Investigation of the anti-pseudorabies virus activity of interferon lambda 3 in cultured porcine kidney epithelial cells

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Abstract

Background: It has been reported Interferon-λ (IFN-λ) has stronger antiviral effect than other interferons. IFN-λ can induce antiviral interferon stimulated genes (ISGs) in epithelia to protect against virus. Pseudorabies virus (PRV) infection in pigs resulting in fatal encephalitis in newborn piglets, respiratory disorders in finishing pigs, reproductive disorders in sows and other symptoms.

Objectives: Since the effect of IFN-λ on inhibiting PRV proliferation is still unknown. In this study, we investigate the relative contribution of porcine IFN-λ3 toward controlling the infection of PRV in vitro. Our findings may provide a new insight for the prevention and treatment of PRV.

Methods: Therefore, the antiviral assay, western blot, qRT-PCR and ELISA assay were used to investigating the contribution of IFN-λ against PRV in PK-15 cells.

Results: Here, we demonstrate that the replication of PRV in PK-15 cells was inhibited after pre-treatment with IFN-λ3, and such inhibition was dose dependent. Overexpression of IFN-λ3 receptor (IFNLR) also restricted virus titre in PK-15 cells. In addition, IFN-λ3 also increased the mRNA and protein expression of interferon-stimulated genes 15 (ISG15), 2′-5′-oligoadenylate synthase 1 (OAS1), IFN-inducible transmembrane 3 (IFITM3) and myxoma resistance protein 1 (Mx1) in PRV-infected PK-15 cells. Other than modulation ISGs, IFN-λ specifically activated IFN-γ mRNA expression not IFN-α or IFN-β.

Conclusions: IFN-λ3 had antiviral activity against PRV and the upregulation of ISGs and IFN-γ mRNA expression may be the mechanism of IFN-λ3’s antiviral activities. Thus, IFN-λ3 has a decisive function that greatly limits PRV replication in PK-15 cells. Our study explores the antiviral activity of IFN-λ3 on PRV for the first time.

KEYWORDS
antiviral activity, IFNLR, IFN-λ3, PK-15, PRV
1 | BACKGROUND

Interferon is a key component of innate immunity against viral infection, usually divided into three types. Type III interferon consist of four members in humans include IFN-λ1, IFN-λ2, IFN-λ3 and IFN-λ4 (O’Brien et al., 2014; Zhou et al., 2018), two members in mice include IFN-λ2 and IFN-λ3 (Bartlett, 2005; Lasfar, 2006) and two members (IFN-λ1 and IFN-λ3) in swine (Shen, 2016; Wang et al., 2011). Compared to type I IFN, IFN-λ is produced earlier after infection, and showed a stronger protective effect in the early stage of infection (Galani et al., 2017).

Upon contact with the receptor (IFNLR1 which comprises the specific subunit IFNL1R1 plus the IL-10R2 subunit), IFN-λ mainly activates the JAK-STAT pathway to induce IFN stimulation gene (ISG) expression, which mediates (Hernández et al., 2015; Kotenko et al., 2003). Type I IFN bind to its receptor (IFNAR) can both activate the antiviral activity and induces the expression of pro-inflammatory cytokines and chemokines (Ivashkiv & Donlin, 2014). Therefore, type I IFN also has the potential to induce inflammatory response, while IFN-λ treatment only induced the expression of antiviral genes (Galani et al., 2017). Thus, the use of IFN-λ in clinical can avoid the side effect of type I IFN (Lazear et al., 2015). In conclusion, IFN-λ has more antivirus advantages than type I IFN. Further explore whether IFN-λ can be a more ideal reagent for the prevention and treatment of viral infection is particularly important.

