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Identification of tripartite motif-containing 22 (TRIM22) as a novel NF-κB activator

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ABSTRACT

Increasing evidence suggests that TRIM family proteins may play important roles in the regulation of innate immune signaling pathways. Here we report TRIM22 is involved in the activation of NF-κB. It was found that overexpression of TRIM22 could dose-dependently activate NF-κB as demonstrated by reporter gene assay and electrophoretic mobility shift assay, but had no effect on the activity of other transcription factors, including NF-AT, AP-1, C/EBP and IRFs. Further study showed that both the N-terminal RING domain and C-terminal SPRY domain were crucial for TRIM22-mediated NF-κB activation. Moreover, our results revealed that TRIM22 overexpression could significantly induce the secretion of pro-inflammatory cytokines by human macrophage cell line U937 in an NF-κB-dependent manner. These data suggested that TRIM22 was a positive regulator of NF-κB-mediated transcription.

1. Introduction

Tripartite motif (TRIM) family proteins are involved in diverse cell processes, including apoptosis, differentiation and transcriptional regulation [1]. Recent studies show that some TRIM proteins, such as TRIM5α, TRIM19, TRIM22 and TRIM28, have antiviral activity [2–6]. Interestingly, there is growing evidence that some TRIM proteins, especially those induced by interferons (IFNs), play an important role in regulating signaling pathways involved in innate immune response [7]. For example, TRIM25 is essential for RIG-I-mediated antiviral activity [8]; TRIM21 is involved in the regulation of some pro-inflammatory cytokines through modulating multiple interferon regulatory factors (IRFs) [9,10]; and TRIM19 has been reported to negatively regulate NF-κB activity through recruiting NF-κB to nuclear bodies [11].

It is well established that regulation of immune signaling is crucial for antiviral and inflammatory responses. Several transcription factors are reported to be critical for regulating immune signaling, including nuclear factor-κB (NF-κB), activator protein-1 (AP-1), nuclear factor of activated T cells (NF-AT), CCAAT/enhancer binding protein (C/EBP) and IRFs [12–16]. Of these transcription factors, NF-κB receives particular attentions. Many studies have shown that NF-κB plays important roles in immune regulation and inflammation through the induction of a large set of downstream genes, including cytokines, chemokines, adhesion molecules and effectors [17].

TRIM22 was originally identified as an IFN-inducible protein in Daudi cells and found to possess antiviral activity against HIV [4]. Our previous data demonstrated that TRIM22 was one of the most strongly induced TRIM family molecules in response to IFNs stimulation in HepG2 cells and could inhibit HBV replication efficiently in a RING-domain-dependent manner [5]. Additionally, TRIM22 was identified as a RING finger E3 ubiquitin ligase in our previous study [18], and its E3 ligase activity was reported to be crucial for its antiviral activity against encephalomyocarditis virus [19]. Furthermore, TRIM22 was also revealed to be implicated in some inflammatory diseases, such as systemic lupus erythematosus (SLE) [20]. In consideration of the importance of TRIM22 in both antiviral responses and inflammatory diseases, we sought to investigate whether the IFN-inducible TRIM22 could contribute to the regulation of immune signaling in this study.

We found that TRIM22 could dose-dependently activate NF-κB, but not other transcription factors, such as AP-1, NF-AT, C/EBP and IRFs. Both N terminal RING domain and C terminal SPRY domain was crucial for the TRIM22-mediated activation of NF-κB. Further study showed that TRIM22 expression could induce the production of pro-inflammatory cytokines in U937, which could be blocked by NF-κB inhibitor PDTC.

2. Materials and methods

2.1. Plasmids

Plasmids expressing myc-tagged TRIM22 and its mutants were described in our previous study [5,18]. The reporter plasmids pNF-κB-Luc, pAP-1-Luc, pNF-AT-Luc, pC/EBP-Luc and pIRF-E-Luc containing firefly luciferase gene were obtained from Stratagene

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(Stratagene, La Jolla, CA). The plasmid pRL-SV40 containing *Renilla* luciferase gene was purchased from Promega (Promega, Madison, WI).

2.2. Cell culture and transfection

Human embryonic kidney cells HEK293 were maintained in DMEM (Gibco, UK) supplemented with 10% fetal calf serum (FCS, Gibco, UK) and 100 U/ml penicillin and 100 μg/ml streptomycin. U937 cells were maintained in RPMI 1640 medium (Gibco, UK) supplemented with 10% FCS and 100 U/ml penicillin and 100 μg/ml streptomycin. 293 cells were transfected with indicated plasmids by the calcium phosphate precipitation method. For the transfection of U937 cells, 1×10^6 cells were resuspended in 100 μl of Cell Line Nucleofector Solution C (Amaxa GmbH, Köln, Germany) and nucleofected with 2 μg of indicated plasmids.

2.3. Luciferase assays

Luciferase reporter plasmids were transfected into cells together with plasmids encoding TRIM22 or its mutants. pRL-SV40 was co-transfected in each experiment as an internal control for transfection. At 24 h after transfection, the cells were harvested with the addition of cell lysis buffer and the luciferase assay was performed according to the manufacturer's instructions (Promega, Madison, WI). The luciferase activity was calculated as percentage of the empty pcDNA vector and the values were normalized to the activity of *Renilla* luciferase in the cell lysates.

