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p53 mediated IFN-β signaling to affect viral replication upon TGEV infection

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ABSTRACT

TGEV can induce IFN-β production, which in turn plays a vital role in host antiviral immune responses. Our previous studies showed that TGEV infection activated p53 signaling to induce host cell apoptosis, which might influence virus replication. However, whether there be an interaction between p53 and IFN-β signaling in the process of TGEV infection is unknown. In the present study, we used low dose of TGEV to infect p53 wild-type PK-15 cells (WT PK-15 cells) and p53 deficient cells (p53-/- PK-15 cells), to investigate the modulation of IFN signaling and virus replication by p53. The results showed that the IFN-β expression and production were notably inhibited in p53-/- PK-15 cells compared with that in WT PK-15 cells at early stage of TGEV infection. In addition, TGEV-induced the changes in mRNA levels of TRIF, TRAM, MDA5, RIG-I, IPS-1, IRF9, IRF3, ISG15 and ISG20 were notably hindered in p53-/- PK-15 cells before 36 h post infection (p.i.). Moreover, TGEV genomic RNA and sub genomic mRNA (N gene and ORF7) levels showed significant increase in p53-/- PK-15 cells compared with WT PK-15 cells after TGEV infection. And viral titers were observably enhanced in p53-/- PK-15 cells. Furthermore, exogenous IFN-β and polyinosinic-polycytidylic acid (poly (I:C)) treatment markedly inhibited the mRNA levels of TGEV gRNA, N and ORF7 in WT PK-15 cells and p53-/- PK-15 cells compared to control. Taken together, these results demonstrated that p53 may mediate IFN-β signaling to inhibit viral replication early after TGEV infection.

1. Introduction

Tumor suppressor p53 could be activated in response to several stimuli, such as oncogenic stress, DNA damage and virus infections, to control cell senescence, cell cycle and cell apoptosis (Levine, 1997). Numbers of studies have showed that p53 plays an important roles in regulating virus replication and infection (Muñoz-Fontela et al., 2008a). For instance, overexpression of p53 inhibited infectious bursal disease virus (IBDV) replication (Ouyang et al., 2017); Influenza virus can promote p53 to inhibit virus replication (Turpin et al., 2005); Early after vesicular stomatitis virus (VSV) infection, viral replication was markedly inhibited by p53-dependent increase of interferon (IFN) production (Muñoz-Fontela et al., 2008a). During host antiviral defense, Type I IFN plays pivotal roles in adaptive and innate immune responses against virus infections (Pitha, 2004; Zhu et al., 2017). RNA virus genomes replication produces double stranded (ds) RNA which can be recognized by RIG-I-like receptors (RLRs) or Toll-like receptors (TLRs), subsequently leading to the type I IFN synthesis and secretion (Narayan et al., 2010). Consequently, the secretion of type I IFN promotes IFN-stimulated genes (ISGs) to act antiviral function (Wong and Chen, 2016). A lot of studies have shown that several ISGs and interferon regulatory factors (IRFs) were directly trans-activated by p53 in response to viral infections (Nakamura et al., 2001; Takaoka et al., 2003). For example, IRF5 (Mori et al., 2002), ISG15 (Park et al., 2016), IRF9 (Muñoz-Fontela et al., 2008b) and retinoic-acid inducible gene-I (RIG-I) (Hsu et al., 2012), which were involved in type I IFN-mediated antiviral response, are p53 target genes.

Transmissible gastroenteritis virus (TGEV) is a well-known etiological agent that cause transmissible gastroenteritis in piglets (Eleouet et al., 1995). It can replicate in enterocytes, leading to vomiting, watery diarrhea, and then dehydration, which produces high mortality in neonatal pigs. (Eléouët et al., 2000). TGEV belongs to the genus *Alphacoronavirus*, which are within the family *Coronaviridae* (Adams et al., 2012). TGEV, as an enveloped virus, possesses a large, single-stranded, positive-sense RNA genome (Curtis et al., 2002). Previous researches indicated that TGEV infection induced IFN-β production which exerts

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antiviral abilities (Naidoo and Derbyshire, 1992; Riffault et al., 2001; Zhu et al., 2017). Beyond that, TGEV infection activated p53, which may play an essential role in regulating antiviral response (Ding et al., 2014; Huang et al., 2013). These observation hint possible cooperation between p53 and type I IFN in regulating immune response against TGEV infection. Here, we investigated the roles of p53 in regulation of IFN-β production and IFN-β signaling, and the effect on viral replication in response to TGEV infection with low viral loads.

2. Materials and methods

2.1. Cells and viruses

The p53 deficient PK-15 cells (p53-/- PK-15 cells) have been constructed according to the description by Xu et al (Ding et al., 2018; Xu et al., 2016). p53-/- PK-15 cells and wild type p53 PK-15 cells (WT) were grown in Dulbecco Minimal Essential Medium (Gibco BRL, MD, USA) including 10% fetal bovine serum (Gibco BRL, MD, USA) at 37 °C in a humidified 5% CO_2. The TGEV strain was used as our previously described (Ding et al., 2012). Virus titers were measured by TCID50 as previously described (Reed and Muench, 1938). Pan apoptosis inhibitor Z-VAD-FMK and RLR-pathway inhibitor BX795 (Wahadat et al., 2018) were purchased from Selleck Chemicals (Selleck Chemicals, TX, USA). TRIF inhibitor resveratrol was purchased from MCE (MedChemExpress, NJ, USA).

