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Characterization of TRIM62 as a RING finger E3 ubiquitin ligase and its subcellular localization

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ABSTRACT

TRIM62, also named DEAR1, is a member of the TRIM/RBCC family, which includes proteins with conserved RING finger, B-box and coiled-coil domains. Several reports have identified a role for this family in cancer, retroviral infection and innate immunity. In this study, the E3 ubiquitin ligase activity and subcellular localization of TRIM62 were characterized. TRIM62, in association with the E2 enzyme UbcH5b, was found to catalyze self-ubiquitination in vitro, a process that required an intact RING finger domain. A ubiquitination assay performed in HEK293T cells further confirmed the E3 ubiquitin ligase activity and self-ubiquitination activity of TRIM62 and the requirement of the RING finger domain. Importantly, the treatment of HEK293T cells with a proteasome inhibitor stabilized poly-ubiquitinated TRIM62, indicating that self-ubiquitination promoted the proteasomal degradation of TRIM62. Additionally, TRIM62 and its two mutants were distinctly localized in the cytoplasm in both HEK293T and HeLa cells. Collectively, our data indicate that TRIM62, a cytoplasmic protein, is a RING finger domain-dependent E3 ubiquitin ligase that catalyzes self-ubiquitination both in vitro and in vivo.

1. Introduction

TRIM (tripartite motif) family proteins, also known as RBCC (RING finger, B-box and coiled-coil) proteins, are defined by the presence of a highly conserved RING (really interesting new gene) finger, one or two B-box domains and a coiled-coil region. Compared with the N-terminus, which has a fixed composition, TRIM proteins have diverse C-terminal domain compositions and equally diverse subcellular localizations and functions [1,2]. To date, approximately 100 human TRIM genes have been identified, and most of these genes originate from a single locus on chromosome 11 [3]. Due to the N-terminal RING finger domain, which predominantly contributes to E3 ubiquitin ligase activity, TRIM/RBCC proteins have been implicated in ubiquitination and are proposed to be one sub-family of E3 ligases (E3s). The members of this family participate in various cellular processes, such as proliferation, differentiation, oncogenesis, apoptosis, intracellular trafficking and innate cellular responses to retroviral infection. Moreover, defects in some TRIM proteins have been shown to be associated with some human diseases [2,4,5].

Ubiquitin is a highly conserved 76-amino acid polypeptide with a difference of only three amino acids between the human and yeast homologs [6]. Ubiquitination is an enzymatic, ubiquitin-mediated post-translational modification process that participates in a number of cellular processes, including endocytosis, DNA repair, transcription, chromatin remodeling, protein transport, inflammation and antigen processing [7–10]. Ubiquitin is conjugated to the substrate protein via a three-step mechanism. First, ubiquitin is processed by a concerted two-step reaction that results in a high-energy thioester linkage between ubiquitin and a single conserved ubiquitin-activating enzyme (E1). The activated ubiquitin is then transferred, through trans-acylation, to one of several ubiquitin-conjugating enzymes (Ubcs or E2s). Last, through the interaction between E2 and a large number of E3s, the ubiquitin molecules are attached to the ε-amino group of the substrate's Lys residues, thus creating a reversible isopeptide bond [11,12]. Subsequent ubiquitin moieties are added through the formation of isopeptide bonds between an internal Lys residue and the C-terminal Gly residue [13]. The poly-ubiquitinated forms can be divided into eight different types. Seven of these types are characterized based on the linkage of one of the seven internal Lys residues in one ubiquitin molecule to the C-terminal diglycine of the next molecule. In the eighth type, the so-called linear ubiquitin chain, the linkage occurs between the N-terminal amino group of Met on a ubiquitin molecule that is conjugated to a target protein and the C-terminal carboxy group of the incoming ubiquitin moiety [14].

E3s confer specificity to ubiquitination by recognizing target substrates and mediating the transfer of ubiquitin to the substrates [15]. These ligases are categorized into two main families:

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RING and HECT (homologous to the E6-AP C-terminus) domain-containing E3s. The E3s that contain the RING domain, which is always found at the N-terminus, serve as scaffolds that facilitate the direct transfer of ubiquitin from E2 to the target protein. In contrast, the HECT domain always occurs at the C-terminus, and these E3s transfer from E2 to an active-site Cys within the HECT domain, forming an E3-ubiquitin thioester complex. There are ~600 RING finger and ~30 HECT ligases in humans. In addition, some smaller families of ligases, such as the U-box, plant homology domain and zinc finger ligases, are known [16,17].

