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Borna disease virus nucleoprotein inhibits type I interferon induction through the interferon regulatory factor 7 pathway

Wuqi Song [a,c,1], Wenping Kao [b,c,1], Aixia Zhai [a], Jun Qian [b], Yujun Li [b], Qingmeng Zhang [a], Hong Zhao [c], Yunlong Hu [c], Hui Li [c], Fengmin Zhang [a,b,c,*]

[a] The Heilongjiang Key Laboratory of Immunity and Infection, Heilongjiang, China
[b] The Key Laboratory of Pathogenic Biology, Heilongjiang Higher Education Institutions, China
[c] Department of Microbiology, Harbin Medical University, China

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ABSTRACT

The expression of type I interferon (IFN) is one of the most potent innate defences against viral infection in higher vertebrates. Borna disease virus (BDV) establishes persistent, noncytolytic infections in animals and in cultured cells. Early studies have shown that the BDV phosphoprotein can inhibit the activation of type I IFN through the TBK1–IRF3 pathway. The function of the BDV nucleoprotein in the inhibition of IFN activity is not yet clear. In this study, we demonstrated IRF7 activation and increased IFN-α/β expression in a BDV-persistently infected human oligodendroglia cell line following RNA interference-mediated BDV nucleoprotein silencing. Furthermore, we showed that BDV nucleoprotein prevented the nuclear localisation of IRF7 and inhibited endogenous IFN induction by poly(I:C), coxsackie virus B3 and IFN-β. Our findings provide evidence for a previously undescribed mechanism by which the BDV nucleoprotein inhibits type I IFN expression by interfering with the IRF7 pathway.

1. Introduction

The expression of type I interferon (IFN-α/β) is one of the most potent innate defences in higher vertebrates against infection with viruses such as coxsackie virus B and herpes simplex virus. Type I interferons can also be induced by poly(I:C) and CpG DNA [1]. The type I IFN system plays an important role in innate resistance to many viruses by inducing direct and indirect antiviral effects. Interferon regulatory factors (IRFs) are a family of transcription factors involved in regulating type I IFN genes and other genes that participate in the early antiviral host response. IRF3 and IRF7 are essential transcriptional factors resulting in the induction of IFN-α/β transcription following viral infection. Activated IRF3 and IRF7 undergo nuclear translocation and subsequently bind to IRF-binding elements [i.e., positive regulatory domains (PRDs) I and III and PRD-like elements (PRD-LEs)] in the IFN-α/β promoter region [2]. Recent studies have shown that IRF7 is the master regulator of IFN gene expression [3,4].

The IFN-α/β can be induced by viral infection. However, IFN induction and production are inhibited by many viruses, including negative-sense single-stranded RNA viruses, such as measles virus, rinderpest virus, vesicular stomatitis virus, Ebola virus, rabies virus (RABV), Nipah virus, and respiratory syncytial virus (RSV). These viruses have developed extraordinarily diverse strategies to impair IFN signalling or to counteract cellular antiviral effectors [5]. The nucleoproteins of lymphocytic choriomeningitis virus (LCMV), classical swine fever virus (CSFV), respiratory syncytial virus (RSV) and rabies virus are involved in the inhibition of IFN expression in host cells [6–9].

Borna disease virus (BDV) is a neurotropic, negative-stranded RNA virus that causes a nonsuppurative meningoencephalomyelitis in a wide range of animals and establishes persistent, noncytolytic infections [10,11]. The nucleoprotein, phosphoprotein and X protein of BDV are the essential constituent elements of the polymerase complex [12]. The nucleoprotein is the most abundant protein in BDV-infected cells and tissues and mediates the cellular immune response [11,13]. BDV phosphoprotein is an essential cofactor of the viral RNA-dependent RNA polymerase [14]. The X protein is a potential negative factor for polymerase activation [15]. Early studies have shown that exogenous IFN does not appear to influence persistent BDV infection in Madin–Daby canine kidney cells (MDCK) and C6 cells and that phosphoprotein associates with and inhibits the TBK1–IRF3 activation pathway in MDCK cells [16]. However, it is unclear whether the BDV nucleoprotein is involved in the regulation of natural immunity.

* Corresponding author. Address: Harbin Medical University, Xuefu Road 194, Harbin, China. Fax: +86 (0)451 86669576.
E-mail address: fengminzhang@ems.hrbmu.edu.cn (F. Zhang).
[1] These authors contributed equally to this work.

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In this study, we designed small interfering RNA (siRNA) plasmids and a BDV protein expression plasmid to evaluate the function of the BDV nucleoprotein in the inhibition of type I IFN expression in oligodendroglia (OL) cells. The results showed that BDV nucleoprotein prevented the nuclear localisation of IRF7 and inhibited endogenous type I IFN induction by poly(I:C), coxsackie virus B3 and IFN-β. Our findings provide evidence for a previously undescribed mechanism by which the BDV nucleoprotein inhibits type I IFN expression by interfering with the IRF7 pathway.

2. Materials and methods

2.1. Cells and viruses

BDV strain 1766, human oligodendroglial (OL) cells, and BDV-persistently infected OL (OL/BDV) cells were kindly donated by Professor Kazuyoshi Ikuta, Department of Virology, Osaka University, Japan. Coxsackie virus B3 (CVB3) Nancy strain was provided by the Department of Microbiology, Harbin Medical University, China. OL and OL/BDV cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 5% and 2% foetal bovine serum, respectively, and 100 U/mL penicillin/streptomycin at 37 °C and 5% CO_2.