Pseudorabies virus (PRV) is a zoonosis in many kinds of animals. Cats, dogs, goats, sheep, cattle and raccoons are susceptible to PRV (Mettenleiter, 2000). Porcine are the storage of PR pathogens and the main source of infection. Once infected with PRV, it will be virulent for a lifetime (Wong et al., 2019). The outbreak of porcine PRV was difficult to control and caused catastrophic economic losses to the pig industry. In 2011, a variant strain of pseudorabies virus from Bartha-K61-vaccinated pig farms appeared in China (Yu et al., 2014). The Bartha-K61 vaccine did not effectively protect against the porcine variant PRV (An et al., 2013) (although this is controversial (Freuling et al., 2017), but since then these novel viruses have continued to circulate in northern, eastern and southern China (Wong et al., 2019). Besides, positive serological PRV antibodies were found in human patients since 2017. Human was thought to be refractory for PRV infection previously. It was noted that all patients continued to suffer from various sequelae after discharge except one patient who was dead (Ou et al., 2020).

Therefore, developing an anti-PRV virus therapy is urgently needed. IFN-λ has been shown to inhibit replication of many viruses in vitro and in vivo (Li et al., 2017). However, there are no reports regarding whether porcine IFN-λ have antiviral effect on PRV. Thus, the current work aims to evaluate the antiviral effect of IFN-λ on PRV in PK-15 cells and provide a theoretical foundation for the treatment of PRV.

2 | RESULTS

2.1 | IFN-λ3 inhibits the replication of PRV-EGFP in a time- and dose-dependent manner in PK-15 cells

To investigate the antiviral effect of IFN-λ on PRV-EGFP. PK-15 cells were primed with IFN-λ3 as indicated concentration for 12 h and then infected with PRV-EGFP at 0.1 MOI for 24 h. As shown in Figure 1a–d, IFN-λ3 inhibited PRV-EGFP replication. The number and size of viral plaques was decreased with the increase in IFN-λ3 concentration (Figure 1e–h). The virus titre was significantly reduced with the increase in IFN-λ3 concentration (Figure 1i). To investigate the time-dependent manner of the IFN-λ3 inhibits the replication of PRV-EGFP, 1000 ng/ml IFN-λ3 were used to treat PK-15 cells and infected with the PRV-EGFP for 12, 24, 36 and 48 h. As shown in Figure 1j, the inhibition of IFN-λ3 on PRV-EGFP decreased with time, but the PRV-EGFP proliferation also slowed down within 12–48 h in PK-15 cells that were treated with IFN-λ3. The above results showed that IFN-λ3 substantially suppressed PRV infection in PK-15 cells. And maintain a potent anti-PRV activity in the later stage.

2.2 | IFN-λ3 receptor IFNLR1 overexpression reduced PRV-EGFP titre in PK-15 cells

IFN-λ mediates antiviral activities upon bind with IFNLR. To better characterise the IFN-λ3 anti-PRV activities in the PK-15 cells, the recombinant plasmid of porcine IFNLR1 and empty vector PLVX-aceEGFP-N1 were transfected into cells, respectively. After 24 h, the cells were treated with PRV-EGFP and 1000 ng/ml IFN-λ3. Then, the virus titre were measured. Our results showed the IFNLR1 protein expression were increased (Figure 2a) and IFNLR1 overexpression reduced PRV-EGFP titre significantly (Figure 2b). These results further verified that IFN-λ can prevent PRV infection in PK-15 cells.

2.3 | IFN-λ3 increased IFN-γ mRNA expression levels in PK-15 cells infected with PRV-EGFP

As shown in Figure 3, the PRV-EGFP did not alter the mRNA expression level of IFN-α but upregulated the IFN-β and IFN-γ mRNA expression level. IFN-λ3 pre-treatment did not increase the IFN-α and IFN-β mRNA expression level when compared to PRV-EGFP infection group. However, the mRNA expression level of IFN-γ was significantly increased by IFN-λ3 when compared with PRV-EGFP infection group. The abovementioned data suggest that IFN-λ3 may help PK-15 cells preferentially produced IFN-γ via immune response to protective against PRV.
IFN-λ3 inhibited PRV-EGFP infection by activating ISGs in PK-15 cells