2.2. Real-time quantitative PCR

RNA extraction and qRT-PCR were performed as our previously described (Ding et al., 2012). Primer sequences for qRT-PCR are listed in Table 1. qRT-PCR was performed in Roche LightCycler® 480II (Roche Diagnostics, Basel, Switzerland) under the requirements of manufacturer's protocol.

2.3. ELISA assay

The production of IFN-β were measured by Porcine Interferon β (IFN-β) ELISA kit (Shenzhen ziker Biological Technogy Co., Ltd, Shenzhen, China) according to the manufacture's recommendations. Briefly, the culture supernatants were added to microelisa stripplate wells to combine with the specific antibody. Next, Horseradish Peroxidase-conjugated antibody was added and incubated. After free components were washed away, each well was added the tetramethylbenzidine (TMB) substrate solution. The optical density (OD) was finally measured spectrophotometrically at 450 nm after the addition of the stop solution. The concentration of IFN-β in the samples were calculated through comparing the OD of samples to the standard curve.

2.4. Statistical analysis

The data are mean ± SEM, which were from three independent experiments in parallel (triplicate). The results were analyzed by one-way analysis of variance. $P < 0.05$ was considered significant.

3. Results

3.1. p53 promotes the IFN-β expression and production in TGEV-infected cells

To investigate the implied possible cooperation between p53 and IFN-β in regulation of host immune response against TGEV infection, we infected WT and p53-/- PK-15 cells with TGEV to examine the mRNA levels of IFN-β. As shown in Fig. 1A, with 0.1 MOI of TGEV infection, the mRNA levels of IFN-β significantly increased at 6 h post infection (p.i.) and reached the peak at 24 h, then decreased at 36 h in WT PK-15 cells, while in p53-/- PK-15 cells, IFN-β mRNA levels increased with infectious time. And IFN-β mRNA levels were markedly impaired in p53-/- PK-15 cells at 6, 12 and 24 h p.i. compared with that in WT PK-15 cells ($P < 0.01$).

To further confirm the contribution of p53 to IFN-β, the levels of IFN-β secreted by infected cells were explored. As shown in Fig. 1B, upon TGEV infection, the secretion of IFN-β showed a time-dependent increase, while the concentration was significantly inhibited in p53-/- PK-15 cells, compared with the levels in WT PK-15 cells. The results were similar to IFN-β mRNA levels. These data indicated that p53 influence IFN-β mRNA expression and secretion in TGEV-infected cells.

3.2. p53 upregulates TRIF, TRAM and RIG-I/MDA-5 expression during TGEV infection

Virus could mediate toll-like receptor (TLRs) and RIG-I/MDA5 pathways to activate type I IFN (Broquet et al., 2011; Eo et al., 2014), which play an important role in antiviral response. To determine the mechanisms that p53 might influence IFN-β, we therefore analyzed TLRs and RIG-I/MDA5 pathways expression by qRT-PCR with TGEV infection at low viral loads. Results showed that with the exception of MyD88, TGEV infection resulted in up-regulation of TRIF, TRAM, MDA5, RIG-I and IPS-1 mRNA levels in both WT and p53-/- PK-15 cells. However, these factors mRNA levels in TGEV-infected p53-/- PK-15 cells were notably hindered before 36 h, compared with the levels in WT cells (Fig. 2A–F).

To confirm the role of p53 in TLRs and RIG-I/MDA5/IPS-1 pathways to activate IFN-β, we used the inhibitors of each pathways to treat WT

Table 1
Sequences of primer pairs used for qRT-PCR.

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)	Accession no.
IFN-β	TGCATCCTCCAAATCGCTCT	ATTGAGGAGTCCCAGGCAAC	NM_001003923.1
MyD88	CCATTCGAGATGACCCCCTG	TAGCAATGGACCAGACGCAG	EU056736.1
TRIF	GCTCCCGAGCTGGAGTTATC	GGTACCTGGAAATCCTCGCA	KC969185.1
TRAM	TCCGTGAACAGACAGCACAA	GCCACGACTTTCTTCCTCCA	NM_001204351.1
MDA5	CACTTGCCCGCGAATTAACA	GTCCGAGACGTCCAGACTTG	NM_001100194.1
RIG-I	GTGTGCGGTGTTTCAGATGC	AGCCTGCTGCTCGGATATTT	EU126659.1
IPS-I	CCTCTGGGACCTCTTCGACA	GCTGTTTGAATTCCGCAGCA	NM_001097429.1
IRF3	GTCACAAGCCTGACGGTGA	GAGCGTCTGCTTCCTTCGAT	EU294308.1
IRF9	ATCCTCCAGGACCCCTTCAA	AACCCTACCTTCCGGAGACT	NM_001078670.1
ISG20	CTATACCATCTACGACACCGCC	TGGCATCTTCCACCGAGTT	NM_001005351
ISG15	CGTGCAAGCTGACCAGTTCT	CACGGTGCACATAGGCTTGA	EU647216.1
TGEV-ORF7	CGTGGCTATATCTCTTCTTTTACTTTAACTAG	AAAACTGTAATAAATACAGCATGGAGGA	AJ271965
TGEV-N	CGTGGCTATATCTCTTCTTTTACTTTAACTAG	TCTTCCGACCACGGGAATT	AJ271965
TGEV-gRNA	GTGAGTGTAGCGTGGCTATA	TCCTTACGATCGCAATCAA	AJ271965
β-actin	GGACTTCGAGCAGGAGATGG	AGGAAGGAGGGCTGGAAGAG	XM_003124280.1