2.2. Plasmids

We constructed the plasmids pmCherry-IRF7 and peGFP-BDV-N for this study. The IRF7 open reading frame was inserted into the multiple cloning site of pmCherry-N1 (TaKaRa, Japan). IRF7 fused with red fluorescent protein (RFP) mCherry was expressed by pmCherry-IRF7. The BDV nucleoprotein open reading frame was inserted into the multiple cloning site of peGFP-N1 (TaKaRa). BDV nucleoprotein fused with enhanced green fluorescent protein (EGFP) could be expressed by peGFP-BDV-N. All plasmids used in this study were confirmed by sequencing. The empty peGFP-N1 plasmid was used as a control.

2.3. RNA interference (RNAi)

The small interfering RNA (siRNA) targeting the mRNA of BDV nucleoprotein was inserted into a plasmid by GenePharma (Shanghai, China). Two siRNA plasmids (sRNAi-N-219 5′-GCCTAGCCTTG TGTTTCTATG-3′ and sRNAi-N-699 5′-GCAGATGACCACGTACAC-TAC-3′) were introduced into OL/BDV cells with Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. A sRNAi-NC plasmid with an unrelated sequence was used as a negative control. Cell cultures were continued for 48 h before detection. The silencing efficiency was monitored by real-time PCR for mRNA levels. OL/BDV cells were co-transfected with pmCherry-IRF7 plasmid and siRNA expression plasmids. Twenty-four hours later, the location of IRF7 was detected in the transfected cells.

2.4. Transfection of plasmids

A 24-well plate was seeded with 5×10^4 cells/well in antibiotic-free DMEM medium supplemented with 5% FCS. After 24 h incubation, cells were transfected with BDV nucleoprotein expression plasmids or empty peGFP-N1 using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. The cultures were continued for 24 h before cells were exposed to treatments with poly(I:C) (100 μg/mL; Sigma, USA) and CVB3 (MOI 0.5). Total RNA was extracted from cells at 4 h post-treatment. OL cells were co-transfected with pmCherry-IRF7 plasmid and BDV nucleoprotein expression plasmids. Twenty-four hours later, the transfected cells were treated with poly(I:C), CVB3 or IFN-β for 4 h. The expression and location of fluorescence was observed by inverted fluorescence microscopy.

2.5. Quantitative reverse transcriptase (RT)-PCR

Total RNA isolated from cells was prepared using TRIzol reagent (Invitrogen, USA) according to the manufacturer's instructions. Total RNA was reverse transcribed into cDNA using M-MLV reverse transcriptase (Promega, USA) and Oligo dT^{18} (TaKaRa). Quantitative PCR was performed on a LightCycle 2.0 (Roche) using SYBR Premix Ex Taq (TaKaRa) according to the manufacturer's protocol. Primers for the measurement of human type I IFN, BDV nucleoprotein and human GAPDH mRNA levels were as follows: IFN-α (F: 5′-GAACTCTACCAGCAGCT-3′, R: 5′-CAGATAGAGAGTGATTC-3′), IFN-β (F: 5′-AAGGCCAAGGAGTACAGTC-3′, R: 5′-AGTTTCGGGGTAACCTG-3′), BDV nucleoprotein (F: 5′-GGTTTAAAACTATGATGGCAGCCTTA-3′, R: 5′-GTGGATTAACATCTGGAGTAGTGTAGC-3'), GAPDH (F: 5′-ACCACAGTACATGCGATCAC-3′, R: 5′-TCCACCACCCTGTTGCTGTA-3′). Analysis of relative change in gene expression was calculated according to the $2^{-\Delta\Delta ct}$ method using the GAPDH gene as the control.

2.6. Statistical analysis

All values are presented as the mean and standard error of the mean (SEM). Statistical analysis was performed using SigmaStat 3.0 (Systat Software, Richmond, CA). The Student's *t* test was used to evaluate the differences between two groups. A *P* value of <0.05 was considered statistically significant. All experiments were repeated at least three times.

3. Results

3.1. BDV inhibits expression of type I IFN in persistently infected OL cells

The level of IFN-α/β mRNA in OL/BDV cells was compared with that in normal OL cells by quantitative-PCR, and the GAPDH gene was used as an internal control. The IFN-α/β level was significantly lower in OL/BDV cells (Fig. 1A), indicating that persistent BDV infection inhibited expression of type I IFN.

It has previously been shown that BDV phosphoprotein counteracts TBK1-dependent IFN-β expression and therefore the establishment of antiviral activity in cells [16]. To identify the function of the BDV nucleoprotein in the inhibition of IFN-α/β expression, we designed siRNA plasmids targeting the nucleoprotein and introduced these plasmids into OL/BDV cells. Forty-eight hours later, we measured the BDV nucleoprotein mRNA, protein and IFN-α/β mRNA levels. The BDV nucleoprotein (N) mRNA level was significantly reduced in OL/BDV cells following siRNA plasmid (sRNAi-N-219 and sRNAi-N-699) transfection, and the IFN-α/β mRNA level was increased compared with control siRNA (sRNAi-NC) transfected OL/BDV cells (Fig. 1B,C,F and G). These results suggest that BDV nucleoprotein is also involved in the resistance to IFN.

Previous studies have shown that IRF3 and IRF7 are key factors in IFN-α/β induction [2–4] and that BDV phosphoprotein counteracts the TBK1–IRF3 pathway to inhibit IFN activation [16]. To measure the activation of IRF7 in OL/BDV cells, a pmCherry-IRF7 plasmid and the siRNA plasmids were co-transfected into OL/BDV cells. Forty-eight hours later, the nuclear localisation of IRF7 was increased in OL/BDV cells co-transfected with siRNA plasmids compared with control cells as analysed by fluorescence microscopy (Fig. 1E and H).