FEBS Letters 589 (2015) 1322–1330

journal homepage: www.FEBSLetters.org

TRIM35 negatively regulates TLR7- and TLR9-mediated type I interferon production by targeting IRF7

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ARTICLE INFO

Article history:
Received 1 November 2014
Revised 6 April 2015
Accepted 13 April 2015
Available online 20 April 2015

Edited by Kazuhiro Iwai

Keywords:
TRIM35
TLR7/9
IRF7
K48-linked ubiquitination
Proteasome-dependent degradation
Feedback regulation

ABSTRACT

Toll-like receptor 7 (TLR7) and TLR9 sense viral nucleic acids and induce type I IFN production, which must be properly controlled to avoid autoimmune diseases. Here, we report the negative regulation of TLR7/9-mediated type I IFN production by TRIM35. TRIM35 expression is induced by TLR7/9 stimulation and then interacts with IRF7, which is the master regulator of type I IFN response. Furthermore, TRIM35 promotes the K48-linked ubiquitination of IRF7 and induces its degradation via a proteasome-dependent pathway. Therefore, TRIM35 is a negative feedback regulator of TLR7/9-mediated type I IFN production due to its ability to suppress the stability of IRF7.

1. Introduction

Following viral infection, the innate immune system utilizes pattern recognition receptors (PRRs) to recognize conserved pathogen-associated molecular patterns (PAMPs). This triggers a series of signaling events that lead to the production of inflammatory cytokines and type I interferons, such as interferon-α (IFN-α) and IFN-β [1–3], which are critical for immunity against viruses. IRF3 and IRF7 are two major transcription factors that induce type I IFN production [4,5]. IRF3 is activated by TLR3 and TLR4, whereas IRF7 is activated by TLR7 and TLR9 [6,7]. Although IRF3 expression is constitutive, the expression of IRF7 is completely dependent on IFN-α/β signaling and is affected by viral infection and IFNs [5]. Therefore, IRF7 expression must be properly controlled to avoid excessive activation of the immune response upon viral infection.

Despite the importance of IRF7 for host defense against viral infection, the regulation mechanism of IRF7 remains unclear. IRF7 has a very short half-life and maintains a low steady-state level, suggesting that IRF7 may undergo ubiquitin (Ub)-mediated degradation via the proteasome pathway [4]. At present, several Ub ligases (E3) that target IRF7 for degradation have been reported. The Kaposi's sarcoma-associated herpes virus (KSHV) immediate-early lytic cycle trigger protein RTA interacts with IRF7, resulting in a powerful inhibitory effect on IRF7 by targeting it for degradation in a proteasome-dependent manner [8]. In addition, RAUL (RTA-associated ubiquitin ligase), a HECT domain ubiquitin (Ub) E3 ligase, limits type I IFN production by directly catalyzing lysine 48-linked polyubiquitination of both IRF7 and IRF3, followed by proteasome-dependent degradation [7]. These studies reveal that a "brake mechanism" for type I IFN production exists and that IRF7 should be tightly regulated by the Ub-proteasome pathway to maintain homeostasis.

Tripartite motif TRIM35 (also termed MAIR and Hls5) is a member of the TRIM family of single-protein E3 ligases and has an apoptosis-inducing function [9]. In addition, TRIM35 is a candidate tumor suppressor due to its inhibition of cell growth, clonogenicity, and tumorigenicity [10]. In this study, we demonstrated that TRIM35 expression is highly induced by TLR7 and TLR9 ligands. Moreover, TRIM35 knockdown promoted type I IFN production

Author contributions: Y.M.W. and B.S. conceived and supervised the study; Y.M.W., B.Y. and B.S. designed experiments; Y.M.W., S.S.Y. and Y.W. performed experiments; Y.M.W., H.Y.Z. and Q.S.L. analyzed data; Y.M.W. and B.S. wrote the manuscript; Y.M.W., S.S.Y. and B.S. made manuscript revisions.

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http://dx.doi.org/10.1016/j.febslet.2015.04.019
0014-5793/

triggered by TLR7 and TLR9 stimulation. This function was further confirmed by the infection of host cells with the RNA virus vesicular stomatitis virus (VSV) and the DNA virus herpes simplex virus type 1 (HSV-1). The mechanism uncovered in this study involves TRIM35 interacting with IRF7 to induce IRF7 degradation via the K48-linked ubiquitin-proteasome pathway. These results suggest that TRIM35 acts as a negative feedback regulator of TLR7- and TLR9-triggered signaling by targeting IRF7.

2. Results

2.1. Upregulation of TRIM35 expression by TLR7 and TLR9 stimulation

TLRs are type I transmembrane proteins, which mediate the recognition of their respective PAMPs [11]. TLR signaling must be well controlled to maintain an appropriate immune response. A number of TRIM family members have been reported to regulate TLR signaling pathway [12]. For example, our lab has identified that TRIM30α is involved in a negative feedback regulation of TLR4-induced inflammatory responses [13]. Besides, TRIM21 interacts with IRF3 and promotes the ubiquitination and degradation of IRF3 to limit IFN-β induction downstream of TLR3, TLR4 and RIG-I [14,15].