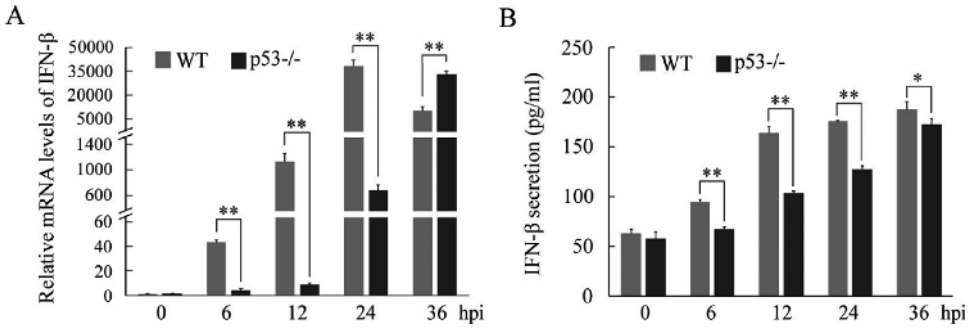

Fig. 1. p53 enhanced the IFN-β mRNA expression and production in TGEV-infected cells. (A) The mRNA levels of IFN-β. WT and p53-/- PK-15 cells were infected with TGEV at MOI of 0.1 for different time. The cells were collected and subjected to qRT-PCR analysis. (B) The secretion of IFN-β. WT and p53-/- PK-15 cells were treated as in A, the supernatants from TGEV-infected cells were collected and then analyzed by ELISA assay. All the data are mean ± SEM. * $P < 0.05$, ** $P < 0.01$ was considered significant.

and p53-/- PK-15 cells, then the mRNA levels of IFN-β were detected. Results showed that resveratrol and BX795 significantly decreased the mRNA levels of TRIF and RIG-I in TGEV-infected WT PK-15 cells, respectively (Fig. 3A and C). And the two inhibitors obviously down-regulated TGEV-induced IFN-β mRNA expression in p53-/- PK-15 cells and WT PK-15 cells (Fig. 3B and D). These findings suggest that p53 might play a broader regulation role in TRIF, TRAM and RIG-I/MDA5/RIG-1 pathways to activate IFN-β.

3.3. p53 enhances the expression of ISGs upon TGEV infection

To test whether p53 mediated IFN-β signaling during TGEV infection, we examined the mRNA levels of IRF9, IRF3, ISG15 and ISG20. Fig. 4A–C demonstrates a higher increase of these IFN-related genes in WT PK-15 cells before 36 h p.i., compared with that in p53-/- PK-15 cells. These results manifest that p53 may regulate ISGs and IRFs expression during TGEV infection.

3.4. p53 suppresses viral replication upon TGEV infection with a low viral load

To test the role of p53 in TGEV replication, we measured the viral genes expression and virus titers in WT and p53-/- PK-15 cells, which were infected with 0.1 MOI of TGEV. Virus genomic RNA (gRNA) and