TRIM62 (tripartite motif containing 62) is a member of the TRIM/RBCC family of proteins. This protein is also known as DEAR1 (ductal epithelium-associated RING chromosome 1) and was identified as a dominant regulator of acinar morphogenesis in the mammary gland and an independent predictor of local recurrence-free survival in early-onset breast cancer [18]. Initially, TRIM62 was discovered to be associated with the entry and release of MLV and HIV-1 in HEK293 cells [19], and it has recently been found to function in the TRIF branch of the TLR4 signaling pathway. TRIM62 knockdown in primary macrophages leads to a defect in TRIF-mediated late NF-κB, AP-1 and interferon production after lipopolysaccharide challenge [20]. Due to the presence of the RING finger domain, TRIM62 may have a similar function as other TRIM/RBCC family members as an E3 ubiquitin ligase. To identify the E3 ubiquitin ligase activity of TRIM62, we performed in vitro ubiquitination assays and found that TRIM62 could function as an E3 ubiquitin ligase and catalyze self-ubiquitination. We further verified this activity of TRIM62 through ubiquitination assays in HEK293T cells. Both the in vitro and in vivo ubiquitination assays showed that TRIM62 ubiquitination was dependent on the presence of the RING finger domain, suggesting that TRIM62 is a RING finger E3 ubiquitin ligase. In addition, we demonstrated that TRIM62 was localized to the cytoplasm in both HEK293T and HeLa cells.

2. Materials and methods

2.1. Plasmid constructs and mutagenesis

Human TRIM62 with a Flag epitope added to its C-terminus was amplified from pCMV6-XL4-TRIM62 (Origene, Rockville, MD) using two primers, 5′-CGGCGATCTGCCATGGCGTGCAGCCTCAAGGAC-3′ (Forward) and 5′-GGCGAATTCCTACTTGTCGTCATCGTCTTTGT AGTCGATGCGGACGGTGTTGATC-3′ (Reverse). The PCR fragment was then cloned into pcDNA3.1(+) (Invitrogen, Carlsbad, CA) using *Bam*HI and *Eco*RI sites to create pTRIM62-F. The RING finger domain of TRIM62 was deleted (pΔRING-F) using the forward primer 5′-GGCAGATCTATGGCGTGCAGCCTCAAGGACGAGCTGCTGGCCGAG CCCGCGCTGGCGCC-3′ (Forward) and the previously mentioned reverse primer. A Cys-to-Ala point mutation in the eleventh amino acid of the RING domain (pC11A-F) was created using the KOD-Plus-Mutagenesis Kit (TOYOBO, Osaka). The fragments containing wild-type TRIM62, TRIM62-ΔRING and TRIM62-C11A sequences were cloned into pMAL-c4E (New England Biolabs, Beverly, MA), a vector designed to produce maltose-binding protein (MBP) fusions, to create pMBP-TRIM62, pMBP-TRIM62-ΔRING and pMBP-TRIM62-C11A, respectively, and pEGFP-C1 (Clontech, Palo Alto, CA) to express GFP-linked proteins. The ubiquitin gene with the HA epitope at its N-terminus was amplified from the cDNA of HeLa cells using two primers: 5′-CGCGGATCCGACATGGACTACCCA-TACGATGTTCCAGATTACGCTCAGATTTTCGT-3′ (Forward) and 5′-GCGCGGCCGCTTAACCACCACGAAGTCTCAACACA-3′ (Reverse). The PCR fragment was cloned into pcDNA3.1(+) using *Bam*HI and *Not*I sites to create pHA-Ub.

2.2. Antibodies

The antibodies were purchased from companies: anti-HA and anti-Flag (Sigma–Aldrich, St. Louis, MO), anti-ubiquitin and goat anti-mouse IgG (HRP-conjugated) (Cell Signaling Technology, Danvers, MA), anti-MBP (New England Biolabs).

2.3. Cell culture and transfection

Human embryonic kidney cells (HEK293T) and human adenocarcinoma cervical cells (HeLa) were maintained in Dulbecco's modified Eagle's medium (Gibco, Auckland, NZ) supplemented with 10% fetal calf serum (Gibco). The cells were transfected with the indicated plasmids using X-tremeGENE HP DNA Transfection Reagent (Roche Applied Science, Indianapolis, IN) or Lipofectamine™ 2000 CD Reagent (Invitrogen) according to the manufacturer's protocols.

