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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

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. [illegible]

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% $CO_2$

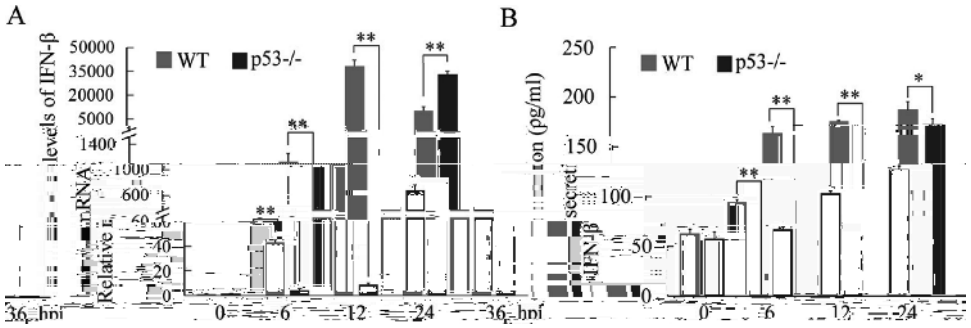



**Fig. 1.** p53 enhanced the IFN-β mRNA expression and production in TGEV-infected cells. (A) The mRNA levels of IFN-β. WT and p53-/- PK-15 cells were infected with TGEV at MOI of 0.1 for different time. The cells were collected and subjected to qRT-PCR analysis. (B) The secretion of IFN-β. WT and p53-/- 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. * < 0.05, ** < 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. 53 [illegible]

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. 53 [illegible]

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

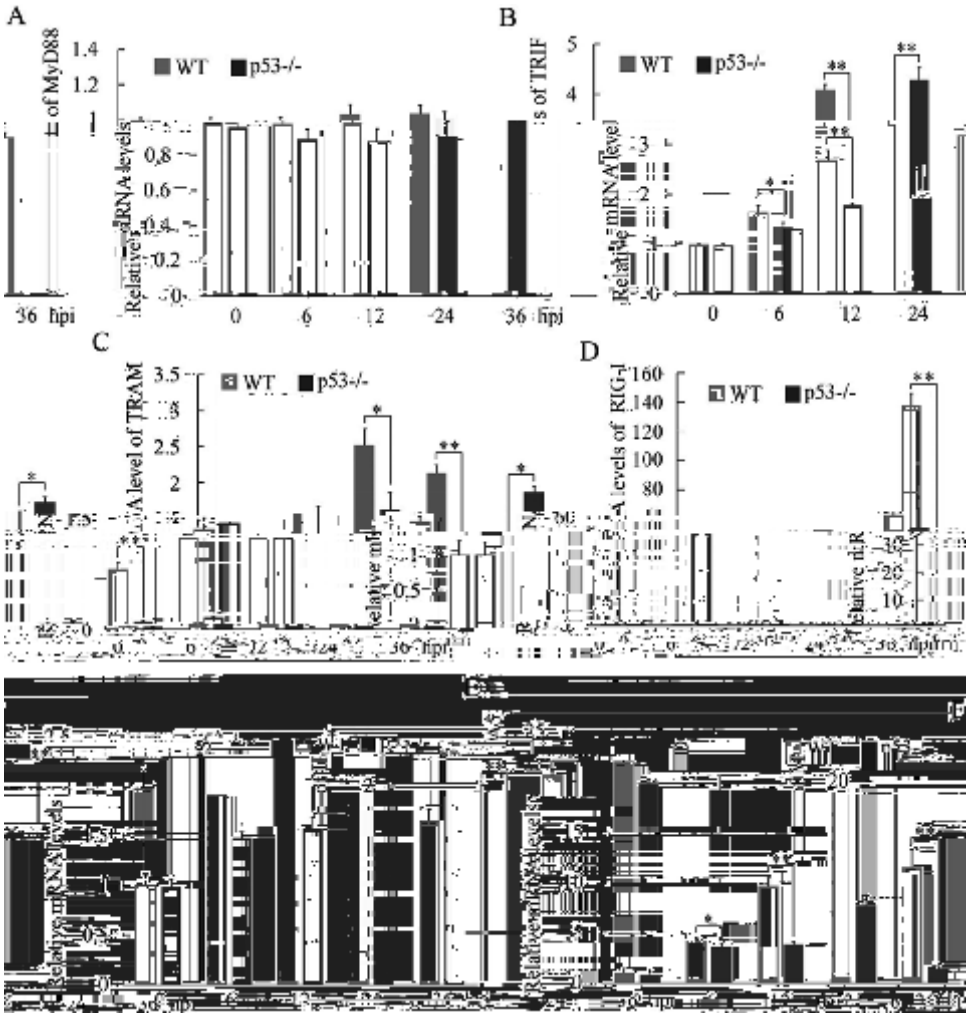



**Fig. 2.** p53 regulated TRIF, TRAM and RIG-I/MDA5/IPS-1 pathways upon TGEV infection. (A–F) The mRNA levels of MyD88, TRIF, TRAM, RIG-I, MDA5 and IPS-1, respectively. WT and p53-/- PK-15 cells were infected with TGEV at MOI of 0.1 for different time. The cells were collected and subjected to qRT-PCR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

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

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 theand virus (

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

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