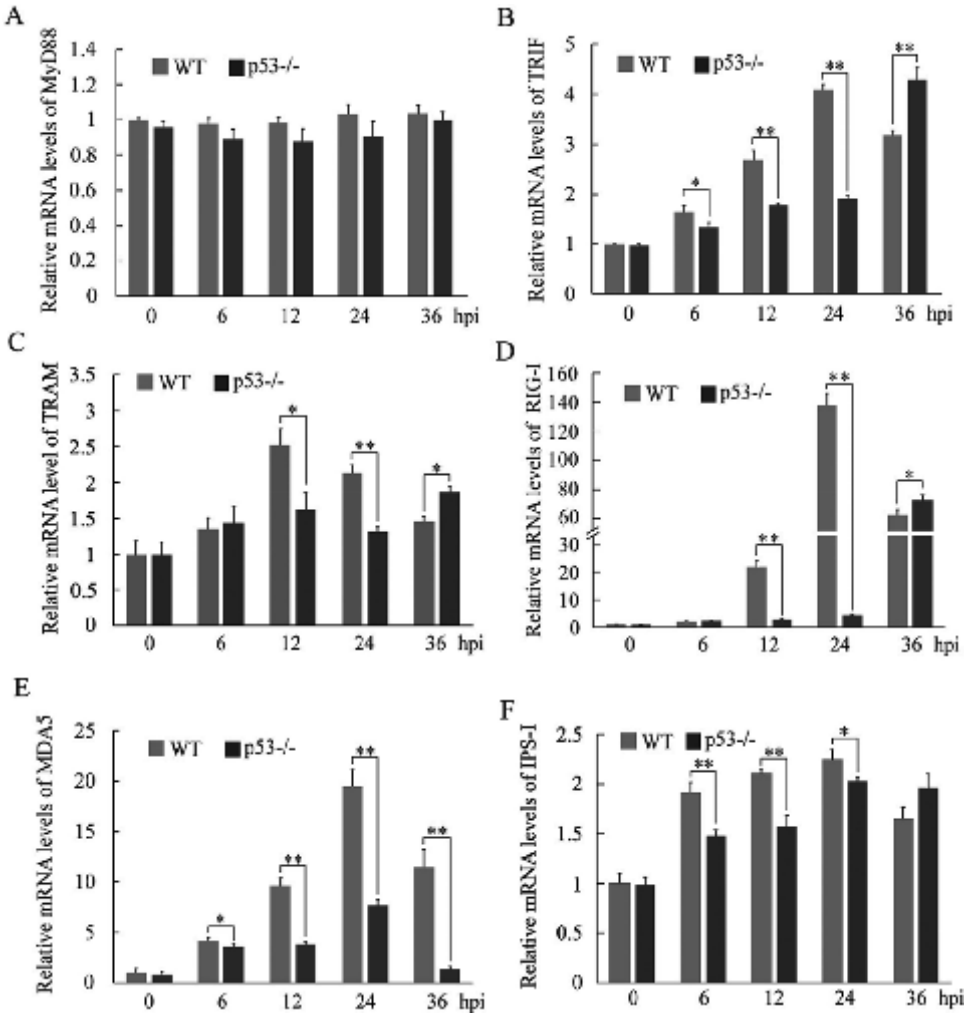

Fig. 2. p53 regulated TRIF, TRAM and RIG-I/MDA5/IPS-1 pathways upon TGEV infection. (A–F) The mRNA levels of MyD88, TRIF, TRAM, RIG-I, MDA5 and IPS-1, respectively. WT and p53-/- PK-15 cells were infected with TGEV at MOI of 0.1 for different time. The cells were collected and subjected to qRT-PCR analysis. Data are mean ± SEM. * $P < 0.05$, ** $P < 0.01$ was considered significant.

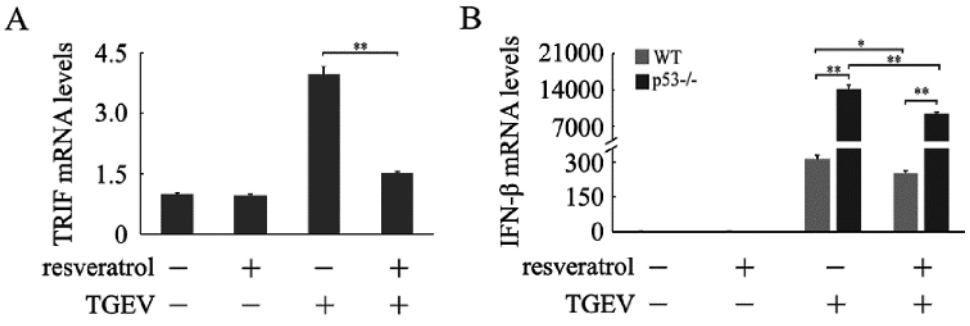

Fig. 3. The effect of TLRs and RLR pathways inhibitors on the mRNA expression of IFN-β upon TGEV infection. WT and p53-/- PK-15 cells were treated with resveratrol (20 μM) (A and B) and BX795 (1 μM) (C and D), and then infected with TGEV at MOI of 0.1 for 24 h. The cells were collected and subjected to qRT-PCR analysis. Data are mean ± SEM. * $P < 0.05$, ** $P < 0.01$ was considered significant.

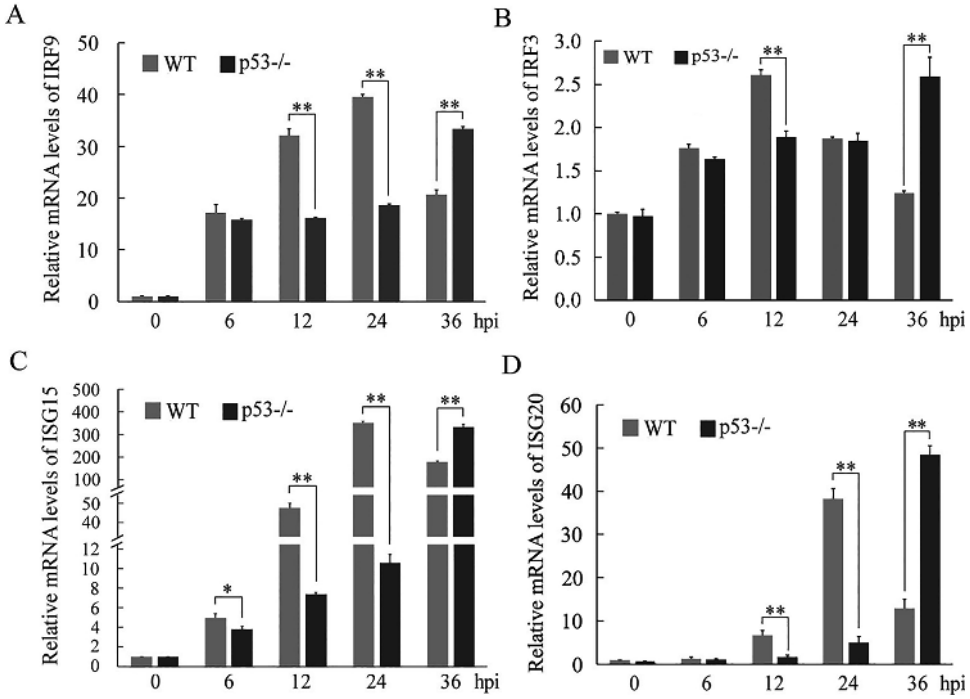

Fig. 4. p53 increased the expression of IFN-related genes upon TGEV infection. (A–D) The mRNA levels of IRF9, IRF3, ISG15 and ISG20, respectively. WT and p53-/- PK-15 cells were infected with TGEV at MOI of 0.1 for different time. The cells were collected and subjected to qRT-PCR analysis. Data are mean ± SEM. * $P < 0.05$, ** $P < 0.01$ was considered significant.

sub genomic mRNA (sgmRNA) levels in WT and p53-/- PK-15 cells were analyzed at 6, 12, 24 and 36 h p.i. by qRT-PCR. Results showed that the levels of TGEV gRNA, sgmRNA-ORF7 and sgmRNA-N were remarkably increased in p53-/- PK-15 cells at 24 and 36 h p.i. compared with that in WT PK-15 cells (Fig. 5A–C). To further confirm the result, we compared TGEV titers in WT and p53-/- PK-15 cells. As expected, viral titers obviously increased in p53-/- PK-15 cells compared with that in WT PK-15 at 24 and 36 h p.i. (Fig. 5D).

