, the EGFP were distributed throughout the cells, however, the fuse protein EGFP-DDX3X was only observed in the cytoplasm. The results indicated that, similarly to the human DDX3X, the porcine DDX3X mainly localizes in the cytoplasm.

3.3. Porcine DDX3X regulates the expression of IFN-β

It has been reported that human DDX3X was involved in the regulation of innate immune responses. To test the role of porcine DDX3X in innate immunity, we carried out reporter gene analysis to see the enhancing effect of porcine DDX3X on IFN-β activation. To this end, PK-15 cells were transfected with a porcine DDX3X expression construct (pCAGGS-DDX3X), together with a luciferase reporter plasmid of porcine IFN-β promoter and pRL-TK. At 24 h post-transfection, the cells were harvested and analyzed by dual-luciferase assay. As shown in Fig. 2A, overexpression of porcine DDX3X in PK-15 cells activated the IFN-β promoter. To support this hypothesis further, the mRNA expression of IFN-β was analyzed by real-time RT-PCR in parallel. We found that overexpression of porcine DDX3X also up-regulated endogenous transcription of IFN-β mRNA (Fig. 2B). To further confirm the role of endogenous porcine DDX3X in mediating the IFN-β induction, we performed RNA interference (RNAi) experiments to knockdown the expression of DDX3X. The oligonucleotides specifically targeting porcine DDX3X led to significant reduction in DDX3X mRNA expression compared to cells transfected with a control oligonucleotide (Fig. 2C). Furthermore, transfection with porcine DDX3X-specific RNAi significantly inhibited SeV- or poly(I:C)-stimulated IFN-β activation (Fig. 2D and E). Taken together, these results indicated the involvement of porcine DDX3X in IFN-β production.

