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

Interleukin-enhanced binding factor 2 interacts with NLRP3 to inhibit the NLRP3 inflammasome activation

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ARTICLE INFO

Article history:
Received 29 March 2018
Accepted 11 April 2018
Available online xxx

Keywords:
Apoptosis-associated speck-like protein containing a CARD
ASC
Caspase-1
Casp-1
Interleukin-1β
IL-1β
Interleukin-enhanced binding factor 2
ILF2
NACHT
LRR and PYD domains-containing protein 3
NLRP3
The NLRP3 inflammasome

ABSTRACT

The activation of the NLRP3 inflammasome is a key process of host immune response that establishes the first line of defense against pathogen infections and cellular stresses, whereas excessive inflammasome activation may damage the hosts, and thus it must be precisely controlled. However, the mechanism underlying the repression of the NLRP3 inflammasome activation remains largely unknown. In this study, by establishing and using a reconstructed NLRP3 inflammasome activation system, we reveal that the NLRP3 inflammasome activation, pro-caspase-1 cleavage, and pro-interleukin-1β (pro-IL-1β) activation are repressed by the interleukin-enhanced binding factor 2 (ILF2). Further studies demonstrate that ILF2 represses the activation of NLRP3 inflammasome through interacting with the NACHT-associated domain (NAD) of NLRP3 and co-localized with NLRP3 in the cytoplasm of HEK293T cells. Finally, by generating a THP-1 cell line stably expressing ILF2 protein using the lentivirus infection system, we demonstrate that ILF2 represses ATP-induced activation of endogenous NLRP3 inflammasome in macrophages. Therefore, this study identifies a new role of ILF2 in the regulation of the NLRP3 inflammasome, and reveals a unique mechanism underlying the repression of the NLRP3 inflammasome activation.

1. Introduction

The host innate immune system identifies pathogens through recognizing pathogen-associated molecular patterns (PAMPs) by corresponding pattern recognition receptors (PRRs) [1,2]. The PRRs are classified into four main categories, including Toll-like receptors (TLRs) [3], Retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) [4], Absent in melanoma 2 (AIM2)-like receptors (ALRs) [5], and Nucleotide-binding oligomeric domain (NOD)-like receptors (NLRs) [6]. A key process of host innate immune response is the activation of inflammasomes [7,8]. The NLRP3 inflammasome is a best characterized inflammasome, which is crucial for acute and chronic inflammatory responses. It consists of three components, intracellular sensor protein (NACHT, LRR and PYD domains-containing protein 3 NLRP3), adaptor protein (Apoptosis-associated speck-like protein containing a CARD, ASC), and effecter protein (Caspase-1, Casp-1), and regulates maturation and secretion of pro-inflammatory cytokines (interleukin-1β, IL-1β and interleukin-18, IL-18) to initiate multiple signaling pathways and drive inflammatory responses [9–11].

Inflammasome activation plays a key role in host immunity, whereas excessive and uncontrolled activation may damage host by causing infectious, inflammatory, and immune diseases [12,13], and thereby it must be tightly controlled. Although negative regulations are employed by host and pathogen to inhibit the NLRP3 inflammasome [14–16], the mechanisms underlying the repression of systematic NLRP3 inflammasome activation remain largely unknown.

Interleukin-enhanced binding factor 2 (ILF2) and interleukin enhancer binding factor 3 (ILF3) form a heterodimer to regulate the transcription of IL-2 [17]. ILF2 regulates the transcription of ribosomal protein HS4-dependent IL13 in T cells [18], DNA repair [19], and mRNA translation [20,21]. ILF2 is also involved in the regulation of virus replication [22–24] and cancer developments [25–28]. More recent study reveals that ILF2 is an autoantigen in canine systemic autoimmune disease [29].

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https://doi.org/10.1016/j.bbrc.2018.04.087
0006-291X/

This study reveals a role of ILF2 in the repression of the NLRP3 inflammasome activation. By establishing and using a reconstructed NLRP3 inflammasome activation system, we reveal that the NLRP3 inflammasome activation, pro-Caspase-1 cleavage, and pro-Interleukin-1β (pro-IL-1β) maturation are significantly repressed by 4 cellular factors, the proteasome 26S subunit non ATPase 10 (PSMD10), the B-cell lymphoma 2-like protein 10 (BCL2L10), the cathepsin S (CTSS), and the interleukin-enhanced binding factor 2 (ILF2). Further studies demonstrate that ILF2 represses the activation of NLRP3 inflammasome by interacts with the NACHT-associated domain (NAD) of NLRP3 and co-localized with NLRP3 in the cytoplasm of HEK293T cells. Finally, by generating a THP-1 cell line stably expressing ILF2 protein using a lentivirus infection system, we demonstrate that ILF2 represses ATP-induced activation of endogenous NLRP3 inflammasome in macrophages.

2. Materials and methods

2.1. Reagents

Lipopolysaccharide (LPS), adenosine triphosphate (ATP) and phorbol-12-myristate-13-acetate (TPA) were purchased from Sigma. RPMI 1640 medium, Delbecco modified Eagle medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco (Grand Island, NY, USA). The anti-Flag (F3165) and anti-GAPDH (G9295) antibodies were purchased from Sigma, anti-NLRP3 (D2P5E), anti-IL-1β (D3U3E) and anti-caspase-1 (catalog no. 2225) from CST, and anti-ASC (sc-271054) and anti-IL-1β (sc-7884) from Santa Cruz. All mice IgG were purchased from Invitrogen Corporation. Protein G agarose, protease inhibitor Cocktail, acrylamide, protein marker and 0.45 μm PVDF membranes were purchased from GE Corporation, Roche, Shanghai Sangon, Fermentas, and Millipore Corporation respectively.