To investigate whether the inhibition of p53 on TGEV replication in WT cells was dependent on proapoptotic signal, we used the pan apoptosis inhibitor Z-VAD-FMK to treat p53-/- PK-15 and WT PK-15 cells, and TGEV genomic RNA and sub genomic mRNA expression were detected. Results showed that Z-VAD-FMK down-regulated the levels of TGEV gRNA and sgmRNA-N, whereas the genes expression in WT PK-15 cells was lower than that in p53-/- PK-15 cells (Fig. 5E). These results suggest that p53 might through activating type I IFN signaling to inhibit viral replication during TGEV infection, and the apoptosis might be benefit to viral replication.

3.5. Antiviral effect of poly (I:C) stimulation is hindered in p53-/- PK-15 cells

Polyinosinic-polycytidylic acid (poly (I:C)), regarded as pathogen-associated molecular pattern, drives interferon stimulated genes (ISGs)

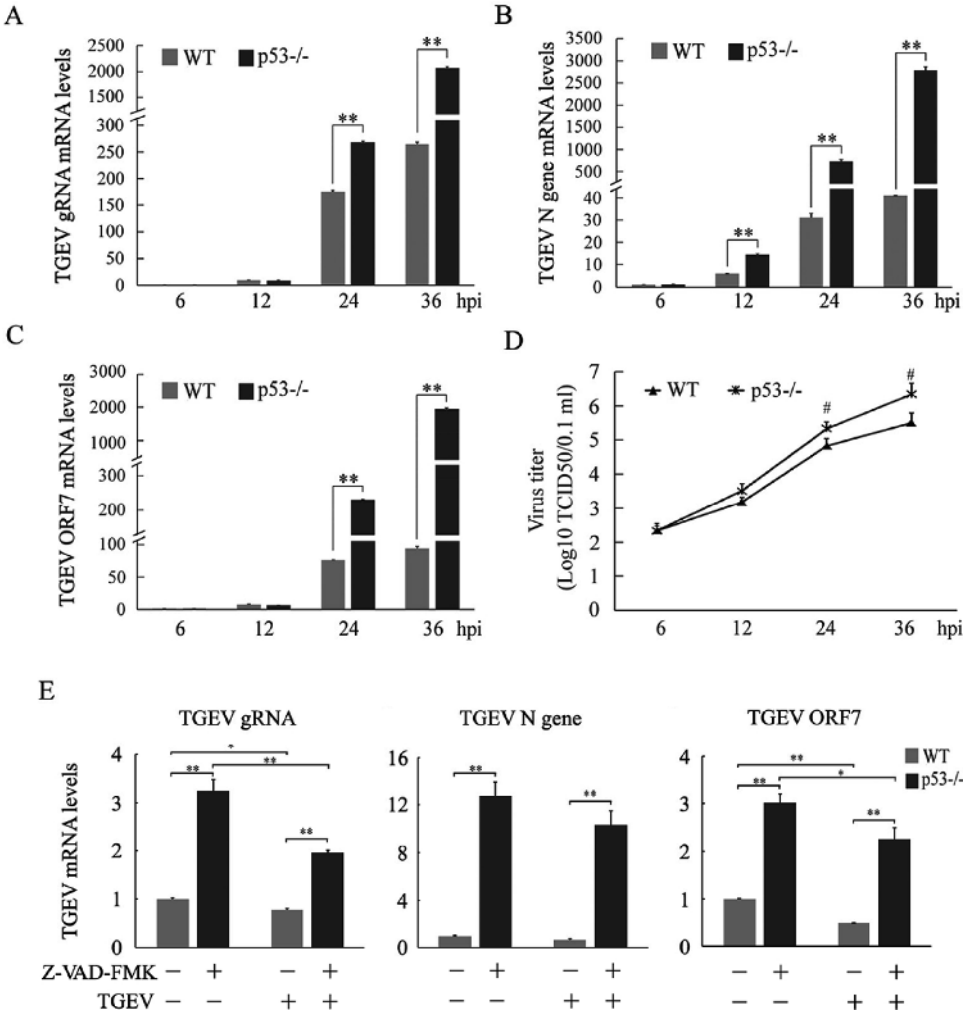

Fig. 5. Effect of p53 on TGEV genes mRNA expression. (A–C) Virus gRNA, sgmRNA-N and sgmRNA-ORF7 levels. WT and p53-/- PK-15 cells were infected with TGEV at MOI of 0.1 for different time. The cells were collected and subjected to qRT-PCR analysis. Data are mean ± SEM. ** $P < 0.01$. (D) Viral titers were detected by TCID50 assays at the indicated times. Data are mean ± SEM. # $P < 0.05$. (E) WT and p53-/- PK-15 cells were treated with pan apoptosis inhibitor Z-VAD-FMK, and then infected with TGEV at MOI of 0.1 for 24 h. The cells were collected and subjected to qRT-PCR analysis for TGEV genomic RNA and sub genomic mRNA. Data are mean ± SEM. * $P < 0.05$, ** $P < 0.01$ was considered significant.

