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 is a positive regulator of type I interferons antiviral activity

Liping Qian [a, b, 1], Yibo Zuo [a, b, 1], Wenjun Deng [c], Ying Miao [a, b], Jin Liu [a, b], Yukang Yuan [a, b], Tingting Guo [a, b], Liting Zhang [a, b], Jun Jin [c], Jun Wang [c, **], Hui Zheng [a, b, *]

[a] Institutes of Biology and Medical Sciences, Soochow University, Suzhou, Jiangsu Province, 215123, China
[b] Jiangsu Key Laboratory of Infection and Immunity, Soochow University, Suzhou, Jiangsu Province, 215123, China
[c] Department of Intensive Care Medicine, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu Province, 215123, China

ARTICLE INFO

Article history:
Received 7 March 2018
Accepted 9 March 2018
Available online xxx

Keywords:
Interferon
Antiviral activity
MCPIP1
ISRE
ISGs

ABSTRACT

Type-I interferons (IFN-I) are widely used for antiviral immunotherapy in clinic. Therefore, identification of the regulators of IFN-I antiviral activity is important for developing novel targets for IFN-based antiviral therapy. Monocyte chemoattractant protein 1-induced protein 1 (MCPIP1) is critical for cellular inflammatory responses. However, the roles of MCPIP1 in interferons (IFNs)-mediated antiviral immunity are unexplored. In this study, we demonstrate for the first time that MCPIP1 is an important positive regulator of IFNs antiviral activity. We found that MCPIP1 can promote innate antiviral immunity independently of both its RNase and deubiquitinase activity. Furthermore, we reveal that MCPIP1 is an IFN-induced positive feedback signal molecule which promotes IFN-I-mediated antiviral efficacy. Mechanistically, MCPIP1 does not affect the activation of JAK/STAT upstream of IFN-I signaling, but significantly promotes IFN-I signaling by enhancing ISRE promoter activity and expression of interferon-stimulated genes (ISGs). And MCPIP1-mediated activation of IFN-I signaling is independently of its RNase and deubiquitinase activity. These findings uncover a novel innate antiviral mechanism mediated by the IFN-MCPIP1 axis, and may provide potential targets for enhancing IFNs antiviral therapy.

1. Introduction

Monocyte chemoattractant protein 1-induced protein 1 (MCPIP1), also known as Regnase-1 or ZC3H12A, belongs to the member of MCPIP family [1,2]. MCPIP1 has a highly conserved CCCH-type zinc finger domain with the RNA-binding ability [3]. The N-terminus of MCPIP1 contains a NYN (Nedd4-BP1, YacP Nuclease) domain, which is responsible for its RNase activity [4,5]. Another important function of MCPIP1 is to remove the ubiquitin moieties from TNF receptor-associated factors (TRAFs) family members as a deubiquitinase [6–10]. Interestingly, MCPIP1 can be rapidly induced by these proinflammatory factors including TNF-α, IL-1β and LPS [1,8,11,12]. However, the roles of MCPIP in the antiviral cytokine interferons (IFNs) signaling remain unknown.

The IFNs family plays key roles in regulating antiviral immunity, cell proliferation, and immunomodulatory functions [13–16]. There are three IFNs families: type I (IFN-I, or IFN-α/β), type II (IFN-γ) and type III (IFN-λ) IFNs [17,18]. Among them, IFN-I family has been widely used for antiviral therapy in clinic. In the classic IFN-I signaling pathway, IFN-α/β binds to IFN receptors (IFNAR1 and IFNAR2) and induces tyrosine phosphorylation of JAK family (JAK1/Tyk2). Subsequently, activated JAK1 and Tyk2 induce phosphorylation and activation of the signal transducers and activators of transcription (STAT1 and STAT2). STAT1 and STAT2, together with IRF9, form a signaling complex known as ISGF3. The complex translocates into the nucleus to bind with interferon-stimulated response elements (ISRE) promoter, and induce the transcription expression of interferon-stimulated genes (ISGs). Finally, these ISGs perform multiple biological functions [18,19]. To date, understanding how IFN-I antiviral signaling is regulated by intracellular proteins remains a major challenge.