2.2. Cell lines

HEK293T cells were purchased from ATCC and THP-1 cells was a gift from Professor Sun Bing of Pasteur Institute of Shanghai. THP-1 cells were cultured in RPMI1640 medium containing 10% inactivated (56 °C for 30 min) FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. HEK293T cells were cultured in DMEM supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin. Both THP-1 cells and HEK293T cells were cultured at 37 °C under 5% CO_2.

2.3. Stimulation of THP-1 cells and differentiation into adherent macrophages

THP-1 cells were stimulated with 60 nM TPA for 12−16 h, following which they differentiated into macrophages. The medium was changed to remove the TPA and cells were cultured for another 24 h. The supernatants were assayed for IL-1β by enzyme-linked immunosorbent assay (ELISA) and cells were harvested for RT-PCR or Western-blotting.

2.4. Plasmid construction

NLRP3, ASC, pro-Casp-1 and IL-1β cDNAs were reverse transcribed from the total RNA sequences of TPA-differentiated THP-1 macrophages using specific primers, and sub-cloned into the pcDNA3.1 (+) vector. The UQCRB, PSMD10, BCL2L10, ATN1, CTSS, DDX5 and ILF2 cDNAs similarly obtained from TPA-differentiated THP-1 macrophages were sub-cloned into the Pcmv-Tag2B vector. The pcDNA3.1(+) − 3 × FLAG vector was obtained by inserting the 3 × FLAG tag between the *Nhe*I and *Hind*III restriction sites.

2.5. Lentivirus construction

The GFP protein on the pLenti CMV GFP Puro vector was replaced with a 3X × FLAG sequence and some stringent restriction sites (*Xba*I, *Eco*RV, *Bst*BI, *Bam*HI) were added before the FLAG tag. The ILF2 protein encoded by the pLenti vector was then transfected into HEK293T cells with psPAX2 and PMD2.G proteins via Lipo 2000. The primers used are shown in Table 1. Cells were cultured under standard conditions and after 36 and 60 h of culture, the supernatants containing lentiviruses were collected and centrifuged for 15 min. THP-1 cells were infected with the lentivirus mixed with 4 μg/ml polybrene (Sigma). After 48 h of culture, the transduced cells were selected with 1.5 μg/ml puromycin (sigma). Transgenic protein expression was analyzed by Western-blotting.

2.6. Enzyme-linked immunosorbent assay (ELISA)

The concentration of IL-1β in the culture supernatant was determined using ELISA as described (BD Biosciences, San Jose, CA, USA).

2.7. Western-blotting

HEK293T cells were lysed using the following lyses buffer: 50 mM Tris-HCl, pH 7.5, 300 mM Nacl, 1% Triton-X, 5 mM EDTA and 10% glycerol. TPA-differentiated THP-1 macrophages were lysed using the following lyses buffer: 50 mM Tris-HCl, pH7.5, 150 mM Nacl, 0.1% NP-40, 5 mM EDTA and 10% glycerol. Protein concentration of the lysates was determined by the Bradford method (Bio-Rad, Hercules, CA, USA). The resulting cell lysate was electrophoresed with 8−12% SDS-PAGE gels for 1.5 h. The isolated protein were transferred to a PVDF membrane (Millipore, MA, USA) using a transfer device. The PVDF membranes were blocked with 5% skim milk in PBST buffer with 0.1% Tween 20, followed by successive incubations steps with the primary antibodies and secondary antibody. Specific bands were then visualized using a chromogenic solution and a chemi-luminescence instrument (Fujifilm LAS-4000).

2.8. Co-immunoprecipitation(Co-IP)

HEK293T cells were lysed as described above and the lysates were incubated overnight with murine IgG (Invitrogen) or anti-FLAG antibody (Sigma, F3165). Protein-G (GE Healthcare) was added to the lysates that were incubated for an additional 2 h, washed 3 times with cell lyses buffer, boiled for 10 min with 2X loading buffer, and detected by Western-blotting as described.

2.9. Confocal microscopy

The HEK239T cells transfected with plasmids were cultured for 36 h and treated with 4% paraformaldehyde for 15 min at room temperature. The cells were washed three times with PBS, permeabilized with 0.2% Triton-X100 for 5 min, and washed again with PBS thrice. The cells were blocked for 1 h with PBS buffer containing 5% BSA, washed three times with PBS, incubated overnight with murine monoclonal HA antibodyand Flag antibody at 4 °C, and washed thrice with PBS. This was followed by a 1 h incubation with FITC-conjugated anti-rabbit and dylight 649-conjugated anti-mouse secondary antibodies and washed three times with PBS. The cells were counterstained with DAPI for 5 min and washed three times with PBS. Positively stained cells were visualized and analyzed using a confocal scanning microscope (Fluo View FV1000;

Please cite this article in press as: J. Jin, et al., Interleukin-enhanced binding factor 2 interacts with NLRP3 to inhibit the NLRP3 inflammasome activation, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.04.087