expression to inhibit virus replication (Liu et al., 2009). To further confirm the role of p53 in regulating IFN signaling and virus replication during TGEV infection, we treated the cells with poly (I:C) (Invivogen, Hong Kong Science Park Shatin, Hong Kong) to detect the expression of IFN-β, ISG15, TGEV gRNA and sgmRNA. We showed that poly (I:C) prominently enhanced the mRNA levels of IFN-β and ISG15, and the levels of ISG15 were remarkably upregulated in TGEV and poly (I:C)-cotreated PK-15 cells compared to poly (I:C) treatment or TGEV infection respectively. However, the response in p53-/- PK-15 cells was lower than that in WT PK-15 cells (Fig. 6A and B). Moreover, Poly (I:C) treatment significantly inhibited the expression of TGEV gRNA, N and ORF7 in WT PK-15 cells and p53-/- PK-15 cells compared to control, but viral gene levels in WT PK-15 cells were much more impaired than in 53-/- PK-15 cells, suggesting that p53 play an essential role in poly (I:C)-inhibited TGEV replication (Fig. 6C–E). To approve the effect of poly (I:C) stimulation, we used the exogenous IFN-β (Medicine Nest Chemdrug, Shanghai, China) to treat WT PK-15 cells and p53-/- PK-15 cells and the expression of TGEV gRNA, N and ORF7 were detected. Results showed that exogenous IFN-β treatment displayed less efficacy to inhibit TGEV replication in p53-/- PK-15 cells compared to WT PK-15 cells (Fig. 6F–H).

4. Discussion

In response to virus infection, host can activate many mechanisms to act antiviral function (Zuniga et al., 2007). For instance, host cells can increase the production of type I IFN and activate type I IFN signaling to mediate immune responses against viral infection (Zuniga et al., 2007). Also, host impairs virus replication through induction of p53 pathways upon some virus infection (Melchert, 2008; Zhu et al., 2014). Much more evidence for cooperation between type I IFN and p53 to their antiviral activities have been confirmed (Melchert, 2008). For example, as a transcription factor, p53 can bind to promoter region of IFN-related genes to transcriptionally regulate the expression (Mori et al., 2002; Takaoka et al., 2003), and type I IFN can also induce p53 expression (Imbeault et al., 2009; Melchert, 2008). In this study, we demonstrated that p53 played an essential role in IFN-β-mediated antiviral activity at the early stage of TGEV infection.

Virus infection commonly triggers the activation of pattern-recognition receptors (PRRs) to motivate innate immune responses (Negishi et al., 2012). Two main classes of PRRs have been identified: membrane-bound receptors, including the Toll-like receptor (TLR) family, and cytosolic receptors, including the RNA helicase RIG-I–like receptor (RLR) family (Honda and Taniguchi, 2006). It is known that TLR- and RLR-mediated signaling events are critical in antiviral immune responses (Blasius and Beutler, 2010; Negishi et al., 2012; Takeuchi and Akira, 2010). To date, many members of TLRs and their respective ligands have been identified. For example, TLR2/4 usually respond to viral protein structures (Kawai and Akira, 2007). TLR3 recognizes double-stranded RNA (dsRNA) (Alexopoulou et al., 2001). TLR7/8 mediates recognition of single-stranded RNA (ssRNA) (Heil et al., 2004; Hemmi et al., 2002). Then, TLR7/8 and TLR9 use myeloid differentiation primary response gene (MyD88), TLR3 utilizes TIR-domain-containing adaptor-inducing IFN-β (TRIF), TLR1/2 and TLR2/6 utilize MyD88 and TIR domain-containing adapter protein (TIRAP)/Mal, TLR4 utilizes four adapters, including MyD88, TIRAP/MAL, TRIF