Recently, an interesting report showed that MCPIP1 can inhibit two members of the flavivirus genus of the *Flaviviridae* family, Japanese encephalitis virus (JEV) and dengue virus (DEN), by binding with viral RNAs, and the RNase activity of MCPIP1 is required for this antiviral potential [20]. However, although this

* Corresponding author. Institutes of Biology and Medical Sciences, Jiangsu Key Laboratory of Infection and Immunity, Soochow University, 199 Ren-ai Road, Suzhou, Jiangsu 215123, China.
** Corresponding author.
E-mail addresses: wjsdfyy@163.com (J. Wang), huizheng@suda.edu.cn (H. Zheng).
[1] These two authors contributed equally to this study.

https://doi.org/10.1016/j.bbrc.2018.03.076
0006-291X/

report also found that MCPIP1 can inhibit some other viruses, MCPIP1 cannot inhibit several viruses including RNA virus enterovirus 71 (EV71) [20], suggesting two possibilities: 1) the binding of MCPIP1 to viral RNAs could be not a broad-spectrum effect, or 2) the binding between MCPIP1 and viral RNAs could be not uniquely important for host antiviral activity. Therefore, it raised the important question whether and how MCPIP1 can regulate host innate antiviral immunity.

Here, we found that MCPIP1 can promote innate antiviral immunity. Surprisingly, this effect is independently of the RNase activity of MCPIP1, suggesting a different regulation with viral-RNA binding. Given that MCPIP1 cannot inhibit some viruses including EV71 [20], which has extremely low ability to produce IFNs, we want to know whether the inconsistent antiviral effects of MCPIP1 on various viruses are associated, at least partially, with IFNs-mediated antiviral activity. We further found that MCPIP1 did not promote IFN-I production, but enhanced IFN-I-mediated signaling pathway and IFN-I antiviral efficacy. And MCPIP1-mediated regulation of IFN-I signaling is also independent of its RNase and deubiquitinase activity. Interestingly, IFN-I can induce the expression of MCPIP1, indicating a positive feedback regulation of IFN-I antiviral signaling. Our findings reveal a novel innate antiviral immunity mechanism mediated by MCPIP1, and could provide new insight into enhancing IFNs antiviral therapeutic efficacy.

2. Materials and methods

2.1. Cells culture

HEK293T, HT1080, 2fTGH, U3A cells were obtained and cultured as described previously [19]. Cells were transfected using Lipo-Trans (Ucallm) or PEI (polyscience). Human IFN-α were purchased from PBL Interferon Source.

2.2. Plasmids

Flag-MCPIP1, Flag-MCPIP1-C157A (CA) and Flag-MCPIP1-D225/226A (DA) were kind gifts from Dr. Wei Xu (Soochow University, China). ShMCPIP1 plasmid was purchased from GENECHEM (Shanghai, China). ISRE-Luc and Renilla plasmids were gifts from Dr. Serge Y. Fuchs (University of Pennsylvania). All plasmids were confirmed by DNA sequencing.

2.3. Virus and viral infection

Vesicular stomatitis virus (VSV) and sendai virus (SeV) were obtained as described previously [19]. Herpes simplex virus (HSV) was a gift from Dr. Chunfu Zheng (Soochow University, China). To determine antiviral effect of IFNα, cells were transfected with shRNAs against MCPIP1. 72 h after transfection, cells were treated with IFNα (30 IU/ml) overnight. After washing twice, cells were infected with viruses at a multiplicity of infection (MOI) of 1.0 for 2 h. Then the infection medium was removed by washing twice. Cells were cultured with fresh medium for continuous 20 h, and then were analyzed by western or real-time q-PCR.

2.4. Western blots

Western blots were performed as described previously [17,19]. The following antibodies were used: anti-Flag (Sigma, F7425), anti-MCPIP1 (Santa Cruz,sc-515275), anti-α-Tubulin (Proteintech, 66031-1-Ig), anti-pY701-STAT1 (Cell Signaling, #9167), anti-pY690-STAT2 (Santa Cruz,sc-21689-R), anti-p-JAK1 (Santa Cruz, sc-16773), anti-p-Tyk2 (Cell Signaling, #9321), anti-VSV-G (Santa Cruz, sc-66180), anti-STAT1 (Santa Cruz,sc-464), anti-STAT2 (Santa Cruz,sc-1668), anti-GAPDH (Proteintech, 10494-1-AP).

