Gene 679 (2018) 212–218

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Porcine ISG15 modulates the antiviral response during pseudorabies virus replication

Huimin Liu[a,1], Shuangshuang Li[b,1], Xia Yang[b], Xinwei Wang[b], Yongtao Li[b], Chuanqing Wang[b], Lu Chen[b,*], Hongtao Chang[b,*]

[a] College of Life Science, Henan Agricultural University, Zhengzhou, Henan Province, China
[b] College of Animal Science and Veterinary Medicine, Henan Agricultural University, Zhengzhou, Henan Province, China

ARTICLE INFO

Keywords:
Pseudorabies virus
Porcine ISG15
Immunodulatory
Antiviral response

ABSTRACT

Pseudorabies virus (PRV) is one of the most vital pathogens of swine, leading to huge economic losses to the pig industry. Functioning in innate immunity, type-I interferon (IFN) plays a vital role in initial stage of viral infection. ISG15, an IFN-stimulated ubiquitin-like protein, is highly increased during virus infection and participates in the IFN-mediated antiviral immune response. However, limited attention has been paid to the functional role of porcine ISG15 (pISG15) in PRV infection. In this study, we generated a PK15 inducible cell line stably expressing the pISG15 gene and investigated the potential anti-PRV response of pISG15. We demonstrated that pISG15 was upregulated in an early stage of PRV infection, and pISG15 overexpression efficiently inhibited PRV replication by reducing the viral titers and mRNA levels of PRV, and also increased expression of IFN-β and activation of the ISRE promoter. However, knockdown of pISG15 by siRNA did not affect PRV replication, and potentiated IFN-I-mediated signaling, resulting in an increase in antiviral response in the process of PRV infection. The results showed that pISG15 has a potential immunodulatory role in cellular antiviral response against PRV.

1. Introduction

Pseudorabies virus (PRV), a member of the family *Herpesviridae*, is an enveloped dsDNA virus and the etiologic agent of Aujeszky's disease in pigs (Yang et al., 2017). PRV infection can cause fatal neurological disorders in newborn piglets, respiratory ailments in fattening pigs, and reproductive failure in sows, which can lead to substantial economic losses to the pig husbandry (Sun et al., 2016). PRV, a pathogen of broad host range, is able to infect nearly all mammals but pigs are the only natural host and reservoir (Müller et al., 2011). Since 2011, outbreaks of pseudorabies in pigs have occurred in many regions of China, and the disease situation continues to worsen.

The type I interferon (IFN-α/β) is an early and critical component of the innate immune response that is triggered by viral infections, and plays a vital role in immunomodulatory effects and viral inhibition (Samuel, 2001a; Katze et al., 2002). Simultaneously, virus can inhibit IFN production and IFN signaling, as well as neutralize the functions of IFN-stimulated genes (ISGs) to counteract the interferon response (Schulz and Mossman, 2016; Su et al., 2016; Zhang et al., 2017). PRV infection impaired the IFN signaling to establish persistent infection in host cells, by suppressing IFN-induced upregulation of STAT1 phosphorylation and various ISGs (Brukman and Enquist, 2006; Su et al., 2016). However, the mechanism of IFN-I-mediated antiviral activity against PRV has not yet fully understood.

Interferon-stimulated gene 15 (ISG15), an ubiquitin-like protein induced by type I IFN, act as a protein modifier with pleiotropic functions (Lenschow et al., 2007; Morales and Lenschow, 2013). ISG15 functions as an important antiviral molecule against herpes, influenza, and Sindbis viral infections (Lenschow et al., 2007; Morales et al., 2015), however, several reports on human showed that ISG15 deficiency increases antiviral activity in humans (Hermann and Bogunovic, 2017). It has been recently reported that ISG15 has an immunomodulatory effect on regulating antiviral activity against viruses (Speer et al., 2016; Sooryanarain et al., 2017).

Abbreviations: PRV, pseudorabies virus; ISG15, interferon-stimulated gene 15; Vero, African green monkey kidney; PK-15, porcine kidney; qRT-PCR, quantitative real-time RT-PCR; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PVDF, polyvinylidene difluoride; PBS, phosphate-buffered saline; DMEM, Dulbecco's Modified Eagle Medium; ISRE, IFN-stimulated response element

* Corresponding authors.
E-mail addresses: chenluhau@126.com (L. Chen), ndcht@163.com (H. Chang).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.gene.2018.09.007
Received 14 July 2018; Received in revised form 26 August 2018; Accepted 6 September 2018
Available online 07 September 2018

Table 1
Primers or sequences used in this study.

Name	Primer	Sequence (5′-3′)	Usage
pISG15	PB-pISG15-F	GC*TCTAGA*CTATGGGTAGGGAACTGAAGGT	PiggyBac-pISG15 construction
	PB-pISG15-R	GC*GGATCC*CTTTATTACTAGCACTCGGTGAGGT	
	qISG15-F	GGTGCAAAGCTTCAGAGACC	qPCR
	qISG15-R	GTCAGCCAGACCTCATAGGC	
PRV	qEP0-F	CGGGCGAAGACAAACAAAGG	qPCR
	qEP0-R	GGGCGGTAGAAGCCAAACATC	
β-actin	*β*-actin-F	CTCCTTCCTGGGCATGGA	qPCR
	β-actin-R	CGCACTTCATGATCGAGTTGA	
IFN-*β*	qIFN-*β*-F	CAGCAGATCTTCGGCATTCT	qPCR
	qIFN-*β*-R	TCCAGGATTGTCTCCAGGTC	
siRNA	si-pISG15-F	CUAUGUGCACCGUGUAUAUTT	Knockdown
	si-pISG15-R	AUAUACACGGUGCACAUAGTT	
	NC-siRNA-F	UUCUCCGAACGUGUCACGUTT	
	NC-siRNA-R	ACGUGACACGUUCGGAGAATT	