2.4. In vitro ubiquitination assay

The plasmids pMAL-c4E, pMBP-TRIM62 and pMBP-TRIM62-ΔRING/C11A were separately transformed into *E. coli* strain K12 TB1 (New England Biolabs). The following procedures were then conducted according to the product's instruction manual to obtain purified MBP and MBP-TRIM62/ΔRING/C11A proteins. First, 2 μg of purified MBP or MBP-TRIM62 protein was incubated with 20 μl of nuclease-treated rabbit reticulocyte lysate (Promega, Madison, WI) and 1 μg of ubiquitin (BostonBiochem, Cambridge, MA) in 50 mM Tris–HCl (pH 7.4), 4 mM ATP (Fermentas, Waltham, MA), 5 mM $MgCl_2$, 2 mM DTT, 10 μM MG132 (Sigma–Aldrich) and ddH_2O to a final volume of 60 μl at 37 °C for 120 min. To determine which E2 enzyme participated in TRIM62-mediated ubiquitination, 2 μg MBP-TRIM62, 1 μg ubiquitin, 100 ng E1 enzyme (BostonBiochem), different E2 enzymes (BostonBiochem), 20 mM Tris–HCl (pH 7.5), 5 mM ATP, 5 mM $MgCl_2$, 2 mM DTT and ddH_2O to a final volume of 50 μl were mixed together and incubated at 37 °C for 120 min. After the reaction, the mixture was incubated with amylose resin (New England Biolabs) at 4 °C for 4 h. The precipitates were washed at least four times with column buffer (20 mM Tris–HCl, 200 mM NaCl, 1 mM EDTA and 1 mM DTT) and analyzed by Western blotting using the indicated antibodies, as previously described [21].

2.5. In vivo ubiquitination assay

We performed a two-step immunoprecipitation assay to evaluate the ubiquitination activity of TRIM62. After the indicated TRIM62 recombinant plasmids were co-transfected with or without HA-tagged ubiquitin for 48 h, the cells were harvested and lysed with lysis buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 1% TritonX-100, 1 mM EDTA, 10 mg/ml aprotinin, 10 mg/ml leupeptin and 1 mM phenyl-methyl-sulfonyl fluoride). The cell lysates were first immunoprecipitated with the indicated antibody and 30 μl of pretreated (washed three times with lysis buffer) protein G-Sepharose (GE Healthcare, Piscataway, NJ) for 4 h. The immunoprecipitates were then re-extracted in lysis buffer containing 1% SDS and denatured by heating at 95 °C for 5 min. The supernatants were diluted with regular lysis buffer until the concentration of SDS was reduced to 0.1% [22], and the diluted supernatants were re-immunoprecipitated with Anti-FLAG M2 Affinity Gel Beads (Sigma–Aldrich). After washing with TBS buffer, the immunoprecipitates were eluted with 3× FLAG peptide (150 ng/μl) and analyzed by Western blotting with the indicated antibodies. For protein stability experiments, the proteasome inhibitor MG132 (20 μM) was added to the culture medium for the last 6 h of a

48 h HEK293 cell transfection. The cells were then harvested and treated according to the procedures described above.

2.6. Immunofluorescence microscopy

HEK293T and HeLa cells transfected with the indicated recombinant TRIM62 or its mutants were plated in a glass-bottom dish. At 48 h post-transfection, the cells were fixed with 4% formaldehyde for 15 min, washed with PBS, permeabilized with PBS containing 0.3% TritonX-100 for 5 min and blocked with PBS containing 1% BSA and 10% FBS for 1 h at room temperature (RT). The cells were then incubated with blocking buffer containing the indicated antibodies (2 μg/ml) for 2 h at RT and Alexa Fluor® 488 Goat Anti-Mouse IgG or Alexa Fluor® 594 Goat Anti-Mouse IgG (Invitrogen) (diluted 1/1000 in binding buffer) for 1 h at RT. The cells were then stained with Hoechst (Beyotime, Shanghai) for 25 min and mounted in Prolong Antifade (Beyotime); the GFP-expressing cells were fixed and stained to visualize nuclei. The cells were examined with a Perkin Elmer Ultraview Spinning disk confocal microscope using the 60× plan objective.

