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Tripartite motif proteins: an emerging antiviral protein family

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INTRODUCTION

Virus must engage with host cells to replicate and spread, which warrants profound changes in the cellular signaling pathways, especially the instigation of host immune responses. Host defenses are complex systemic immune responses at different levels, including intrinsic, innate and adaptive immunity. Recent studies show that the tripartite motif (TRIM) containing proteins play a versatile role in all layers of host immune defenses. TRIM proteins are a family of ubiquitin E3 ligases which share a characteristic N-terminal tripartite motif (also known as RBCC motif) consisting of a highly conserved order of three domains: the really interesting new gene (RING) domain, one or two B-box domains and a coiled coil region [1]. The RING domain confers TRIM proteins the ubiquitin E3 ligase activity. Ubiquitination is a post-translational modification that alters target protein stability, trafficking, subcellular localization, enzymatic activation and protein recruitment [2]. TRIM proteins have been found to promote host immune response by ubiquitination of critical signaling molecules in immunity and interfere with viral infection by directly targeting viral proteins for degradation.

TRIM proteins are known for a long time to participate in various biological processes, such as development and carcinogenesis; however, we are just now discovering their importance in recent years. Notably, the study of TRIM proteins in host defense is one of the fastest growing subjects in the field of immunity. Thus, systematic and comprehensive understanding of the role of TRIM proteins in multiple layers of host defense will not only help elucidate the antiviral mechanisms of TRIM proteins but also provide potentials for developing new antiviral therapeutics. In this review, we focus on the role and the antiviral mechanisms of TRIM proteins in each layer of host immune defense, elaborate the viral evasion strategy by targeting TRIM proteins and discuss the future study direction.

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TRIM PROTEIN STRUCTURE, CLASSIFICATION, AND EXPRESSION

TRIM protein family consists of 76 members, which all share the RBCC motif. RBCC domain comprises three motifs which are RING, B-box and coiled coil [3]. The RING is a 40–60 amino acid long zinc finger motif, which is essential for the ubiquitin E3 ligase activity and the binding with the E2 conjugating enzyme. RING domain plays a crucial role in TRIM proteins-mediated ubiquitination. B-box domain presents in either single or paired form and plays a role in self-assembly of TRIM proteins and interaction with their substrates. Coiled coil is a common hyper-helical structure that involves protein-protein interaction, including protein dimerization. Similarly, the coiled coil of TRIM proteins mediates dimerization and self-association of TRIM proteins. Additionally, the coiled coil sequesters TRIM proteins in the cytoplasm or the nuclear bodies [4].

The RBCC motif is followed by one or more domains located on the C-terminus of TRIM proteins. The TRIM superfamily is classified into eleven groups according to the C-terminal domains (Fig. 1). Group 1 is featured with multiple domains, including the C-terminal subgroup one signature (COS), fibronectin type 3 (FN3) and SPRY domain. COS box domain is responsible for microtubule binding. FN3 binds heparin and DNA. The SPRY domain (also termed as B30.2) is involved in protein-protein interaction and RNA binding. Group 2 has a single COS domain whereas Group 3 has both COS and FN3 domains. Group 4 is the largest subfamily of TRIM proteins characterized by a SPRY domain at the C terminus. Group 5 has no defined motif in the C-terminal region. Group 6 has the PHD-BROMO domains while group 7 possesses the NHL repeats. Bromodomains bind acetylated lysine residue on the N-terminal of histones and repress transcription. NHL repeats mediate protein-protein interaction. Groups 8–10 are featured with MATH, ARF and filamin domain, respectively. Filament type immunoglobulin domains are responsible for dimerization and actin crosslinking whereas ADP ribosylation factor-like (ARF) domains stimulate vesicular trafficking. MATH domain is essential for receptor-TRAF protein interaction. Group 11 comprises a transmembrane domain (TM) in their C-terminal region. Taken together, the variable C-terminal domains of TRIM proteins contribute to the versatile binding ability to their substrate.