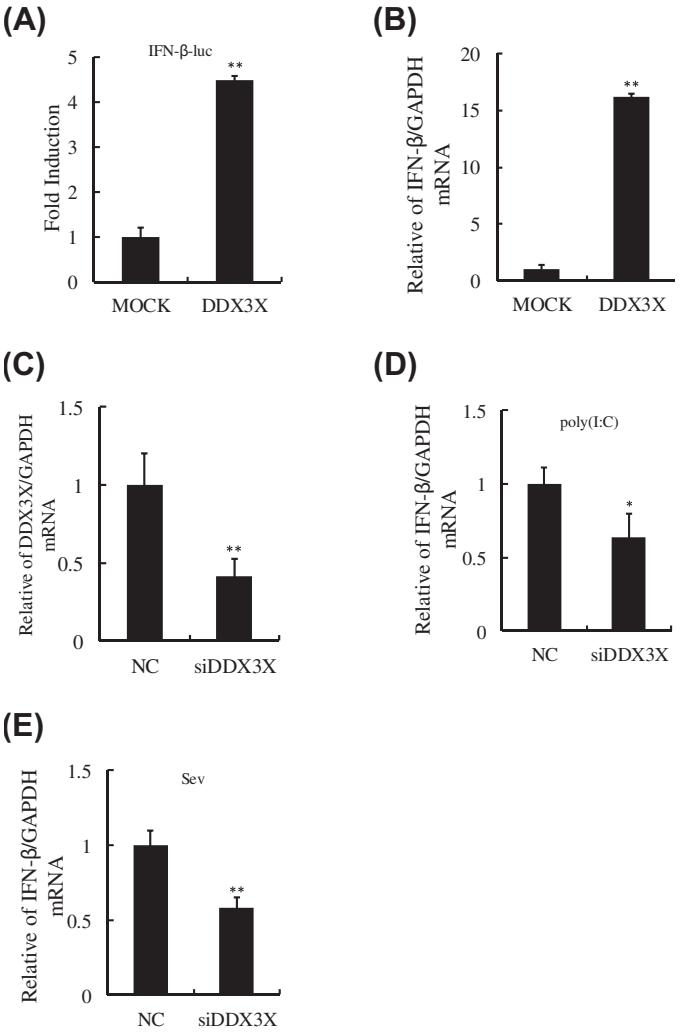


Fig. 2. Porcine DDX3X is involved in the regulation of IFN-β production. (A) PK-15 cells were transfected with 300 ng of plasmid encoding full-length DDX3X (pCAGGS-DDX3X) along with IFN-β-Luc and pRL-TK using Lipofectamine 2000. Luciferase assays were performed 24 h after transfection. (B and C) pCAGGS-DDX3X (B) and indicated siRNA (C) was transfected into PK-15 cells respectively, at 24 h post-transfection, total RNA was extracted and the expression of porcine IFN-β (B) and DDX3X (C) were evaluated by real-time RT-PCR. (D and E) Porcine DDX3X siRNA was transfected into PK-15 cells, 24 h after the initial transfection, the cells were transfected with poly(I:C) (D) or infected with Sev (E), cells were harvested separately 12 h later, subjected to porcine IFN-β-specific real-time RT-PCR. Results are representative of those from three independent experiments. Statistical significance was assessed based on the *P*-value: $^{*}P < 0.05$ and $^{**}P < 0.01$.

3.4. Porcine DDX3X co-localizes with TBK1/IKKε and enhances TBK1/IKKε-mediated IFN-β induction

Didier et al. have demonstrated that human DDX3X enhances IFN production via its interaction with TBK1 [9]. Additionally, Gu et al. recently showed that DDX3X mediates phosphorylation of interferon regulatory factor 3 (IRF3) by the kinase IKKε [22]. Wang and Ryu also demonstrated that HBV polymerase inhibits IFN induction by disrupting the interaction between IKKε and DDX3X [13]. It is well known that both TBK1 and IKKε are critical kinases in IFN-β signaling. Because porcine DDX3X overexpression activated IFN-β production, we tested if this activation associated with TBK1 and IKKε. To this end, a recombinant plasmid expressing the fusion protein of EGFP and full-length porcine DDX3X (pEGFP-DDX3X) was co-transfected with pDsRed2-TBK1 or pDsRed2-IKKε, which encodes a fusion protein of RFP and TBK1 or IKKε, respectively. Fluorescences were examined under confocal laser scanning microscope. As shown in Fig. 3A and C, the green fluorescence almost completely overlapped with red fluorescent profile. These results indicated that the porcine DDX3X have a co-localization with TBK1 or IKKε in PK-15 cells.

Forced expression of TBK1 or IKKε causes the activation of transcription from the IFN-β [23]. As a kinase substrate of TBK1/IKKε, human DDX3X can augment the TBK1/IKKε-mediated IFN-β induction. On the basis of our finding that porcine DDX3X co-localized with TBK1 and IKKε, we wondered whether porcine DDX3X has a role in this pathway. To test this hypothesis, PK-15 cells were transfected with DDX3X- and/or TBK1/IKKε-encoding plasmids (pCAGGS-DDX3X, pCMV-TBK1, pCMV-IKKε) together with the