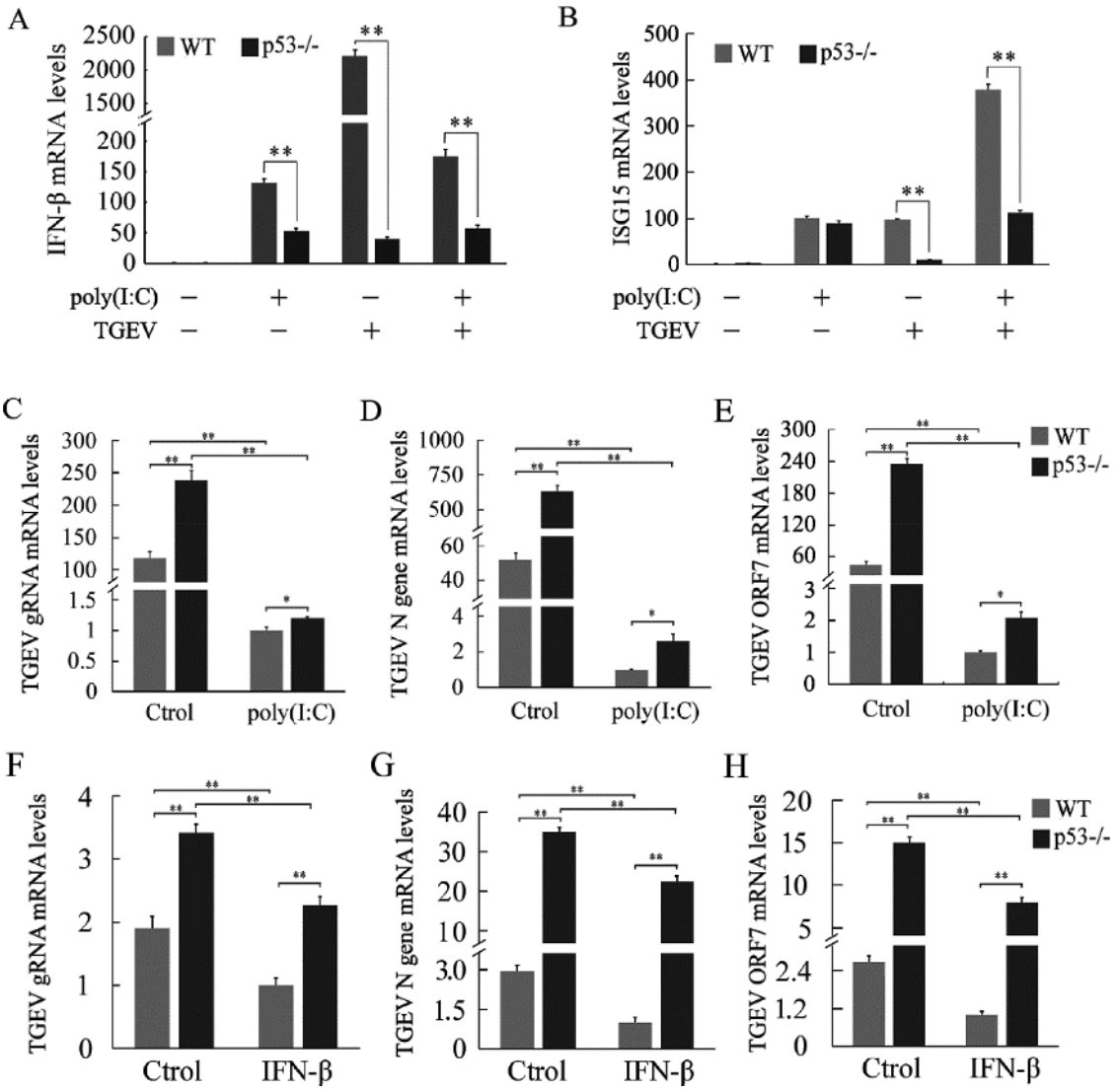

Fig. 6. The effect of poly (I:C) and exogenous IFN-β on expression of IFN-β and TGEV genes. WT and p53-/- PK-15 cells were pretreated with 5 μM of poly (I:C), then the cells were infected with TGEV for 24 h. The cells were collected and subjected to qRT-PCR analysis for IFN-β (A) and ISG15 (B) expression. (C–E) TGEV gRNA, TGEV N and ORF7 expression. WT and p53-/- PK-15 cells were treated as in (A and B), the cells were collected and subjected to qRT-PCR analysis. Data are mean ± SEM. * $P < 0.05$, ** $P < 0.01$. (F-H) WT and p53-/- PK-15 cells were treated with exogenous IFN-β (1000IU/ml), and then infected with TGEV at MOI of 0.1 for 24 h. The cells were collected and subjected to qRT-PCR analysis for TGEV genomic RNA and sub genomic mRNA. Data are mean ± SEM. ** $P < 0.01$ was considered significant.

and TRIF-related adapter molecule (TRAM), to induce type I IFN to exert antiviral effects (Bauer and Hartmann, 2008; Kawai and Akira, 2007). In our study, TRIF and TRAM mRNA expression levels were remarkably impaired in p53-/- PK-15 cells compare with that in WT PK-15 cells. In addition, resveratrol significantly down-regulated TGEV-induced IFN-β mRNA expression in p53-/- PK-15 cells and WT PK-15 cells, suggesting that p53 plays a vital role in regulation of TRIF and TRAM-induced IFN-β production upon TGEV infection.

The RIG-I-like receptors (RLRs), RIG-I and MDA5, recognize ssRNA and dsRNA to initiate innate antiviral immune responses (Brunen et al., 2013; Huang et al., 2017; Jr and Medzhitov, 2002; Takaoka et al., 2003). RIG-I and MDA5 also contain two N-terminal caspase recruitment domains (CARDs) (Satoh et al., 2010), and the N-terminal CARDs of RIG-I and MDA5 trigger intracellular signaling pathways via IFN-β promoter stimulator (IPS-1) (also known as MAVS), which activates TANK-binding kinase 1 (TBK1) and IKK-I, subsequently induce the transcription of type I IFN and IFN-inducible genes (Satoh et al., 2010). In response to various RNA viruses infection, host could activate RIG-I to induce the production of type I IFN, such as vesicular stomatitis virus (VSV) (Das et al., 2014), Sendai virus (SeV) (Okano et al., 2011), Japanese encephalitis virus (JEV) (Jiang et al., 2014), and influenza virus (Ichinohe, 2010), whereas MDA5 is critical for the detection of infection bursal disease virus (IBDV) (Lee et al., 2014) and encephalomyocarditis virus (EMCV) (Pichlmair et al., 2009). Also, some RNA viruses such as Measles virus (Ikegame et al., 2010), West Nile virus (Fredericksen et al., 2008), Rhinovirus (Slater et al., 2012) and Reovirus (Sherry, 2009) are recognized by both RIG-I and MDA5. Studies showed that RIG-I and IPS-1 are p53-regulated genes, for example, p53 expression was associated with higher basal mRNA and protein levels of RIG-I in response to IFN treatment (Muñoz-Fontela et al., 2008a). Over-expression of p53 could inhibit IBDV replication and up-regulate the expression level of IPS-1 (YANG et al., 2016). In the present study, RIG-I, MDA5 and IPS-1 mRNA expression levels were remarkably impaired in p53-/- PK-15 cells compare with that in WT PK-15 cells. In addition, inhibition of RLR pathway by BX795 significantly down-regulated TGEV-induced IFN-β mRNA expression in p53-/- PK-15 cells and WT PK-15 cells, suggesting that p53 plays an important role in regulation of RIG-I/MDA5/IPS-1 -induced IFN-β production upon TGEV

infection.