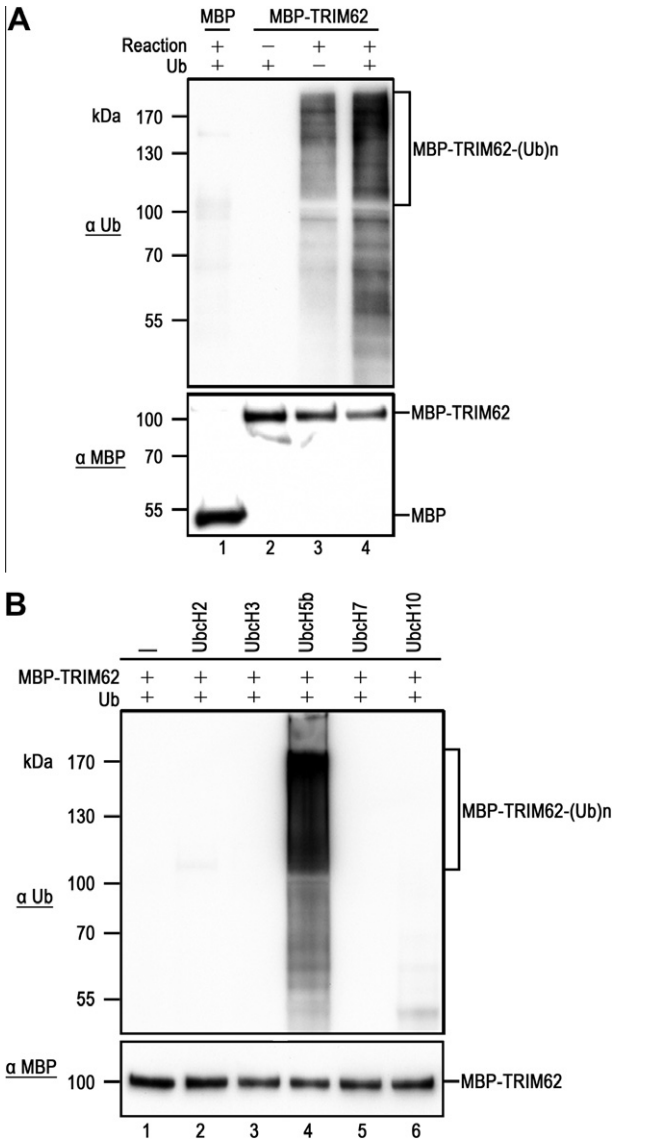

Fig. 1. In vitro self-ubiquitination of TRIM62. (A) Purified MBP or MBP-TRIM62 was incubated with reticulocyte cell lysate and ubiquitin (lanes 1 and 4) or MBP-TRIM62 was incubated with ubiquitin alone or reticulocyte cell lysate (lanes 2 and 3) at 37 °C for 120 min. (B) Purified MBP-TRIM62 was incubated with ubiquitin, recombinant E1 enzyme and different recombinant E2 enzymes (UbcH2, UbcH3, UbcH5b, UbcH7 and UbcH10) at 37 °C for 120 min. (A and B) After the reaction, the mixture was incubated with amylose resin beads at 4 °C for 4 h. Then, MBP or MBP-TRIM62 immobilized on the beads was washed to remove the reaction mixture and solubilized in SDS solution. MBP and MBP-TRIM62 were analyzed by Western blotting using an anti-Ub antibody to detect ubiquitinated MBP-TRIM62 (upper panel) and anti-MBP to monitor both MBP and MBP-TRIM62 (lower panel).

3. Results

3.1. TRIM62 acts as an E3 ubiquitin ligase in vitro

Rabbit reticulocyte lysate was used to evaluate the ubiquitination activity of TRIM62 because it contains most of the E1s and various E2s and is capable of initiating the ubiquitination reaction under certain conditions. The MBP and MBP-TRIM62 proteins expressed in *E. coli* strain K12 TB1 were purified using amylose resin and incubated with or without reticulocyte lysate in the presence or absence of ubiquitin. After binding to the amylose resin beads, the precipitates were analyzed by Western blotting using anti-Ub and anti-MBP antibodies. As shown in Fig. 1A, MBP-TRIM62 produced multi-ubiquitinated products in the presence of the lysate (lane 3), whereas the controls were negative (lanes 1 and 2). Furthermore, excess ubiquitin improved the generation of multi-ubiquitinated products (lane 4). These results indicated that TRIM62 might function as an E3 ubiquitin ligase.