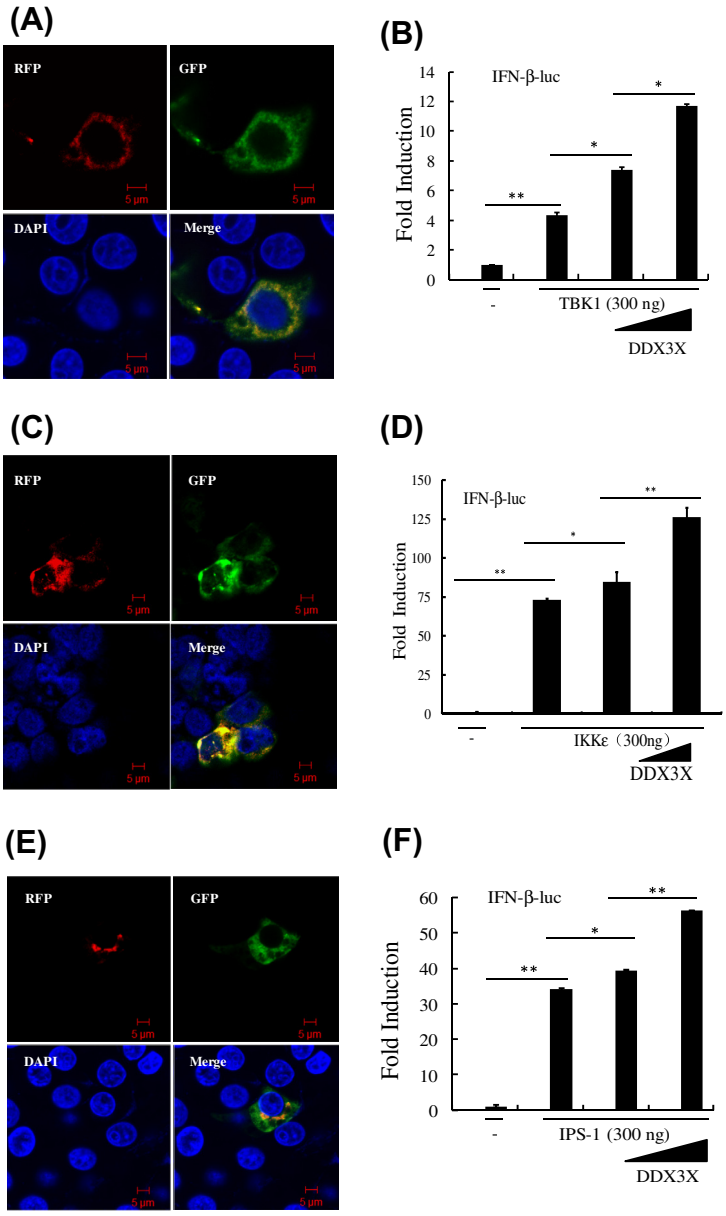


Fig. 3. Porcine DDX3X co-localizes with TBK1, IKKε and IPS-1, and enhances the TBK1-, IKKε- and IPS-1-mediated IFN-β activation. (A, C and E) GFP-tagged porcine DDX3X was co-transfected with RFP-tagged TBK1 (A), IKKε (C) or IPS-1 (E). After 24 h, cells were fixed and stained nuclei with DAPI, and observed on confocal fluorescence microscope. Scale bar: 5 μm. (B, D and F) PK-15 cells were transfected with porcine DDX3X-(0, 100, 300 ng), IFN-β-Luc and pRT-TK, together with TBK1-(B), IKKε-(D) or IPS-1(F)-encoding plasmids, cell lysates were prepared at 24 h post-transfection and subjected to a dual-luciferase assay. Results are representative of those from three independent experiments. Statistical significance was assessed based on the *P*-value: $^{*}P < 0.05$ and $^{**}P < 0.01$.

porcine IFN-β promoter and control plasmid (pRL-TK), following, luciferase reporter assays were performed. As shown in Fig. 3B and D, the promoter activation was more augmented by co-expression of DDX3X and TBK1 or IKKε than by overexpressed TBK1 or IKKε alone. Based on these results, we suspected that porcine DDX3X enhances the TBK1/IKKε mediated-signal pathway via the interaction.