TRIM proteins are found in many types of cells, such as immune cells, epithelial cells, endothelial cells, fibroblasts, and neuronal cells. Interestingly, TRIM proteins are expressed differentially in B-cells, T-cells, dendritic cells, mast cells, NK-cells, and macrophages, suggesting a regulatory mechanism for TRIM. In addition, TRIM proteins show distinct subcellular localizations. Many of them form cytoplasmic bodies when overexpressed. Mutations in the RING, coiled coil domain or B-box have been reported to cause aberrant localization. More importantly, viral infection results in cytosolic TRIM proteins trafficking into the nucleus [5].

TRIM PROTEINS AND UBIQUITINATION

Ubiquitin is a highly conserved and ubiquitously expressed 76-amino acid protein [6]. The ubiquitination process is a reversible covalent conjugation of ubiquitin to substrate by a three-stepwise enzymatic system (Fig. 2). In the first step, ubiquitin is activated by a ubiquitin-activating enzyme (E1) in an ATP-dependent reaction, in which the C-terminus of

ubiquitin links to the E1 via a thioester bond. In the second step, the activated ubiquitin is transferred to a ubiquitin-conjugating enzyme (E2), forming an E2-Ub thioester. The E2 then co-opts with the ubiquitin ligase (E3) to access to the substrate protein. The E2-E3 enzyme complex conjugates the ubiquitin to the substrate via an isopeptide bond between the C-terminal glycine of ubiquitin and the lysine of the substrate protein. To date, two E1s (UBA1 and UBA6), about 35 E2s and more than 600 E3 ligases have been identified in humans, suggesting a single E2 can interact with multiple E3s. Regarding TRIM proteins, the Cys₃-His-Cys₄ zinc finger in the RING domain binds several E2s, including UBE2D and UBE2E.

The ubiquitin moiety can be conjugated to the substrate by a single molecule (mono-ubiquitination) or by adding polyubiquitin chain (polyubiquitination). The polyubiquitin chains are grouped into different linkages determined by the conjugation via one of the seven lysines (K6, K11, K27, K29, K33, K48, K63) between ubiquitins. The linkage of polyubiquitin can influence the fate of the substrate, adding another layer of complexity to the outcome of ubiquitination. The majority of TRIM proteins mediate either K48- or K63-linked polyubiquitination of target proteins. The K48-linked ubiquitination usually directs proteins for proteasome-dependent degradation while the K63-linked ubiquitination involves non-proteolytic processes, such as subcellular localization, signalosome stability and activation, and trafficking. For example, TRIM32 targets influenza PB1 protein for K48-linked polyubiquitination, which results in PB1 protein degradation and viral restriction [7]. In contrast, TRIM25 mediates K63-linked ubiquitination of RIG-I, which facilitates RIG-I multimerization and activation [8].

Although most TRIM proteins are ubiquitin E3 ligases, several TRIM proteins can act as an E3 ligase for ubiquitin-like molecules, such as SUMO and ISG15. For instance, TRIM28 mediates sumoylation of IRF7, which leads to inhibition of IRF7 transactivation and IFN expression [9]. It is also reported that TRIM25 acts as an ISG15 E3 ligase for 14–3–3 sigma protein; however, the biological consequence is not clear [10]. These TRIM proteins, including TRIM25 and TRIM28, are well-known ubiquitin E3 ligases. But how these TRIM proteins work on two or more different post-translational modifications is unknown. Future direction of this field is to pinpoint the underlying molecular mechanisms, which will deepen our understanding of the role of TRIM proteins in host defense.

ROLE OF TRIM PROTEINS IN HOST DEFENSE

1. TRIM proteins and intrinsic immunity—Intrinsic immunity is the instant antiviral response by a group of constitutively expressed host factors through diverse mechanisms. The “preexisted” expression of these host factors guarantees the rapid and direct inhibition of viruses at almost every step of viral life cycle. A group of TRIM proteins belongs to these intrinsic factors that have been reported to curb viral infection through direct interaction with viral proteins. These TRIM proteins employ distinct mechanisms to inhibit viral entry, replication or dissemination. The intrinsic antiviral mechanism of each of these TRIM proteins will be discussed below (Table 1).

