ARTICLE IN PRESS

Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

MCPIP1 negatively regulate cellular antiviral innate immune responses through DUB and disruption of TRAF3-TBK1-IKKε complex

Xiaojuan Chen, Qian Zhao, Qing Xie, Yaling Xing**, Zhongbin Chen*

Division of Infection and Immunity, Department of Biological Technology, Beijing Institute of Radiation Medicine, Beijing, 100850, China

ARTICLE INFO

Article history:
Received 6 June 2018
Accepted 16 June 2018
Available online xxx

Keywords:
MCPIP1
Antiviral innate immune
Interferon
IRF3
TRAF3-TBK1-IKKε complex

ABSTRACT

IFNβ innate immune plays an essential role in antiviral immune. Previous reports suggested that many important regulatory proteins in innate immune pathway may be modified by ubiquitin and that many de-ubiquitination (DUB) proteins may affect immunity. Monocyte chemotactic protein-inducing protein 1 (MCPIP1), one of the CCCH Zn finger-containing proteins, was reported to have DUB function, but its effect on IFNβ innate immune was not fully understood. In this study, we uncovered a novel mechanism that may explain how MCPIP1 efficiently inhibits IFNβ innate immune. It was found that MCPIP1 negatively regulates the IFNβ expression activated by RIG-I, STING, TBK1, IRF3. Furthermore, MCPIP1 inhibits the nuclear translocation of IRF3 upon stimulation with virus, which plays a key role in type I IFN expression. Additionally, MCPIP1 interacts with important modulators of IFNβ expression pathway including IPS1, TRAF3, TBK1 and IKKε. Meanwhile, the interaction between the components in TRAF3-TBK1-IKKε complex was disrupted by MCPIP1. These results collectively suggest MCPIP1 as an innate immune regulator encoded by the host and point to a new mechanism through which MCPIP1 negatively regulates IRF3 activation and type I IFNβ expression.

© 2018 Elsevier Inc. All rights reserved.

1. Introduction

The innate immune system stands the first line of the defense that protects the host from viral intrusion, depending on pattern recognition receptors (PRRs) and the corresponding pathways [1–4]. The pathogen-associated molecular patterns (PAMPs) of the invading viruses may be recognized by PRRs. Then, the adaptor proteins (TRIF forTLR3, MyD88 for TLR7/8/9, MAVS/IPS-1 for RIG-I) would be recruited, and the infecting signals would be transmitted to the downstream kinase complexes, followed by the activation of transcription factors, such as interferon regulatoryfactor-3 (IRF-3), nuclear factor kB (NF-kB) and ATF-2/c-jun [2,5–7]. Upon activated, the transcription factors may regulate the expression of type I Interferons which induce the expression of IFN-stimulated genes (ISGs) and ultimately establish the antiviral function of the host [4,8–10].

Many important regulatory proteins in innate immune pathways may be modified by ubiquitin [11–13]. On the other hand, Many de-ubiquitination (DUB) proteins may affect immunity. We have found that the papain-like proteases (PLPs) encoded by coronavirus (CoV) reduce the ubiquitinated modification of essential regulatory molecules of IFN innate immune pathway, such as RIG-1, MAVS, STING, TRAF3, TBK1 and IRF3. Additionally, CoV PLPs negatively regulate IFN expression of the host, acting as both deubiquitinases and IFN antagonists [14–16].

The C-terminal phosphorylation and activation of IRF3 requires noncanonical IκB kinases, TBK1 or IKK, which form signaling complexes with TRAF family members that transmit upstream signals to downstream effectors resulting in the expression of type I IFN. Previous studies suggest that TRAF family members are involved in the regulation of antiviral immune responses [17–20]. We have reported that SARS-CoV PLP blocks the ubiquitination of STING-TRAF3-TBK1 complex and disrupts STING-TRAF3-TBK1 complex [21].