3.5. Porcine DDX3X co-localizes with IPS-1 and enhances IPS-1-mediated IFN-β induction

As DDX3X plays several roles in RNA metabolisms, such as RNA translocation or mRNA translation, it was not surprising that DDX3X is involved in RIG-I signaling at multiple steps. Unlike previous reports, Oshiumi et al. demonstrated that DDX3X can bind to the IPS-1 directly and help IPS-1 up-regulate IFN-β promoter activation [11]. Hence, we examined whether porcine DDX3X was associated with IPS-1. DNA expression constructs RFP-tagged IPS-1 (pDsRed-IPS-1) and pEGFP-DDX3X were co-transfected into PK-15 cells together. After 24 h, cells were observed by laser scanning confocal microscopy. As shown in Fig. 3E, three-color imaging analysis indicated that porcine DDX3X in part co-localized with IPS-1 in PK-15 cells. Furthermore, porcine DDX3X was co-expressed with IPS-1, and IFN-β promoter activation was examined. Similar to co-expression with TBK1 and IKKε, porcine DDX3X can also enhance the IPS-1-mediated IFN-β promoter activation (Fig. 3F). The results suggested a possible interaction between IPS-1 and porcine DDX3X.

More recently, Cruciat et al. reported that human DDX3 is required for Wnt–β-catenin signaling in mammalian cells [24]. We also investigated whether ectopic expression of porcine DDX3X enhances Wnt–β-catenin signaling in PK-15 cells. To this end,