However, it is possible that MBP-TRIM62 may be ubiquitinated by other E3s in the reticulocyte lysate. To exclude such a possibil-

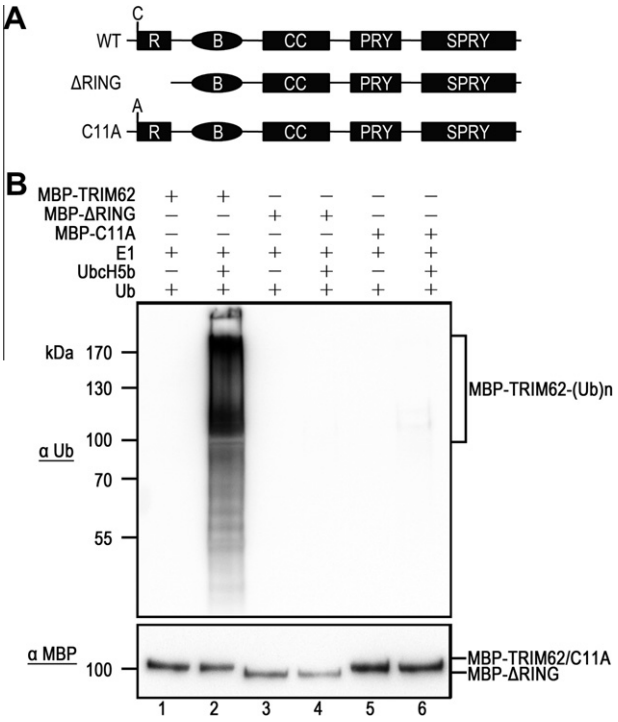

Fig. 2. The RING finger domain-dependent self-ubiquitination of TRIM62 in vitro. (A) Schematic representation of wild-type TRIM62, the RING domain deletion mutant (ΔRING) and the point mutant (C11A). (B) Purified MBP-TRIM62 (lanes 1 and 2), MBP-ΔRING (lanes 3 and 4) and MBP-C11A (lanes 5 and 6) were incubated with recombinant E1 enzyme and ubiquitin in the presence or absence of recombinant E2 enzyme (UbcH5b) at 37 °C for 120 min. After the reaction, the mixture was incubated with amylose resin beads at 4 °C for 4 h. The protein immobilized on the beads was washed to remove the reaction mixture and solubilized in SDS solution. MBP-TRIM62, MBP-ΔRING and MBP-C11A were analyzed by Western blotting using an anti-Ub antibody to detect ubiquitinated TRIM62/ΔRING/C11A (upper panel) and anti-MBP to monitor these three proteins (lower panel).