We also detected intracellular IFN-β mRNA and the extracellular secretion of IFN-β at indicated time. As respected, the mRNA and the protein levels of IFN-β consistently maintained low levels in p53-/- PK-15 cells, including 28 h and 32 h of TGEV infection (data not shown). However, at 36 h p.i., p53-/- PK-15 cells have higher levels of IFN mRNA but lower in protein levels. We infer that the secretion of IFN-β in cell supernatant might lag behind the gene expression, which may be the reason why p53-/- PK-15 cells have lower protein levels at 36 h p.i. To further investigate why the mRNA levels of many genes (TRIF, RIG-I and TRAM) were higher at 36 h p.i. in p53-/- PK-15 cells compared with WT PK-15 cells, we detected the apoptotic gene expression at 36 hpi. The results showed that Bcl-2 mRNA levels were down-regulated in WT PK-15 cells compared to p53-/- PK-15 cells (Supplement Fig. 1). Hence, we speculated at late stage of TGEV infection, p53 might mainly regulate apoptosis while not antiviral genes, suggesting that p53 might play different role at different stage of TGEV infection.

When type I IFN was released from infected cells, it bind to its receptor to trigger a series of signaling factors which form a heterotrimeric complex ISGF3 (Harada et al., 2010). The latter then bind to the IFN stimulated response elements (ISREs), which present in the promoters of IFN-stimulated genes (ISGs), leading to the activation of antiviral responses (Harada et al., 2010). Many researches have been confirmed that p53 directly transcribed several target genes, to influence type I IFN signaling, such as ISG15, IRF9 and IRF3 (Choi et al., 2014; Melchert, 2008; Muñoz-Fontela et al., 2008a, b; Park et al., 2016). In our study, IRF9, IRF3, ISG15 and ISG20 mRNA expression levels were remarkably impaired in p53-/- PK-15 cells compare with that in WT PK-15 cells, suggesting that p53 plays a vital role in regulation of IFN production and IFN signaling upon TGEV infection.

In response to virus infection, host induce or enhance the type I IFN to inhibit the replication of virus or the progeny production (Muñoz-Fontela et al., 2008a). In this study, as above, p53 played a vital role in IFN-β induction and IFN-β signal pathways during TGEV infection. We further confirm the regulatory role of p53 on TGEV replication and production at low titer of virus infection. Results showed that virus gRNA and sgmRNA levels enhanced in p53-/- PK-15 cells compared to that in WT PK-15 cells when infected with TGEV (0.1 MOI). In addition, the pan apoptosis inhibitor down-regulated the levels of TGEV gRNA and sgmRNA-N, whereas the genes expression in WT PK-15 cells was lower than that in p53-/- PK-15 cells. While in our previous research, we indicated that when 10 MOI of TGEV infected cells, the virus genes mRNA levels in p53 inhibitor-treated cells were higher than control cells at 12 h p.i., but it did not appear significant increase (Huang et al., 2013). These results and difference suggest that p53 might be through activating type I IFN signaling to inhibit viral replication during TGEV infection with lower virus titers, and the apoptosis might be benefit to viral replication. However, in high viral titer infection, p53 might mainly act its proapoptotic functions. In this condition, the occurrence of cell apoptosis might play some role in virus assembled and live virus liberation. These results were similar to influenza A virus (IAV) and human immuno-deficiency virus type 1 (HIV-1) (Mukerjee et al., 2010; Shi et al., 2018; Zhu et al., 2014). In addition, as a strong IFN inducer, poly (I:C) co-treated WT PK-15 cells with TGEV, showed a sharp up-regulation in the expression of IFN-β and IFN-simulated gene compared to poly (I:C) treatment only. And TGEV gRNA and sgmRNA levels showed a significant decrease in poly (I:C) and exogenous IFN-β-treated WT PK-15 cells. These findings indicate that p53 effectively enhance TGEV- and poly (I:C)-induced IFN signal, and was crucial for heightening antiviral activity mediated by type I IFN.

In conclusion, we elaborated a mechanism of p53-mediated the enhancement of TRIF, TRAM and RIG-I/MDA5/IPS-1 pathways, resulting in IFN-β production, which in turn activated IFN-β signals to the induction ISGs and IRF9, resulting in a marked decrease of viral replication at 0.1 MOI of TGEV infection. Our results demonstrated that p53 played a crucial role in innate immunity through enhancing type I IFN-dependent antiviral activity.

Authors contribution

Li Ding designed the experiments and wrote the article. Li Ding and Jiawei Li interpreted the data. Meiling Hong revised the manuscript. Jiawei Li performed the experiments with assistance and advice from Li Ding, Weihao Li, Zhenhua Fang, Na Li, Qiqi Guo, Haoyue Qu, Dan Feng, Jiangyue Li and Meiling Hong. All authors read and approved the final manuscript.

Conflict of interest

There is no conflict of interest of any authors in relation to the submission.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:10.1016/j.vetmic.2018.10.025.

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