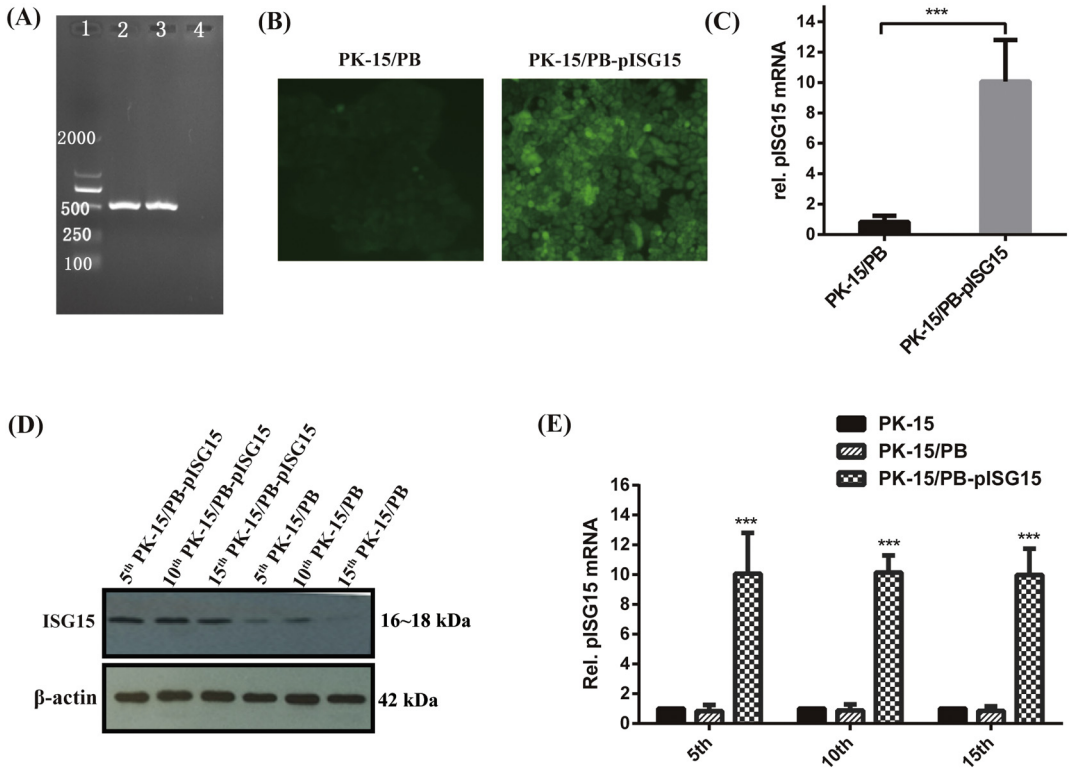

Fig. 1. PK-15 cells stably expressing pISG15 were established: (A) RT-PCR of pISG15 in the PK-15/PB-pISG15 cell line. Lane 1: DL2000 marker; Lane 2: PB-pISG15 as positive control; Lane 3: PK-15/PB-pISG15 cells; Lane 4: PK-15 without pISG15 as negative control. (B) PK-15 cells transfected with PB-pISG15 were treated with puromycin, and the puromycin-positive cell clones designated as PK-15/PB-pISG15 were imaged by fluorescence microscopy; ×200. (C) The mRNA level of pISG15 was measured by qRT-PCR. (D) The protein level of pISG15 from the 5th, 10th, and 15th generations of the PK-15/PB-pISG15 cell line and the PK-15/PB cells were detected by western blot, and *β*-actin was detected as a loading control. (E) The mRNA levels of pISG15 from different generations of PK-15, PK-15/PB, and PK-15/PB-pISG15 cells were measured by qRT-PCR. ***, $p \leq 0.001$.

Here we sought to investigate the role of porcine ISG15 (pISG15) in PRV replication and determine whether pISG15 plays a potential antiviral activity during PRV infection. Our results suggest that pISG15 has a potential immunomodulatory role in cellular antiviral activity against PRV infection.

2. Materials and methods

2.1. Cells, viruses and plasmids

PK-15 and Vero cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen, CA) supplemented with 10% fetal bovine serum and antibiotics, and were maintained at 37 °C with 5% CO_2. High-titer stock of PRV (PRV-QXX) was prepared by inoculating PK-15 cells with PRV-QXX, which have been isolated in our previous report (Li et al., 2015). The viral titers were determined using plaque assays.

Porcine ISG15 (pISG15) was amplified from PK-15 cells by PCR using specific primers (Table 1) and cloned into the PiggyBac (PB) transposon system (Mountain View, CA, USA) to generate the plasmid PB-pISG15. The porcine promoter reporter plasmid IFN-*β*-luc has been described previously (Li et al., 2015). Rabbit monoclonal antibody against *β*-actin was used for western blotting. The sequences of target genes and negative control are presented in Table 1.