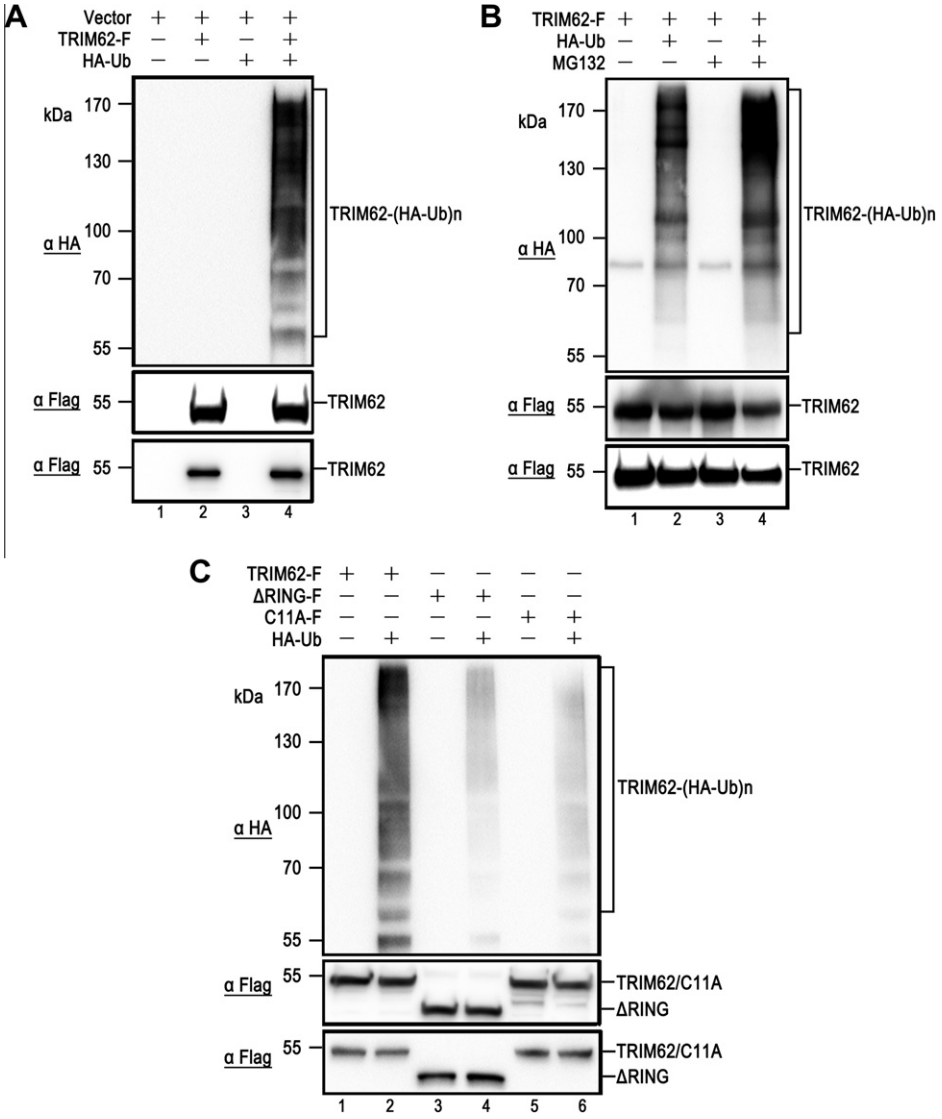

Fig. 3. In vivo self-ubiquitination of TRIM62. (A) TRIM62-F was co-transfected with or without HA-tagged ubiquitin (lanes 4 and 2) and empty vector was co-transfected with or without HA-tagged ubiquitin (lanes 3 and 1) in HEK293T cells for an in vivo ubiquitination assay. The cells were harvested at 48 h post-transfection. (B) HEK293T cells co-transfected with TRIM62-F with or without HA-tagged ubiquitin. After 42 h, the proteasome inhibitor MG132 was added to (lanes 3 and 4) or omitted from (lanes 1 and 2) the culture medium, and the cells were harvested at 48 h. (A and B) The cells harvested as described above were lysed for use in a ubiquitination assay using the two-step immunoprecipitation strategy. First, the cell lysates were immunoprecipitated with anti-Flag antibody. The immunoprecipitates were then denatured and re-immunoprecipitated with 'Anti-FLAG M2 Affinity Gel Beads' and analyzed by Western blotting using an anti-HA antibody to detect ubiquitinated TRIM62 (upper panel) and an anti-Flag antibody to detect Flag-tagged TRIM62 in the immunoprecipitates (middle panel) and cell lysates (Input, lower panel). (C) HEK293T cells were co-transfected with TRIM62-F, ΔRING-F and C11A-F with or without HA-tagged ubiquitin. The cells were harvested at 48 h post-transfection for the ubiquitination assay using the two-step immunoprecipitation strategy (described above). Ubiquitinated TRIM62/ΔRING/C11A was detected by the anti-HA antibody (upper panel) and Flag-tagged TRIM62/ΔRING/C11A was detected by the anti-Flag antibody in immunoprecipitates (middle panel) and cell lysates (Input, lower panel).

ity, MBP-TRIM62 was incubated with recombinant E1 enzyme and different E2 enzymes (UbcH2, UbcH3, UbcH5b, UbcH7 and UbcH10) in the presence of ubiquitin. As shown in Fig. 1B, MBP-TRIM62 displayed ubiquitination activity only in the presence of UbcH5b. These results further suggested that TRIM62 functions as an E3 ubiquitin ligase in cooperation with UbcH5b, one type of E2 ligase.

3.2. An intact RING finger domain of TRIM62 is essential for its self-ubiquitination

To evaluate the importance of the RING finger domain of TRIM62 in its E3 ubiquitin ligase activity, TRIM62 with a RING domain deletion (ΔRING) and a point mutant (C11A), a Cys-to-Ala mutation to reduce the stability of the RING domain, were constructed (Fig. 2A). MBP-TRIM62, MBP-ΔRING and MBP-C11A were separately incubated with E1, ubiquitin and/or UbcH5b. After binding to the amylose resin, the precipitates were analyzed by Western blotting using anti-Ub and anti-MBP antibodies. As shown in Fig. 2B, a high-molecular-weight smear was detected only in the presence of MBP-TRIM62 and UbcH5b. This result showed that the in vitro ubiquitination of TRIM62 is dependent on its RING finger domain, suggesting that TRIM62 is a RING finger E3 ubiquitin ligase that can catalyze self-ubiquitination.