2.5. RNA isolation and real-time PCR

Total RNAs were extracted as described previously [19]. The primer sequences are as following:

MCPIP1 (5′-CTGGAGAAGAAGAAGATCCTGG-3′ and 5′-TGACGAAGGAGTACATGAGCAG-3′); IFIT1 (5′-CACAAGCCATTTTCTTTGCT-3′ and 5′-ACTTGGCTGCATATCGAAAG-3′); ISG15 (5′-GGGACCTGACGGTGAAGATG-3′ and 5′-CGCCGATCTTCTGGGTGAT-3′); HSV-UL46 (5′-CTTGCCGGTCTGCCACAG-3′ and 5′-CTCCAATCGCCGGTTCCTCC-3′); HSV-ICP27 (5′-ATCGCACCTTCTCTGTGGTC-3′ and 5′-GCAAATCTTCTGGGGTTTCA-3′); VSV (5′-ACGGCGTACTTCCAGATGG-3′ and 5′-CTCGGTTCAAGATCCAGGT-3′); SeV (5′-ATGACCGCCTACATCCAGCG-3′and 5′-CGCAGCCGTTCTCCAGGTC-3′); IFNβ (5′-CATTACCTGAAGGCCAAGGA-3′ and5′-CAGCATCTGCTGGTTGAAGA-3′); STAT1 (5′-ACTCAAAATTCCTGGAGCAG-3′ and 5′-ACGCTTGCTTTTCCTTATGTT-3′); STAT2 (5′-ATGCTGCAGAATCTTGACA-3′ and 5′-TAGTTCAGCTGATCCAAGAAG-3′); IRF9 (5′-GATGATACAGCTAAGACCA-3′ and 5′-AACTCTTGTTGAGTGCACAG-3′); β-actin (5′-CAACTGGGACGACATGGAGAAA-3′ and5′-AGCACAGCCTGGATAGCAACG-3′).

2.6. Reporter gene assay

Cells were transfected with the ISRE-Luciferase and Renilla plasmids, together with either Flag-MCPIP1 or shMCPIP1. After 48 or 72 h, cells were treated with or without IFN-α overnight. Then the medium was removed by washing twice. The Luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega, #E1910).

2.7. Statistical analysis

Comparison between different groups was analyzed by two-tailed Student's t-test. Data were shown as the mean ± SD. All values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. MCPIP1 can promote innate antiviral immunity independently of its RNase and deubiquitinase activity

To explore the function of MCPIP1 in innate antiviral immunity, vesicular stomatitis virus (VSV), as a sensitive virus model, was firstly chosen to assess the cellular antiviral response. Cells were transfected with two short hairpin RNAs against human MCPIP1 (shMCPIP1-1# and shMCPIP1-2#), respectively. The result showed that VSV infection was substantially enhanced by depletion of MCPIP1 using both shRNAs constructs (Fig. 1A). Moreover, overexpression of MCPIP1 inhibits VSV infection in a dose-dependent manner, as shown by decreased VSV protein VSV-G (Fig. 1B). To further confirm the possible broad-spectrum effects of MCPIP1 on innate antiviral response, we employed several other viruses, including RNA virus sendai virus (SeV) and DNA virus herpes simplex virus (HSV). Consistent with our data from VSV infection, knockdown of MCPIP1 also promoted SeV infection (Fig. 1A). Interestingly, MCPIP1 knockdown also facilitated HSV infection, as shown by increased viral RNAs of two HSV-encoded proteins (ICP27, UL46) in shMCPIP1 groups (Fig. 1A). These data suggest that MCPIP1 is able to promote host innate antiviral immunity.

Given that MCPIP1 can inhibit infection of JEV and DEN, and RNase activity of MCPIP1 is required for this antiviral ability [20], we next ask whether the effect of MCPIP1 on host innate antiviral immunity is dependent on its RNase activity. To this end, we

Please cite this article in press as: L. Qian, et al., MCPIP1 is a positive regulator of type I interferons antiviral activity, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.03.076