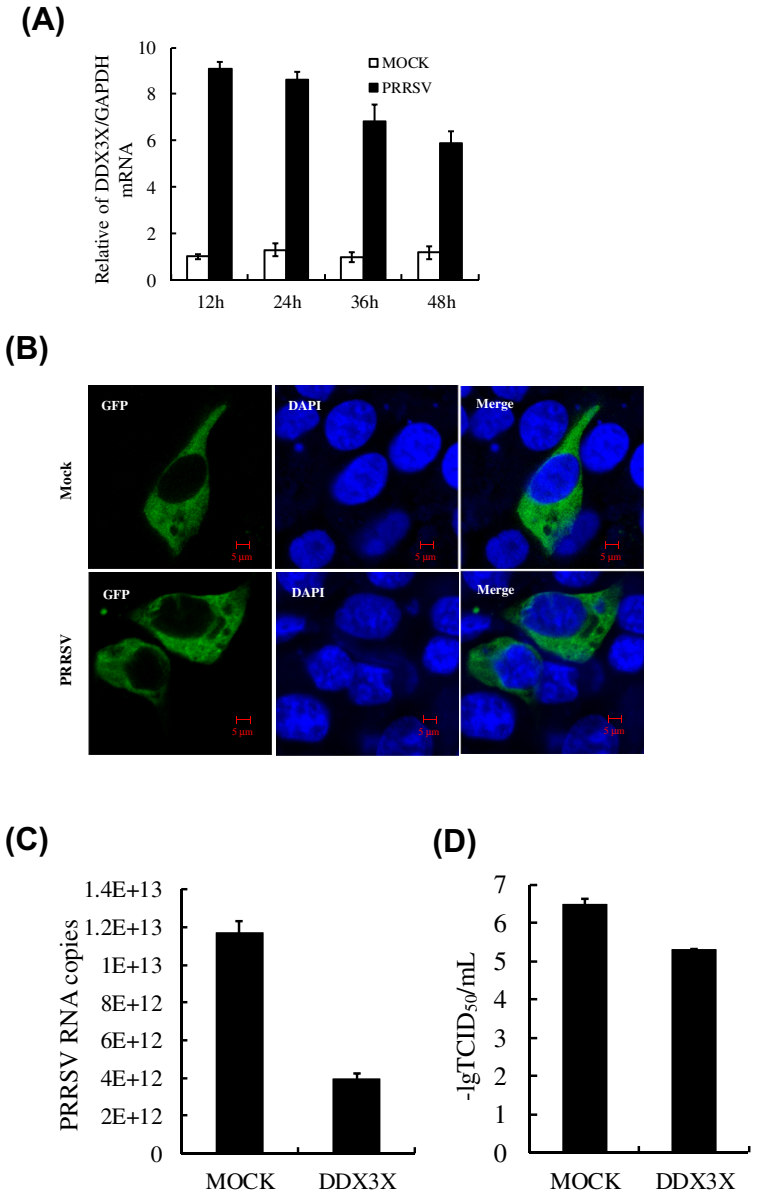


Fig. 4. Activation of porcine DDX3X expression plays an antiviral role during PRRSV infection. (A) MARC-145 cells were mock-infected or infected with PRRSV at a multiplicity of infection (MOI) of 0.5. Cells were collected at the indicated time points, and subjected to real-time RT-PCR to analyze the expression of porcine DDX3X. (B) MARC-145 cells were transfected with plasmid encoding EGFP-DDX3X fusion protein. Twenty-four hours later, the transfected cells were infected or mock-infected with PRRSV (MOI 0.5). At 36 post-infection, cells were fixed and counterstained cell nuclei with DAPI. Fluorescent images were examined under confocal laser scanning microscope. (C and D) MARC-145 cells were transfected with pCAGGS-DDX3X or empty vector. Twenty-four hours after transfection, cells were infected with PRRSV (MOI 0.5). The infected cells were collected at 24 h post-infection, viral RNA loads were tested by qPCR, and PRRSV-containing samples were tested by $TCID_{50}$ assay. All data represent the means and standard deviation of three independent experiments.

PK-15 cells were co-transfected with pCAGGS-DDX3X, pRL-TK, and reporter plasmids harboring wild type Tcf (T cell factor) binding sites (TOPflash) or a mutant Tcf-binding site (FOPflash) (Upstate Biotechnology, NY), respectively. By comparing the ratio of TOP/FOP as described previously [25], no significant difference could be observed between cells transfected with pCAGGS-DDX3X and empty vector control (data not shown), indicating that porcine DDX3X does not associate with Wnt–β-catenin signaling in PK-15 cells.

3.6. Porcine DDX3X plays an antiviral role during PRRSV infection

Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically important diseases in pigs worldwide characterized by severe reproductive failure in sows and respiratory distress in piglets and growing pigs [26,27]. The etiological agent, PRRSV belongs to the *Nidovirales* order, *Arteriviridae* family of positive-sense single stranded RNA viruses [28]. So far, four different viruses have been demonstrated to interact with human DDX3X and to manipulate its function in different ways [29]. To develop a much better understanding of porcine DDX3X, we investigated the relationship between PRRSV and porcine DDX3X. MARC-145 cells were infected with PRRSV strain WUH3 at multiplicity of infection (MOI) of 0.1. Real-time RT-PCR was performed to detect DDX3X mRNA. As shown in Fig. 4A, MARC-145 cells infected with PRRSV exhibited significantly increased expression of DDX3X mRNA, with maximal production at 12 h post-infection. Further, we detected the localization of porcine DDX3X during PRRSV infection, and found that in PRRSV-infected cells the porcine DDX3X still localized in the cytoplasm (Fig. 4B). These results indicated

that PRRSV infection activates the expression of DDX3X mRNA, however, does not induce DDX3X translocation.

In addition, we evaluated the impact of porcine DDX3X on PRRSV proliferation *in vitro*. To this end, MARC-145 cells were transfected with pCAGGS-DDX3X or control plasmid (pCAGGS-HA), followed by infected with PRRSV. The treated cells were collected and virus titer was determined by qRT-PCR and $TCID_{50}$. As shown in Fig. 4C and D, overexpression of porcine DDX3X significantly inhibits the replication of PRRSV. Hence, we concluded that the porcine DDX3X plays an antiviral role during PRRSV infection. The signaling pathway and the antiviral mechanism of porcine DDX3X remain to be fully elucidated.

In summary, the present study clearly demonstrated that porcine DDX3X was involved in the innate immune signaling pathways to contribute to the induction of type I interferon. Furthermore, porcine DDX3X co-localized with TBK1 and IPS-1, and functions as a positive regulator of the TBK1- and IPS-1-induced type I IFN pathway. In addition, we found that porcine DDX3X plays an antiviral role during PRRSV infection. Understanding the role of porcine DDX3X should help to develop more effective disease control strategies against PRRSV.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.12.098.

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