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Foot-and-mouth disease virus non-structural protein 2B negatively regulates the RLR-mediated IFN-β induction

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ABSTRACT

Foot-and-mouth disease virus (FMDV) is the causative agent of Foot-and-mouth disease (FMD), which is an acute and highly contagious disease affecting pigs, cattle and other cloven-hoofed animals. Several studies have shown that FMDV has evolved multiple strategies to evade the host innate immune response, but the underlying mechanisms for immune evasion are still not fully understood. In the current research, we have demonstrated that FMDV utilizes its non-structural protein 2B to sabotage the host immune response. Over-expression of the FMDV 2B inhibited Poly(I:C)-induced or SeV-triggered up-regulation of IFN-β, IL-6 as well as ISG15. When HEK293T cells were transfected with FMDV 2B, the phosphorylation of TBK1 and IRF3 was inhibited. Co-immunoprecipitation and pull-down experiments indicated that FMDV 2B protein could interact with host RIG-I and MDA5. Moreover, FMDV 2B also inhibited the expression of the RIG-I and MDA5. Thus, FMDV 2B negatively regulates the RLR-mediated IFN-β induction by targeting RIG-I and MDA5.

1. Introduction

Foot-and-mouth disease (FMD) is a highly contagious disease to cloven-footed animals, which is notifiable to the Office International des Epizooties (OIE) and of great socioeconomic consequence. The causative agent of FMD is the foot-and-mouth disease virus (FMDV) [1]. The genome of FMDV is a positive sense ssRNA with about 8500 bases in length [2]. The genome is composed of a 5′ untranslation region (5′ UTR), a protein-coding region and a 3′ untranslation region (3′ UTR) [3]. The open reading frame (ORF) of FMDV is approximately 6.5 kb and consists of the L region, the P1 region, P2 region and P3 region. The P1 region encodes four viral structural proteins including VP4, VP2, VP3, and VP1, and the P2 and P3 regions encodes the viral non-structural proteins 2A, 2B, 2C, 3A, 3B, $3C^{pro}$ and $3D^{pol}$ [4].

The interferons (IFNs) are glycoproteins with strong antiviral activities. Based on the structure of their receptors on the cell membrane, IFNs are divided into three types [5]. Among them, type I IFNs (IFN-α and IFN-β) could be induced during virus infection, and then activate the signaling pathway to promote the transcription of downstream interferon-stimulating genes (ISGs), which further shape the host antiviral state and impact the adaptive immune response [6–8]. The Retinoic acid inducible gene-I like RNA helicases receptors (RLRs) are pattern-recognition receptors that reside in the cytosol. Which consist of retinoic acid-inducible gene I (RIG-I), melanoma differentiation associated factor 5 (MDA5) and laboratory of genetics and physiology 2 (LGP2) [9–13]. Both RIG-I and MDA5 could recognize RNA viruses, but differ in their RNA recognition specificity, while LGP2 plays regulatory roles in antiviral immunity [14–16]. The engagement of RNA with RIG-I or MDA5 trigger the activation of MAVS, eventually leading to the production of type I interferons and pro-inflammatory factors [17–19].

Several studies have shown that FMDV has evolved the ability to inhibit the host innate immune response. Previous studies have proved that FMDV L^{pro} could disrupt NF-κB signaling, inhibit the expression of type I interferons and cleave LGP2 [20–23]. In addition, FMDV L^{pro} also counteracts host innate antiviral responses via its DUB activity [24]. FMDV VP1 can interact with soluble

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resistance-related calcium-binding protein (sorcin), then block the involvement of calcium ions required for activation of immune signaling pathways [25]. FMDV 3A inhibits the RLR-mediated IFN-β induction by engagement with, as well as down-regulating the expression of RIG-I, MDA5, and VISA [26]. FMDV 2B is recently reported to interact with and decrease the expression of RIG-I [27]. In the current research, the role of FMDV 2B in regulating the RLR-mediated IFN-β induction is further explored.

2. Materials and methods

2.1. Cells and viruses

HEK293T cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) in 5% CO2 incubator (37 °C). Cells were infected with SeV at the MOI (multiplicity of infection) of 10.

2.2. Reagents

EndoFree Plasmid Maxi Kit was purchased from QIAGEN GmbH. The restriction enzymes, MiniBEST Universal RNA Extraction Kit, SYBR Rremix DimerEraseTm (Perfect Real Time) and PrimeScript RT reagent Kit were from TaKaRa (China). Transfection reagent jetPRIME was from Polyplus-transfection Biotechnology company (France). TransDetect Double-Luciferase Reporter Assay Kit was from TRANSGEN BIOTECH (China). Poly(I:C) High Molecular Weight was from InvivoGen (USA), HRP-conjugated goat anti-mouse and anti-rabbit IgG antibodies were from Qiangxin Biorepublic (Beijing, China). Anti-RIG (D14G6), anti-MDA5 (D74E4), anti-MAVS (D5A9E), anti-TBK1/NAK (D1B4), anti-IRF3 (D9J5Q), anti-Phospho-IRF3 (Ser396), anti-Phospho-TBK1 and anti-FLAG M2 monoclonal antibody were from Cell Signaling Technology. Anti-EGFP antibody was from Abnova. Pierce Co-Immunoprecipitation Kit and SuperSignal West Femto Maximum Sensitivity Substrate were from Thermo Scientific.