2.4. Electrophoretic mobility shift assay (EMSA)

Nuclear protein extracts were made as described previously [21]. A total of 8 μg of nuclear proteins was preincubated on ice with 2 μg of poly(deoxyinosine–deoxycytosine) as an unspecific competitor and 1 μg of sonificated sperm DNA in band shift buffer (50 mM Tris, 150 mM KCl, 5% glycerol, 10 mM $MgCl_2$ and 0.1% NP-40, 5 mM EDTA, 2.5 mM DTT) for 15 min. Biotin-labeled oligonucleotides containing the NF-κB binding sequence (5′-GGGGACTTTCCC-3′) were then added in a total volume of 20 μl, incubated on ice for 20 min, and loaded onto 6% native polyacrylamide gels in 0.5× Tris–borate–EDTA buffer. The gels were blotted on nylon membrane, and the blot was cross-linked by UV irradiation. Biotin-labeled probe was detected by a Lightshift Chemiluminescent EMSA kit (Pierce, Rockford, IL) according to the manufacturer's recommendations.

2.5. Immunofluorescence microscopy

293 cells were transfected with plasmids expressing myc-tagged TRIM22 and its mutants. At 48 h after transfection, cells on coverslips were fixed in 4% formaldehyde for 10 min, permeabilized with 0.2% Triton X-100, 1% BSA in PBS for 10 min, and blocked with 1% BSA in PBS for 1 h at room temperature. Cells were then incubated with anti-myc antibody (9E10, 1:500 dilution) (Santa Cruz, CA) overnight at 4 °C, followed by appropriate secondary antibodies conjugated with fluorescein isothiocyanate (FITC) (eBiosicences, San Diego, CA). After stained with 4′,6-diamidino-2-phenylindole (DAPI) (Santa Cruz, CA), cells were examined under a confocal laser-scanning microscope (Leica, Germany).

2.6. ELISA

The concentrations of TNF-α, IL-6, IL-1β and IL-10 in the cell culture supernatants of U937 were determined by commercially available ELISA Kits (eBiosicences, San Diego, CA).

2.7. Statistics

The data represent the means ± SD of three independent experiments performed in triplicate. Statistical analysis was performed using Student's *t* test. A $P < 0.05$ was considered to be significant.

3. Results

3.1. TRIM22 selectively activates NF-κB

To investigate the possible role of TRIM22 in the regulation of immune signaling, we examined the effect of TRIM22 expression on the activity of several transcription factors, including NF-κB, AP-1, NF-AT, C/EBP and IRFs, by reporter gene assay. As shown in Fig. 1A, overexpression of TRIM22 could activate NF-κB in a dose-dependent manner, while had slight or no effect on the activity of other transcription factors, suggesting that TRIM22 could selectively activate NF-κB. To further investigate the effect of TRIM22 on NF-κB activity, we performed EMSA by incubating nuclear extracts from empty vector or TRIM22 expression plasmid (pTRIM22)-transfected cells with biotin-labeled NF-κB consensus oligonucleotide. The EMSA results also showed that TRIM22 could dose-dependently activate NF-κB (Fig. 1B).

3.2. The contribution of N-terminal RING domain and C-terminal SPRY domain to the TRIM22-mediated NF-κB activation

TRIM22 contains a cluster of a RING-finger domain, a B box/coiled coil domain (B Box/CCD) and a SPRY domain [1]. Our previous investigation demonstrated that TRIM22 was a RING finger E3 ligase [18], and both its RING and SPRY domain were crucial for its anti-HBV activity [5]. We next investigated the role of RING or SPRY domain in TRIM22-mediated activation of NF-κB. Results showed that its RING domain deletion mutant (TRIM22-ΔRING) and SPRY domain deletion mutant (TRIM22-ΔSPRY) lost the ability to activate NF-κB (Fig. 2B). We also investigated the intracellular localization of wild-type or mutant TRIM22 in 293 cells using immunofluorescence staining. It was found that wild-type TRIM22 and TRIM22-ΔRING were predominantly localized in the nucleus, while TRIM22-ΔSPRY was localized exclusively in the cytoplasm, consistent with what we reported in HepG2 cells [5].

The conserved cysteine residue at position 15 is critical for RING domain-mediated biological functions [4,5]. To further investigate the role of RING domain in the TRIM22-mediated NF-κB activation, the 15th cysteine of RING domain was substituted into the alanine (TRIM22-C15A) (Fig. 2A). Just as the TRIM22-ΔRING, the TRIM22-C15A was also localized in the nuclei of 293 cells (Fig. 2C) and lost the ability to activate NF-κB (Fig. 2B), further demonstrating the crucial role of the nuclear-located RING domain in TRIM22-mediated NF-κB activation.

3.3. TRIM22 induces the secretion of pro-inflammatory cytokines

It is well documented that NF-κB plays a central role in the inflammatory processes by augmenting the expression of inflammatory genes. We therefore investigated whether TRIM22 itself could induce the production of pro-inflammatory cytokines by macrophages. We transfected human macrophage cell line U937 with plasmids expressing TRIM22 or its mutants. Post-transfection (12 h), cells were differentiated with phorbol myristate acetate (PMA) for another 24 h. Cell supernatants were then assayed for the pro-inflammatory cytokines, including TNF-α, IL-6 and IL-1β. As shown in Fig. 3, we found that the secretion of TNF-α, IL-6 and IL-1β from pTRIM22-transfected U937 cells was significantly higher than that from the control groups