1.1. TRIM proteins-mediated intrinsic immunity to HIV-1: TRIM5 α is the prototype of TRIM proteins in intrinsic immunity. TRIM5 α is a well-known cytosolic host restriction factor to the cross-species transmission of retroviruses, which protects the Old World monkeys including rhesus macaques from infection of HIV-1 [11]. The restriction specificity of TRIM5 α is dependent on its C-terminal PRY/SPRY domain that mediates the interaction with HIV-1 capsid [12]. On the HIV-1 capsid surface, TRIM5 proteins assemble into a hexagonal net with the SPRY domains centered on the edges and the B-box and RING domains at the vertices. The conformational flexibility of the hexagonal net allows TRIM5 α to accommodate the variable curvature of retroviral capsids. TRIM5 α -capsid interaction results in premature uncoating, thereby halting reverse transcription and transport to the nucleus of the viral genome [12] (Fig. 3). The ubiquitin E3 ligase activity of TRIM5 α is required for restriction; however, TRIM5 α -mediated ubiquitination of HIV-1 capsid has not been detected. TRIM5 α also binds and restricts N tropic mouse leukemia virus (N-MLV) [13], but the precise mechanism is yet to be determined. In addition, it also reported that TRIM5 α inhibits retroviral infection by activating innate immune signaling. We discuss it in the below section of TRIM proteins and Innate Immunity.

Several other TRIM proteins also exhibit intrinsic anti-retroviral activity, including TRIM11 [14], TRIM28 [15], TRIM22 [16] and TRIM19 [17] (Fig. 3). Like TRIM5 α , TRIM11 also interacts with HIV-1 capsid. Similarly to TRIM5 α , TRIM11 restricts HIV-1 reverse transcription by promoting premature viral uncoating [14]. TRIM28 (also known as KAP1) limits HIV-1 by binding the acetylated integrase [15]. The acetylation of integrase is essential for the integration of HIV-1 cDNA into the host genome. TRIM28 mediates the deacetylation of viral integrase through the formation of a protein complex that includes the deacetylase HDAC1 [15]. TRIM22 restricts HIV infection by two distinct mechanisms. First, TRIM22 controls the trafficking of the gag protein to the plasma membrane and interferes with the assembly of new viral particles [16]. Secondly, TRIM22 co-opts with class II transactivator (CIITA) and Sp1 to inhibit viral gene transcription initiation and elongation [18]. TRIM19, also known as promyelocytic leukemia protein (PML), restricts the HIV-1 by reducing the reverse transcription products in the host and interfering with viral transcription [17]. It is not surprising that multiple TRIM proteins restrict HIV-1 as each TRIM protein employs a different mode of action. However, most work is based on cell lines and lack of animal models. Further animal work will elucidate the in vivo role of these TRIM proteins.

1.2. TRIM proteins-mediated intrinsic immunity to influenza A virus: Similar to HIV-1, influenza A virus (IAV) is targeted by multiple TRIM proteins. TRIM32 and TRIM41 are host intrinsic immune factors to IAV (Fig. 3). TRIM32 senses and targets IAV PB1 polymerase protein for ubiquitination and protein degradation [7]. TRIM41 inhibits the IAV by its interaction with viral nucleoprotein through the SPRY domain, which leads to K48-linked polyubiquitination and subsequent protein degradation [19]. TRIM22, an interferon-stimulated gene, also ubiquitinates and degrades IAV nucleoprotein [20] (Fig. 3). TRIM56 inhibits the replication of influenza viruses by specifically targeting viral RNA synthesis through its C-terminal tail [21] (Fig. 3). Furthermore, TRIM25, a critical TRIM in innate immunity (discussed in below section), inhibits IAV through a direct mechanism that

is independent of its ubiquitin ligase activity and the interferon pathway. TRIM25 binds IAV ribonucleoproteins and inhibits viral RNA synthesis [22] (Fig. 3). These recent studies show that TRIM proteins either target viral proteins for protein degradation or inhibit viral RNA synthesis. It is clear that TRIM proteins mediate viral protein degradation through K48-linked polyubiquitination; however, the mechanism for the inhibition of viral RNA synthesis is elusive. As the ubiquitin E3 ligase activity is dispensable [22], these TRIM proteins might disrupt IAV polymerase complex by directly binding NP or other subunit of viral RNP.