MCPIP1 (monocyte chemotactic protein-induced protein 1), a negative regulator of macrophage activation, was also found to negatively regulate JNK and NF-κB activity by removing ubiquitin moieties from proteins including TRAFs [22]. In this study, we observed that MCPIP1 inhibits the IFNβ expression activated by

* Corresponding author. Beijing Institute of Radiation Medicine, 27 Taiping Rd, Haidian District, Beijing, 100850, China.
** Corresponding author. Beijing Institute of Radiation Medicine, 27 Taiping Rd, Haidian District, Beijing, 100850, China.
E-mail addresses: yalingxing@126.com (Y. Xing), chenzb@bmi.ac.cn, chenzhongbin2013@163.com (Z. Chen).

https://doi.org/10.1016/j.bbrc.2018.06.083
0006-291X/© 2018 Elsevier Inc. All rights reserved.

Please cite this article in press as: X. Chen, et al., MCPIP1 negatively regulate cellular antiviral innate immune responses through DUB and disruption of TRAF3-TBK1-IKKε complex, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.06.083

RIG-I, STING, TBK1, IRF3. Additionally, MCPIP1 inhibits the nuclear migration of IRF3. Furthermore, MCPIP1 interacts with TRAF3 and disrupts TRAF3-TBK1-IKKε complex which is essential for the activation of IFNβ production pathway. This report suggests that MCPIP1 may act as an IFN antagonist antiviral protein encoded by the host and uncovers the mechanism undergoes by MCPIP1 to inhibit IFNβ innate immune pathway.

2. Materials and methods

2.1. Cell and plasmids

HEK293T and HeLa cells were cultured in Dulbecco's modified Eagle's medium containing 10% (v/v) FCS supplemented with penicillin (100 U/ml) and streptomycin (100 mg/ml).

The plasmid expressing V5-MCPIP1 was cloned according to NCBI Reference SequenceNM_025079.2. The reporter plasmids IFNβ-Luc, PRD (III-I)4-Luc, NF-κB-Luc, and plasmids NL63-PLP2-TM, Myc-IRF3, Flag-IRF3, Flag-IRF7, Flag-IPS-1, Flag-STING, Flag-TBK1, Flag-IKKε, Flag-RIG-IN, A20, HA-TRAF3 were described previously [14,21,23,24].

2.2. SiRNA preparation

MCPIP1-target siRNA sequence (5′-CCAGCGUGUAUA-CUAAGCUTT-3′) were designed and chemically synthesized by Genescript Co. A siRNA with the sequence of 5′-UUCUCCGAACGUGUCACGU-3′ was selected as the negative control siRNA (NC-siRNA) as described previously [25,26].

2.3. Luciferase reporter gene assay

HEK293T cells were transfected with the indicated stimulator plasmid DNA (Flag-IPS-1, Flag-STING, Flag-TBK1, Flag-IKKε, Flag-RIG-IN), reporter plasmid DNA (pRL-TK, IFNβ-Luc, or PRD (III-I)4-Luc) and either V5-MCPIP1 or NL63PLP2-TM/A20 using Lipofectamine 2000 (Invitrogene) according to the manufacturer's protocol and incubated for 24 h. Then, firefly luciferase and renilla luciferase activities were assayed using the Dual Luciferase Reporter Assay Kit (Promega). Data were shown as mean relative luciferase (firefly luciferase activity divided by Renilla luciferase activity) with standard deviation from a representative experiment carried out in triplicate. The luciferase assay was performed as described previously [14,15].

2.4. Immunofluorescence assay

HEK293T or Hela cells were grown to confluence in a six-well plate. Plasmid DNA expressing V5-MCPIP1 and Flag-IRF3 (1.2 μg per well) were transfected. Twenty-four hours later, the cells were infected or mock-infected by SeV (100 HAU) and incubated for 18 h. Fluorescence was examined by using a confocal microscope. Immunoflurescence assay was performed as described previously [15].