2.3. Plamids

FMDV 2B (strain O/MYA/01/1998, GenBank: KR401154.1) was synthesized and cloned into the pEGFP-C1 vector. Human RIG-I, MDA5, VISA, TBK1, and IRF3 genes were originally cloned from HEK293T cells by RT-PCR using specific primers, then cloned into the p3×FLAG-CMV-9 expression plasmid. IFN-β, NF-κB and ISRE promoter luciferase reporter plasmids and pGMR-TK Renilla luciferase reporter vector were purchased from Genomeditech company (Shanghai, China). All the primers used in this experiment (Table 1) were synthesized by GENEWIZ company (Suzhou, China).

2.4. Cell culture and transfection

HEK293T cells were cuktured until 80–90% confluent, then seeded in 6-well plate or 12-well plate. Cell transfection was performed following the protocol of jetPRIME reagent. After transfection, cells were cultured in DMEM supplemented with 2% fetal bovine serum (FBS).

2.5. RNA extraction and qRT-PCR

The HEK293T cells (2×10^5) were seeded into 6-well plates and transfected with pEGFP-C1 or pEGFP-C1-2B. Twelve hours post-transfection, the cells were treated with SeV (MOI = 10), Poly(I:C) (30 ng/ml) or medium as control for another 12 h. Total RNA was extracted uing MiniBEST Universal RNA Extraction Kit (TaKaRa, China), and reverse transcribed to cDNA with PrimeScript RT reagent Kit (TaKaRa, China).

Specific primers were designed based on the sequences of human RIG-I, MDA5, VISA, TBK1, TRAF3, TRAF6, IRF3, IL-6, ISG15 and IFN-β. The relative transcriptional level of these cytokines was detected by fluorescent quantitative real-time PCR. Each reaction was performed in 20 μL including 10 μL SYBR Premix DimerErase (2×), 0.6 μL each gene-specific primer (10 μM), 0.4 μL ROX

Table 1
All the primers used in this experiment.

Primers	Sequences(5′-3′)	Use
2B-F	GGGGTACCATGCCTTTCTTCTTCTCTGACGT	Amplification of 2B
2B-R	CGGGATCCTTACTGTTTCTCCGCTCTCTCAAGGT	
hRIG-I-F	ATAAGAATGCGGCCGCTATGACCACCGAGCAGCGACG	Amplification of RIG-I
hRIG-I-R	CGGGGTACCTCATTTGGACATTTCTGCTGGAT	
hMDA5-F	ATAAGAATGCGGCCGCTATGTCGAATGGGTATTCCAC	Amplification of MDA5
hMDA5-R	CGGGGTACCCTAATCCTCATCACTAAATAAAC	
hVISA-F	ATAAGAATGCGGCCGCTATGCCGTTTGCTGAAG	Amplification of VISA
hVISA-R	CGGGGTACCCTAGTGCAGACGCCGCCGGTAC	
hTBK1-F	ATAAGAATGCGGCCGCTATGCAGAGCACTTCTAATC	Amplification TBK1 of
hTBK1-R	CGGGGTACCCTAAAGACAGTCAACGTTGCGAAGGC	
hIRF3-F	ATAAGAATGCGGCCGCTATGGGAACCCCAAAGCCAC	Amplification of IRF3
hIRF3-R	CGGGGTACCTCAGCTCTCCCCAGGGCCCTGGAAAT	
hRIG-I-q-F	CTGACTGCCTCGGTTGGTGTTG	Q-PCR
hRIG-I-q-R	CTTGCTCCAGTTCCTCCAGATTGTG	
hMDA5-q-F	TCCGCTATCTCATCTCGTGCTTCA	Q-PCR
hMDA5-q-R	TCCAACCAAGGTGCCAGACTCC	
hVISA-q-F	GCATCAGGAGCAGGACACAGAAC	Q-PCR
hVISA-q-R	GGAGAAGGAGGTGCCAGTAGATACA	
hTBK1-q-F	TGGAAGCGGCAGAGTTAGGTGAA	Q-PCR
hTBK1-q-R	TTCGGATGAGTGCCTTCTTGATGTG	
hTRAF3-q-F	TACAAGCGGCGGAAGCAGGA	Q-PCR
hTRAF3-q-R	CCAAGGAAGCAGGGCATCATATTCT	
hTRAF6-q-F	GAGATCCATGACCAGAACTGTCCTT	Q-PCR
hTRAF6-q-R	GGCGTGCCAAGTGATTCCTCT	
hIRF3-q-F	AGAGGCTCGTGATGGTCAAGGTT	Q-PCR
hIRF3-q-R	AGTGGGTGGCTGTTGGAAATGTG	
hIL-6-q-F	GATGGATGCTTCCAATCTGGATTCAATG	Q-PCR
hIL-6-q-R	TCTGGCTTGTTCCTCACTACTCTCA	
hISG15-q-F	GGTGGTGGACAAATGCGACGAA	Q-PCR
hISG15-q-R	CGAAGGTCAGCCAGAACAGGTC	

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