IFN-λ bind to IFNLR and induce the expression of the IFN-stimulated genes (ISGs) to mediate antiviral activity. Here we investigate whether IFN-λ3 induce the ISGs expression in PK-15 cells infected with PRV-EGFP. As shown in Figure 4. The PRV decreased OAS1, ISG15, MX1 and IFITM3 mRNA and protein expression level in PK-15 cells. IFN-λ3 treatment significantly increased OAS1, ISG15, MX1 and IFITM3 mRNA and protein expression level in PK-15 cells with dose dependent. The results proved that IFN-λ3 mediate anti-PRV activity via inducing the ISGs expression in PK-15 cells.
that IFN-λ play a pivotal role in inhibiting viral infections such as porcine epidemic diarrhoea virus (PEDV) (Li et al., 2019), influenza virus (Sun et al., 2018), hepatitis E virus (HEV) (Li et al., 2019) and so on. However, there is no information available about whether porcine IFN-λ inhibits swine pseudorabies virus (PRV). PRV, a herpesvirus subfamily of the family Herpesviridae, is a kind of virus that can infect various animals and has strong pathogenicity. In this study, we investigated the relative contribution of porcine IFN-λ3 toward controlling the infection of PRV in vitro.

IFN-λ preferentially acts on epithelial cells, because its receptor IFNLR1 is mainly expressed in epithelial cells (Mordstein et al., 2010; Pott et al., 2011). Here, we report that IFN-λ3 have strong antiviral activity against PRV infection in PK-15 cells. Our results showed that 100 and 1000 ng/ml IFN-λ3 treatment inhibited PRV proliferation in PK-15 cells. IFN-λ3 treatment significantly reduced the PRV titre and virus plaques with time and dose dependent. Consistent with these results, treatment with 10, 100 and 1000 ng/ml doses of recombinant human IFN-λ3 protected against PRRSV infection in MARC-145 cells.
(Robek et al., 2005). Nice et al. (Nice et al., 2015) demonstrated that IFN-λ can prevent and cure the enteric persistent infection of murine norovirus without the help of adaptive immunity. Pott et al. (2011) study confirms that IFN-λ not type I IFN reduced rotavirus infection rate in vivo. All of these suggest that IFN-λ provides a key antiviral defence of the epithelium. To better verified the anti-PRV activity of IFN-λ, we overexpress IFNLR1 in PK-15 cells. The results showed over-express IFNLR reduced PRV titre. Consistent with Klinkhammer et al. (2018) results, mice lacking functional IFNLR1 no longer restricted virus dissemination from the upper airways to the lungs. Together, these data show that IFN-λ effectively limits the replication of PRV viruses in PK-15 cells.

Interferon (IFN) is divided into three categories. Type I IFN includes IFN-α, IFN-β, IFN-ε and IFN-ω produced almost in all kinds of cells. Type II IFN, INF-γ, is produced by immune cells and forms a dimer that acts on a receptor complex consisting of two IFNRI and two IFNRII (Lasfar et al., 2016). Previous study have proved that IFN-λ affects plasmacytoid dendritic cells (pDCs)-specific functions: it can stimulate pDCs to produce type I IFNs particularly (Finotti et al., 2016). Jaeger et al. (2012) also found that IFN-λ may help NK cells preferentially produced IFN-γ to protective against airway inflammation via affecting DCs-mediated skewing of the immune response. Our results demonstrated that PRV-EGFP induced IFN-λ1 and IFN-λ3 mRNA expression levels but did not elevate IFN-α concentration in PK-15 cells. That means PRV-EGFP may inhibited IFN-λ translation. IFN-λ3 treatment in PRV-EGFP-infected PK-15 cells elevated IFN-λ1 and IFN-λ3 mRNA expression levels and the concentration of IFN-λ1 and IFN-λ3. Enhanced IFN-γ mRNA expression levels when compared with PRV-EGFP-infected PK-15 cells. However, in PK-15 cells, IFN-λ3 did not increased IFN-α and IFN-β mRNA expression levels. Abovementioned results may suggest that IFN-λ3 specifically induced type II IFN, INF-α and INF-γ to activate immune response or antiviral activity in PK-15 cells.