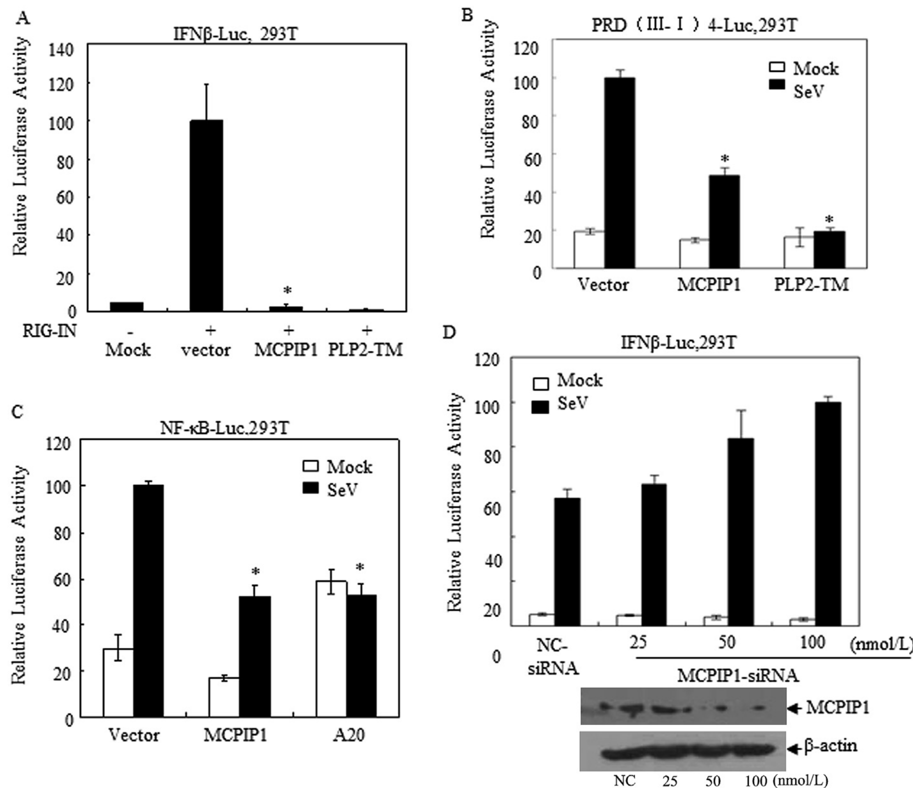

Fig. 1. MCPIP1 inhibits the expression of IFNβ in cells. **a** HEK293T cells were co-transfected with the plasmids which expressing IFNβ-Luc and either MCPIP1 or NL63 PLP2-TM (positive control). Twenty-four hours later, cells were harvested and subjected to a Dual-luciferase assay. **b** HEK293T cells were transfected with PRD (III-I) 4-Luc and either MCPIP1 or NL63 PLP2-TM expressing plasmids (positive control). Twenty-four hours later, cells were harvested and subjected to a Dual-luciferase assay. **c** HEK293T cells were transfected with the plasmids which expressing NF-κB -Luc and either MCPIP1 or A20 (positive control). Twenty-four hours later, cells were harvested and subjected to a Dual-luciferase assay. **d** HEK293T cells were respectively co-transfected with the plasmids which expressing IFNβ-Luc and MCPIP1-siRNA (25, 50, 100 nmol/L). Nc-siRNA (sequence described in the text) was used as control. Twenty-four hours later, cells were harvested and subjected to Dual-luciferase assay. The expression of MCMIP1 was also detected by Western-blotting assay. The results were expressed as mean relative luciferase (firefly luciferase activity divided by Renilla luciferase activity) with standard deviation from a representative experiment carried out in triplicate. Data were presented as mean ± SEM, n = 3. $^{*}p < 0.05$.

Please cite this article in press as: X. Chen, et al., MCPIP1 negatively regulate cellular antiviral innate immune responses through DUB and disruption of TRAF3-TBK1-IKKε complex, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.06.083

Download English Version:

https://daneshyari.com/en/article/8292340

Download Persian Version:

https://daneshyari.com/article/8292340

Daneshyari.com