Considering the important roles of TRIM family in TLR signaling regulation, we determined to find other members involved in this pathway. So we knocked down the expression of several TRIM family members in RAW264.7 cells and treated these cells with TLR7 ligand Resiquimod (R848) and the TLR9 ligand unmethylated DNA with CpG motifs. Interestingly, we found that TRIM35 knockdown dramatically enhanced IFN-β induction downstream of R848 and CpG-B DNA stimulation in comparison to other TRIM molecules (Fig. S1). To further verify the function of TRIM35, RAW264.7 cells were stimulated for different periods of time with R848 and CpG-B DNA, the results showed that TRIM35 expression was highly induced on both mRNA and protein levels (Fig. 1). These results suggest that TRIM35 may play an important role in regulation of TLR7/9 signaling pathways.

2.2. Knockdown of TRIM35 promotes type I IFN and ISGs production

To determine whether TRIM35 is involved in the regulation of TLR7 and TLR9 signaling, we utilized the knockdown approach to examine the potential role of TRIM35 in type I IFN production. Three pairs of siRNA molecules for TRIM35 (T1, T2 and T3) and a control siRNA (SC) were designed. After transfection of these siRNAs, T3 was found to efficiently inhibit TRIM35 expression in HEK293T cells transfected with TRIM35 (Fig. 2A). Furthermore, we also silenced endogenous TRIM35 expression in RAW264.7 cells and obtained similar results (Fig. 2B). We next selected siRNA T1 and T3 to knock down TRIM35 in RAW264.7 cells and then stimulated these cells with R848 or CpG-B DNA for 6 h. The data showed that TLR7/9 stimuli resulted in a much higher level of type I IFN and ISGs (IP-10 and Isg56) in cells transfected with the TRIM35-specific siRNA (T3) than in those transfected with the control siRNA or T1 (Fig. 2C). TLR7 and TLR9 also recognize RNA virus VSV and DNA virus HSV-1, respectively. So we silenced TRIM35 expression and infected the cells with VSV or HSV-1 for 6 h. Virus infection significantly promoted type I IFN and ISGs induction when TRIM35 was knocked down, which was consistent with R848 or CpG-B DNA stimulation (Fig. 2D). By contrast, we detected no difference in the mRNA expression or secreted protein level of TNF-α, which is a downstream cytokine induced by transcription factor NF-κB (Fig. 2E and F). These data indicate that TRIM35 plays a negative role in regulating TLR7- and TLR9-triggered type I IFN and ISGs production.

2.3. Knockdown of TRIM35 promotes IFN-β and ISGs production in pDCs

Plasmacytoid DCs (pDC) selectively express endosomal TLR7 and TLR9. So we determined to investigate the function of TRIM35 in pDCs. We obtained pDCs by culturing bone marrow cells in conditioned media containing Flt3 ligand (Flt3L). After 6–8 days, Flt3L-DCs, which contain largely pDCs, were collected and transfected with control siRNA or TRIM35-specific siRNA T3 for 24 h. Two classes of synthetic oligodeoxynucleotides (ODNs), CpG-A and CpG-B both activate TLR9, but CpG-A induces IFN-α production much more efficiently than CpG-B in pDCs [16,17]. So the TLR9 ligand CpG-A DNA was used to stimulate Flt3L-DCs. The knockdown and induction effect of TRIM35 were measured at protein level. The results indicated that endogenous TRIM35 expression has been reduced by siRNA T3 in comparison to the controls and CpG-A DNA obviously induced TRIM35 expression (Fig. 3A and B). Type I IFN, pro-inflammatory cytokines and ISGs in the above DCs were determined by Q-PCR analysis. We observed that TRIM35 knockdown promoted the levels of type I IFN and IP-10 than that treated with control siRNA after CpG-A DNA stimulation.

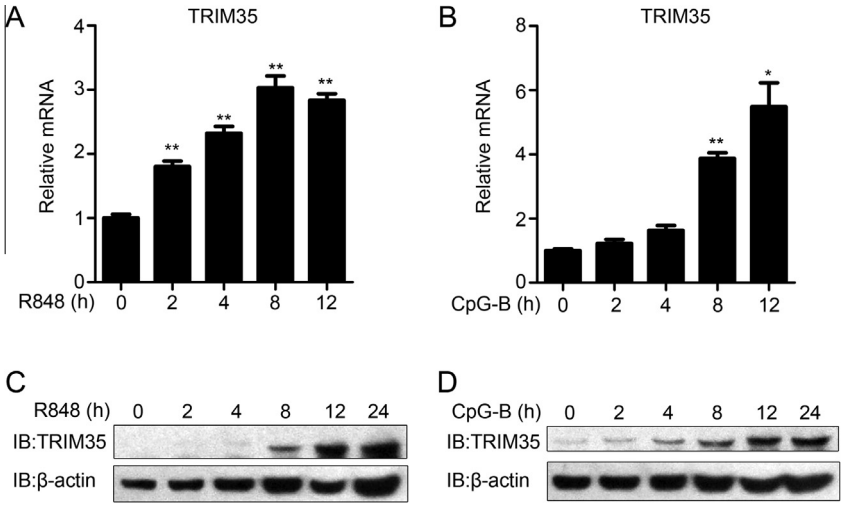

Fig. 1. TRIM35 is induced by R848 and CpG-B DNA. (A–D) Real-time PCR (A and B) or immunoblot analysis (C and D) of TRIM35 in RAW264.7 cells treated with R848 (0.1 μg/ml) or CpG-B DNA (1 μg/ml) for various times. β-Actin served as a loading control throughout the experiment. The mRNA results are relative to those of the untreated cells. The values represent the mean ± S.E.M. of more than three experiments. $^{*}P < 0.05$ and $^{**}P < 0.01$.