3.3. In vivo self-ubiquitination of TRIM62 in HEK293T cells

The E3 ubiquitin ligase activity of TRIM62 was determined using an in vitro ubiquitination assay, as described in the preceding sections, and we also performed the ubiquitination assays in HEK293T cells to confirm the observations. HEK293T cells were co-transfected with TRIM62-F with or without HA-Ub, and a

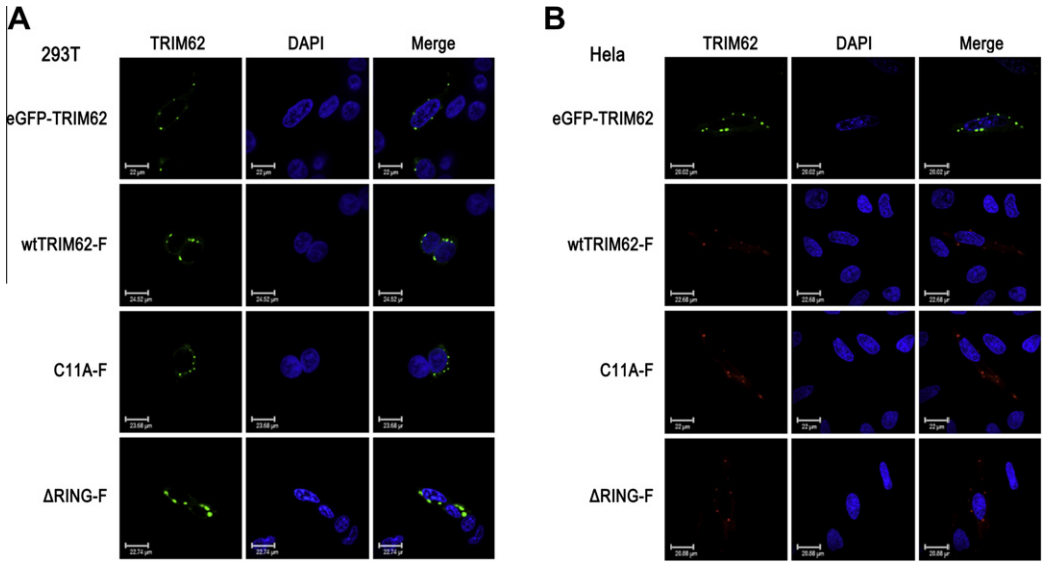

Fig. 4. Subcellular localization of TRIM62. HEK293T (A) and HeLa (B) cells were transfected with the indicated plasmids, pEGFP-TRIM62 and pcDNA3.1-TRIM62/ΔRING/C11A. The cells were prepared for immunostaining at 48 h post-transfection. The cells were fixed, and the cells that were transfected with TRIM62-F, ΔRING-F or C11A-F were incubated with anti-Flag antibody, followed by FITC-conjugated anti-mouse antibodies, as indicated (left – green or right – red). The nucleus was stained with Hoechst (blue), and the cells were mounted in Prolong Antifade. The cells were then examined under a confocal laser-scanning microscope (original magnification, 60×).

two-step immunoprecipitation assay was conducted. As shown in Fig. 3A, the smear was only detected in the presence of both TRIM62-F and HA-Ub. To confirm whether TRIM62 ubiquitination led to the degradation of the protein, the proteasome inhibitor MG132 was added to the culture medium at 42 h post-transfection for 6 h. As shown in Fig. 3B, the smear products were enhanced in the presence of MG132. These results indicated that TRIM62 also exhibited ubiquitination activity in HEK293T cells, which subsequently led to its degradation in the proteasome.

It has been demonstrated that the RING finger domain plays a critical role in the E3 ubiquitin ligase activity of TRIM62 in vitro. To further confirm this result in vivo, HEK293T cells were co-transfected with TRIM62-F, ΔRING-F or C11A-F with or without HA-Ub. As shown in Fig. 3C, compared with TRIM62-F (lane 2), ΔRING-F and C11A-F displayed a significant decrease in the smear products (lanes 4 and 6), which demonstrated that TRIM62 self-ubiquitination was dependent on the presence of a RING finger domain. However, it is possible that the low level of ubiquitination of these two mutants might be catalyzed by endogenous TRIM62 or other E3 ubiquitin ligases in HEK293T cells.