1.3. TRIM proteins-mediated intrinsic immunity to other viruses: In addition to IAV, TRIM22 restricts many other viruses (Fig. 3). First, TRIM22 inhibits HBV by suppressing the core promoter responsible for viral pre-genomic RNA synthesis [23]. Secondly, TRIM22 also restricts hepatitis C virus (HCV) by targeting NS5 α for ubiquitination and degradation [24]. Lastly, TRIM22 inhibits encephalomyocarditis virus (EMCV) by targeting viral 3C protease for ubiquitination and degradation [25]. These studies indicate that TRIM22 is a broad-spectrum antiviral protein by mediating K48-linked ubiquitination and subsequent proteasomal degradation of viral proteins; however, whether these viral proteins share a common motif for TRIM22 interaction needs further clarification.

Like TRIM22, several other TRIM proteins are reported to have broad-spectrum antiviral activity (Fig. 3). For example, TRIM19 inhibits HIV-1 (discussed above), VSV, herpes virus and adeno-associated virus [26]. TRIM56 restricts influenza B virus, yellow fever virus and dengue virus by reducing the vRNA levels through its C-terminal domain [27, 28], however, the mechanism is not clear. Additionally, TRIM25 mediates K63-linked ubiquitination of ZAP (zinc finger antiviral protein) to promote ZAP antiviral activity to retroviruses, alphaviruses, filoviruses and HBV [29, 30]. ZAP has been shown to recruit RNA exosome to degrade viral RNA, thereby limiting viral infection, which suggests a general antiviral mechanism.

In addition, several viruses are targeted by TRIM proteins through ubiquitination-mediated protein degradation (Fig. 3). TRIM41 inhibits HBV enhancer II activity, which is dependent on its E3 ubiquitin ligase activity and the integrity of the C-terminal domain [31]. Similarly, TRIM14 inhibits the HCV infection by targeting the NS5 α protein for degradation [32]. Japanese encephalitis virus (JEV), another virus from the family Flaviviridae, is inhibited by TRIM52. TRIM52 interacts with the nonstructural protein 2A of JEV and mediates NS2A ubiquitination and protein degradation [33].

2. TRIM proteins and innate immunity—Innate immune responses are triggered by exogenous microbial products that are common to many pathogens but are absent in the host [34]. These microbial products, also known as pathogen-associated molecular patterns (PAMPs), engage with the pattern-recognition receptors (PRRs) which either present on the cell surface or inside the cells [34]. The engagement activates a series of signal cascades to stimulate the expression of a cohort of chemokines, cytokines, and genes critical for host defense. The PRRs consist of several subgroups, including Toll-like receptor (TLR), RIG-I-like receptor (RLR), cyclic GMP-AMP synthase (cGAS) and NOD-like receptor (NLR). TLR, RLR, and cGAS signaling pathways converge to the hub, TANK-binding kinase 1 (TBK1) and IKK epsilon (IKK ϵ). TBK1 and IKK ϵ phosphorylate the interferon regulatory

factors (IRFs). For example, TBK1 phosphorylates serines in the C-terminal domain of IRF3, which triggers the dimerization and nuclear translocation of IRF3. In the nucleus, IRFs form active transcriptional complexes that bind to interferon stimulation response elements (ISRE) and activate type I IFN genes expression [34]. Type I IFN is the master cytokine in innate immunity and induces mRNA expression of > 300 IFN-stimulated genes (ISGs), such as Myxovirus resistance protein 1 (Mx1). Interestingly, some TRIM proteins are ISGs, including TRIM22, TRIM25, and TRIM5. In this section, we discuss the role of TRIM proteins in the following innate immune signaling pathways (Table 2).