All three kinds of interferons activate JAK/STAT pathway, therefore leads to transcription of ISGs (Chow & Gale, 2015) results in inhibition of virus infection. IFN-λ bind with their receptor which is heterodimeric complex composed of IFNLR1 and IL-10Rβ restricted to epithelial cells to induce ISGs (Broggi et al., 2017). In our study, IFN-λ3 treatment upregulated PRV reduced OAS1, ISG15, Mx1 and IFITM3 mRNA expression and protein levels. Consistent with Li et al.’s (2017) results, they found that pIIIFN-λ1 induced high levels of antiviral gene expression (ISG15, OASL and MxA) in IPEC-J2 cells indicating that the ISGs induced by IFN-λ confer an antiviral state in IPEC-J2. Luo et al. (2011) also proved that IFN-λ3 was able to induce the expression of ISG56, 2′,5′-OAS and Mx1 in MARC-145 cells to protect cells against PRRSV. These data demonstrate IFN-λ3 had antiviral activity against PRV via mediating ISGs and animal studies of any possible therapeutic effect are feasible.

4 | CONCLUSION

Our study demonstrated that IFN-λ3 inhibited PRV proliferation via upregulating IFN-γ mRNA expression levels and inducing the expression of antiviral proteins, including OAS1, ISG15, Mx1 and IFITM3. Our findings suggest that IFN-λ3 displayed robust antiviral activity against PRV infection in PK-15 cells.

5 | METHODS

5.1 | Cells and virus

PK-15 cells were maintained in Dulbecco’s Modified Eagle’s Medium Nutrient Mixture (DMEM) (Gibco, USA), supplemented with antibiotics (100 units/ml penicillin and 100 μg/ml streptomycin), and 10% foetal bovine serum (FBS) (Gibco, USA). The recombinant virus PRV-EGFP (Yin et al., 2017) was propagated in PK-15 cell with 2% FBS (Gibco, USA) added in the DMEM.

5.2 | Transfection

Porcin IFNLR1 was cloned into vector PLVX-acEGFP-N1. For transfection, the cells were transiently transfected with vector PLVX-acEGFP-N1 or plasmids encoding IFNLR1 using Lipofectamine 3000 (Invitrogen, 2185325) following the manufacturer’s protocols. Cell lysates were collected after 24 h to verify overexpression efficiency by western blot.

5.3 | Antiviral assay

To investigate the anti-PRV-EGFP activity of E. coli-derived porcin IFN-λ3 prepared in our laboratory, PK-15 cells pre-treated with the indicated concentrations of IFN-λ3 (100 and 1000 ng/ml) for 12 h or transfected with plasmid encoding IFNLR1 for 24 h. The cells were then infected with PRV-EGFP at 0.1 MOI for 2 h and then cultured with medium containing indicated IFN-λ for 12, 24, 36 and 48 h. The cytopathic effect (CPE) units in culture plates were counted after 48 h, and the viral titre was analysed using Reed-Muench method.

5.4 | Western blot analysis

Western blot process was followed by Deng et al. (2020). After PK-15 cells were lysed, total proteins were extracted with RIPA buffer (Thermo Fisher Scientific) with Thermo Scientific Halt protease inhibitor cocktail. Protein concentration was measured by BCA protein assay kit (Thermo Fisher Scientific). Equal amounts of protein sample were loaded into 12% SDS-PAGE and transferred to nitrocellulose filter membranes. Then, the membrane was blocked in nonfat dry milk (5%) for 1 h at RT. Membranes were incubated with the primary antibodies overnight at 4°C followed by 1 h of incubation using proper secondary HRP-conjugated antibodies (Bio-Rad) and development with ECL detection kit (GE Healthcare, Piscataway, NJ, USA). Then, the membranes were detected with Bio-Rad ChemiDoc XRS+ System (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The primary antibodies of ISG15 (No: ab233071), OAS1 (NO: ab86343), Mx1
TABLE 1  Primer sequences of genes selected for analysis ISGs