3.4. Subcellular localization of TRIM62

The proteins of the TRIM family display various subcellular localizations in eukaryotic cells that relate to their biochemical functions in vivo. To determine the subcellular localization of TRIM62, we transfected HEK293T and HeLa cells with pcDNA3.1-TRIM62/ΔRING/C11A and pEGFP-TRIM62 and found that GFP-TRIM62 formed aggregates only in the cytoplasm of the cells (Fig. 4). Additionally, an indirect immunofluorescence assay showed that TRIM62-F, ΔRING-F and C11A-F formed only cytoplasmic aggregates in both cell lines. These results showed that TRIM62 and its mutants were enriched in the cytoplasm of both HEK293T and HeLa cells in the form of aggregates.

4. Discussion

TRIM62, which belongs to the TRIM/RBCC family, contains a RING finger domain. Previous studies have demonstrated that TRIM62 is associated with diverse cellular processes, such as negatively regulating acinar morphogenesis, acting as an independent predictor in early-onset breast cancer, interfering with the life cycle of MLV and HIV-1 and participating in the regulation of innate immunity [18–20]. Here, we report that TRIM62 is an E3 ubiquitin ligase whose activity is dependent on the presence of a RING finger domain both in vitro and in vivo. Our results also showed that TRIM62 underwent self-ubiquitination, but the absence or disruption of its RING finger domain decreased or abolished this activity.

The ubiquitination process is described as the conjugation of ubiquitin to other cellular proteins, and it regulates a broad range of eukaryotic cell functions. This reaction occurs via a three-step mechanism that involves three major components, E1, E2 and E3, in addition to ubiquitin [7]. Different E3 ubiquitin ligases require interaction with different E2s to deliver ubiquitin to the substrates [1]. Previous reports have shown that TRIM22, TRIM5α and TRIM21 (Ro52) cooperate with UbcH5b and that TRIM69 interacts with several different E2s (UbcH6, UbcH2, UbcH5a, UbcH5c and UbcH13/UeV1a) [23–26]. In our study, we found that TRIM62 also requires UbcH5b to initiate the ubiquitination process in vitro (Fig. 1B). Additionally, both in vitro and in vivo ubiquitination assays using two types of mutants (ΔRING and C11A) indicated that an intact RING finger domain is essential for the ubiquitination mediated by TRIM62 (Fig. 2B and Fig. 3C). The ubiquitin modification of many cellular proteins usually results in the substrates being targeted for proteasomal degradation, although the modification may serve non-proteolytic functions. Some ubiquitinated TRIM proteins eventually undergo degradation, whereas others do not; this difference is mainly caused by the different forms of ubiquitin chains attached to the substrates. For example, K48 ubiquitination predominantly leads to protein degradation, whereas K63 ubiquitination always results in various cellular activities [13,27]. To determine the consequence of self-ubiquitinated TRIM62, we treated the cells with the proteasomal inhibitor MG132 and found an increased accumulation of ubiquitinated TRIM62. This result indicated that self-ubiquitinated TRIM62 could promote its own degradation via the proteasomal pathway (Fig. 3B).

Members of the TRIM/RBCC family exhibit various subcellular localizations and appear to define novel subcellular compartments,

which might relate to their biological functions. Mono-ubiquitinated TRIM5α is dispersed in the cytoplasm, whereas TRIM21 (Ro52) is distributed in both the nucleus and cytoplasm. Several reports claim that TRIM22 displays different subcellular localizations [24,25,28]. In our study, GFP-tagged TRIM62 (GFP-TRIM62), Flag-tagged TRIM62 and its mutants (ΔRING and C11A) were all located only in the cytoplasm of both HEK293T and HeLa cells (Fig. 4). Possible hypotheses for this cytoplasmic distribution of TRIM62 may be that poly-ubiquitinated TRIM62 undergoes proteasomal degradation or that TRIM62 interacts with other cytoplasmic cellular proteins; however, these hypotheses require further investigation.

In conclusion, we identified that TRIM62 is a RING finger domain-dependent E3 ubiquitin ligase that can catalyze self-ubiquitination; we also found that it was localized in the cytoplasm. Although TRIM62 has been shown to participate in the TRIF branch of the TLR4 signaling pathway [20], the detailed mechanism remains unclear. Since TRIM62 is an E3 ubiquitin ligase, finding its potential substrates should be the key point of the following studies, which will help to clarify the biological functions of TRIM62.

Acknowledgments

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