2.1 TRIM proteins and TLR signaling: TLRs are membrane proteins residing on cell surface or endosomes. The TLRs on endosomes recognize viral nucleic acids [34]. For example, TLR3 detects viral double-strand RNA while TLR7 and TLR8 sense viral single-strand RNA. TLR9 recognizes unmethylated CpG motifs within viral DNA. Although all TLRs activate TBK1/IKK ϵ and downstream IRF signaling, they recruit different adaptors. TLR3 recruits TRIF whereas TLR7, 8 and 9 relay signals through MyD88.

Several TRIM proteins regulate TLR signaling pathways (Fig. 4). TRIM21 (also known as Ro52) has been reported to negatively regulate IFN production by targeting IRFs, including IRF3, IRF7, and IRF5 for ubiquitination and degradation [35]. However, another study presented an opposite result by showing that TRIM21 interferes with the interaction between Pin1 (peptidyl-prolyl cis/trans isomerase, NIMA-interacting 1) and IRF3, thus preventing IRF3 ubiquitination and degradation [36]. It will need further investigation to clarify the role of TRIM21 in IRF3 signaling although these observations are context-dependent. TRIM protein also regulates TLR adaptors. For example, TRIM56 promotes TLR3 activation by interacting with TRIF, but its E3 ligase activity is not required [37]. By contrast, TRIM38 impairs TLR3 signaling by targeting TRIF for proteasomal degradation [38]. The discovery of TRIM protein as a negative regulator of host immune response is important because it represents a new self-control mechanism to prevent over-activation of immune response.

2.2. TRIM proteins and RLR signaling pathway: The RLRs, including RIG-I, MDA5, and LGP2, recognize the double-stranded RNA (dsRNA) or 5' triphosphate RNA generated by viral replication of RNA viruses in the cytoplasm. Engagement of viral RNA activates RIG-I and MDA5, which binds the mitochondrial antiviral signaling protein (MAVS) and initiates the TBK1-IRF3 signaling cascade to induce type I IFN expression [34].

2.2.1. TRIM proteins and the RIG-I-like receptors.: TRIM proteins play both positive and negative role in modulating the RLR signaling pathway (Fig. 4). TRIM25 mediates K63-linked ubiquitination of RIG-I [8] and the polyubiquitin chain stabilizes the oligomerization of RIG-I, which promotes MAVS aggregation and subsequent activation. Similarly, TRIM4 conjugates K63-linked polyubiquitin chains the CARD domain of RIG-I [39]. Interestingly, TRIM25 also synthesizes unanchored K63-linked polyubiquitin chain, which helps RIG-I oligomerization, and stability to supports the interaction with MAVS [40]. MDA5, another RLR, also undergoes K63-linked ubiquitination; however, TRIM65 is the cognate ubiquitin E3 ligase. Similar to RIG-I ubiquitination, the K63-linked ubiquitination of MDA5 is critical for MDA5 oligomerization and activation [41]. By contrast, TRIM13 negatively regulate the MDA5-dependent IRF activation although it not clear whether the ubiquitin E3 ligase

activity is required [42]. In addition, TRIM38 positively regulates both RIG-I and MDA5 by sumoylation, which prevents K48-linked polyubiquitination and degradation [43]. It is clear that TRIM proteins regulate the activity of the cytosolic RNA sensor, RIG-I and MDA5, however, how these TRIM proteins are regulated upon external signal stimulation, such as RNA virus infection, is elusive. Furthermore, whether there is functional redundancy of these TRIM proteins and how these TRIM proteins coordinate need further investigation.

2.2.2. TRIM proteins and MAVS.: Multiple TRIM proteins have been found to regulate MAVS on the mitochondria with distinct outcomes (Fig. 4). TRIM31 mediates K63-linked polyubiquitination of MAVS and promotes the formation of prion-like aggregates, the active form of MAVS [44]. TRIM14 bridges NEMO to MAVS by the polyubiquitin of TRIM14 to activate IRF3 and NF- κ B pathways [45]. Additionally, TRIM44 has been shown to prevent K48-linked polyubiquitination of MAVS [46]. Unexpectedly, one study reported that TRIM25 mediated K48-linked polyubiquitination of MAVS, which permits IRF3 signaling but inhibits NF- κ B activity, another branch of MAVS signaling [47]. As TRIM25 is well known as one of the ubiquitin E3 ligases critical for the K63-linked polyubiquitination and subsequent activation of RIG-I, it should require a more careful experimental design to study the role of TRIM25 on MAVS.