| Target gene | Primer | Primer sequence (5′->3′) | Product size | Tm (°C) |
|-------------|--------|--------------------------|--------------|---------|
| OAS1        | Forward | TCCGAACGCAGGTCAAGG       | 136 bp       | 60      |
|             | Reverse | AAGACGACGAGGTCAGCA       |              |         |
| ISG15       | Forward | TGGGGGACTGCATGATGGCC     | 197 bp       | 60      |
|             | Reverse | CAGGATGCTCATGGTGTCA      |              |         |
| Mx1         | Forward | CATCAACTTGGTGGTGTC       | 200 bp       | 60      |
|             | Reverse | CAATCATGTAGCCCTTCTTC     |              |         |
| IFITM3      | Forward | GCTTCCACGCCCCTCTTC       | 142 bp       | 60      |
|             | Reverse | TCTCGCTTGGATGTTGAT       |              |         |
| β-actin     | Forward | GCTGTGCTATTTGTGCTCTAG    | 179 bp       | 60      |
|             | Reverse | CGCTCAGTGCACCAATAGTG     |              |         |
| IFN-α       | Forward | TCACAGAGTCACCCACC        | 182 bp       | 60      |
|             | Reverse | CATTGTGGCACGGAGC         |              |         |
| IFN-β       | Forward | ACCAACAAAGGAGCCAG        | 222 bp       | 60      |
|             | Reverse | TTCCATCCAGCGCAT          |              |         |
| IFN-γ       | Forward | AACCAGGCCATTCAAGGAGC     | 149bp        | 60      |
|             | Reverse | TCAGTGATGGCCTTGCGCTG     |              |         |

(No: ab95926) and β-actin (No: ab179467) was purchased from Abcam and IFITM3 (No: abx008340) was purchased from Abbexa. At least three biological replicates were analysed for each experiment.

5.5 | RNA extraction and quantitative real-time PCR (qRT-PCR)

RNAiso Plus (9109; Takara, China) was used for extracting the PK-15 cells total RNA following protocols provided by the manufacturer. RNA (1 μg) was used to synthesise cDNA through Prim-ScriptTM RT reagent Kit (RR047A, Takara, China) following specific instructions. Primers were designed and synthesised by Sangon (Shanghai, China). The mRNA expression was measured with SYBR® Premix Ex TaqTMII (RR820A, Takara, China). The reactions protocol was 95°C for 10 min, followed by under 95°C for 10 min, under 60°C for 20 s and under 72°C for 20 s. The qRT-PCR data were analysed using the 2^−ΔΔCT method (Thomas et al., 2008). At least three biological replicates were analysed for each experiment. The qRT-PCR primers were shown in Table 1.

5.6 | ELISA assay

Test was carried out according to the kit brochures operation (Enzyme Industrial Co., Ltd, China) and the spectrophotometric absorbance was assessed at 450 nm for IFN-λ1 and IFN-λ3.

5.7 | Statistical analysis

Data are expressed as mean ± standard deviation. All statistical analyses were analysed by GraphPad Prism (GraphPad Software, Inc). Unpaired t test and ANOVA were used to investigate the significance of differences between the experimental groups and the control group. p < 0.05 indicates statistical significance of the difference.

AUTHOR CONTRIBUTIONS

HD, LZ and ZX conceived and designed the experiments. HD and ZJ performed the experiments. JZ, JD and FL performed the analysis. HD drafted the manuscript. ZX and XS substantively revised this manuscript. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data generated or analysed during this study are included in this published article.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The Sichuan provincial laboratory animal management committee [License No: SYXK (chuan) 2019-187] approval has been received. The ‘Guidelines for Experimental Animals’ of the Ministry of Science and Technology (Beijing, China) were followed.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/vms.3.933.
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