2.2.3. TRIM proteins and the TBK1 protein complex.: Several TRIM proteins target the TBK1 kinase complex for IFN regulation (Fig. 4). First, TRIM26 undergoes autoubiquitination upon viral infection and the polyubiquitin of TRIM26 bridges NEMO to TBK1, thus facilitating TBK1 activation [48]. Secondly, the short form of TRIM9 (TRIM9s) also undergoes K63-linked auto-polyubiquitination upon viral infection. The polyubiquitin of TRIM9s serves as a platform to bridge GSK3 β to TBK1, leading to the activation of IRF3 signaling [49]. Thirdly, TRIM11 inhibits TBK1 activity by blocking TBK1 interaction with its binding partners, NAP1 and TANK [50]. Lastly, TRIM38 facilitates K48 linked polyubiquitination and degradation of NAP1, resulting in reduced activity of IRF3 [51]. As the TBK1 kinase complex is the hub of various innate immune signaling pathways, whether these TRIM proteins are specific to the RLR signal pathway or also work on other innate signaling pathways need to be clarified in the future.

2.3. TRIM proteins and cGAS signaling: Cytosolic viral DNA is recognized by the recently identified DNA sensor, cyclic GMP-AMP synthase (cGAS, also known as MB21D1) [52]. After binding to DNA, cGAS produces cyclic GMP-AMP (cGAMP). cGAMP is a second messenger and binds to an endoplasmic reticulum membrane protein, the stimulator of interferon genes (STING, also known as TMEM173, MPYS, MITA, and ERIS), which leads the dimerization of STING. Subsequently, STING recruits TBK1 to the endoplasmic reticulum and activates TBK1. Activated TBK1 phosphorylates interferon regulatory factors (IRFs).

TRIM56 and TRIM32 have been reported to catalyze K63-linked polyubiquitination of STING, which is essential for activation of IFN β expression and restriction of DNA viruses [53, 54] (Fig. 4). In contrast, TRIM30 α and TRIM29 promote the degradation of STING via K48-linked ubiquitination through a proteasome-dependent pathway [55, 56] (Fig. 4). Interestingly, TRIM38-mediated sumoylation of STING counteracts the K48-linked

ubiquitination of STING and prevents STING from protein degradation [57] (Fig. 4). Similarly, TRIM38 also mediates sumoylation of cGAS, thus preventing cGAS polyubiquitination and degradation [57]. Recently, the ubiquitin E3 ligase for STING, TRIM56, was found to catalyze monoubiquitination of cGAS, which promotes cGAS dimerization, DNA-binding activity, and cGAMP production [58] (Fig. 4). Notably, TRIM38 and TRIM56 regulate both cGAS and STING. However, STING is a membrane protein on the ER while cGAS is a cytosolic and nuclear protein. The subcellular localization of these TRIM proteins and the intracellular trafficking of these proteins are the questions need to be addressed.

2.4. TRIM proteins and NLR signaling: NLRs comprise a family of intracellular sensors that recognize microbial products and mount proinflammatory and antimicrobial immune response. Upon stimulation, NLR forms inflammasomes, a large and highly ordered cytosolic complex, which leads to caspase-mediated proteolytic activation and IL-1 β and IL-18 [34]. Several TRIM proteins have been reported to regulate NLR signaling by targeting the signaling proteins for ubiquitination and protein degradation (Fig. 4). For example, TRIM33 binds and ubiquitinates DHX33, a cytosolic receptor upstream of NLRP3. The ubiquitination of DHX33 facilitates inflammasome activation [59]. Coiled Coil domain of TRIM31 interacts with NACHT domain of NLRP3 and mediates K48-linked ubiquitination that leads to the degradation of NLRP3 [60]. TRIM30 α negatively regulates NLRP3 inflammasome activation by modulating reactive oxygen species production [61]. In addition, TRIM27 degrades NOD2 and inhibits the NOD2 receptors during viral infection [62].

2.5. TRIM proteins and other innate immune signaling pathways: The NF- κ B transcription factors play a critical role in various antiviral immune signaling pathways [34]. A wide range of soluble and membrane-bound extracellular ligands activate NF- κ B, such as LPS and IL-1. For example, TLR4 recruits a series of intermediary adaptors after LPS stimulation. These adaptors further recruit and activate the IKK kinase complex consisting of IKK α , IKK β , and NEMO. The activated IKK complex phosphorylates I κ B α , which leads to I κ B α protein degradation and subsequent release and activation of NF- κ B. These pathways are negatively regulated by several TRIM proteins (Fig. 4). For example, TRIM30 α and TRIM38 mediate protein degradation of TAB2/3, the adaptors of TAK1, thus inhibiting TAK1 activity and downstream IKK kinase activation [63, 64]. TRIM21 inhibits the NF- κ B pathway through mono-ubiquitination of IKK β [65]; however, the molecular mechanism is not clear. TRIM38 catalyzes K48-linked polyubiquitination of TRAF6 (TNF receptor-associated factor 6) to negatively regulate NF- κ B signaling pathway [66]. In addition, TRIM27 inhibits NF- κ B and IRF3 via interaction with IKK, but the mechanism is not clear [67]. TRIM29 also suppresses both pathways by degradation of NEMO [68]. By contrast, TRIM23 binds NEMO through the C terminal ARF domain and targets NEMO for K27-linked ubiquitination, which leads to increased activation of NF- κ B signaling [69]. The mechanism for K27-linked ubiquitination-mediated NEMO activation is unknown. The K27-linked polyubiquitin doesn't lead to NEMO protein degradation. It is plausible that the K27-linked polyubiquitin provides a signaling platform for the recruitment of the downstream signaling molecules or facilitates NEMO dimerization.

IFN binds IFN receptors to activate JAK (Janus kinase)-STAT (signal transducer and activator of transcription) pathway, which stimulates hundreds of ISGs to execute the antiviral actions. TRIM6 is found to catalyze unanchored K48-linked polyubiquitin chains, which activates IKK ϵ to phosphorylate STAT1 (Fig. 4). The phosphorylation results in the induction of a subset of ISGs essential for the antiviral response [70].

3. TRIM proteins and adaptive immunity—Most TRIM proteins are found to regulate intrinsic and innate immunity; however, there are also several TRIM proteins that regulate adaptive immunity. TRIM5 couples innate viral sensing and CD8⁺ T-cell activation to increase species barriers against retrovirus infection [71]. TRIM8 is a Th17-specific HIV dependency factor and an important anti-HIV factor [72]. TRIM24 regulates the expression of Th2-type cytokines [73]. TRIM27 inhibits the activation of CD4⁺ cells through K48-linked polyubiquitination of PI3K-C2 β [74]. Additionally, TRIM21 plays an important role in the detection of antibody-opsonized viruses, such as the foot and mouth disease virus (FMDV). TRIM21 binds to the highly conserved Fc region of immunoglobulins and targets the virus for degradation by the proteasome [75]. Overall, there are only a few TRIM proteins are discovered to regulate adaptive immunity because of the limits of research tools. Nonetheless, the list will grow as more animal models for TRIM proteins are generated.

TARGETING TRIM PROTEINS: A NEWLY DISCOVERED VIRAL EVASION STRATEGY

- **Targeting TRIM proteins by RNA virus**—The arms race against host defense has forced viruses to evolve new strategies for evasion. Thus, it is not a surprise to find some viruses that target the TRIM proteins to dampen host defense. Firstly, several RNA viruses target TRIM25, the regulator of RIG-I. The non-structural protein 1 (NS1) of influenza A virus directly interacts with the coiled coil domain of TRIM25, thereby preventing its multimerization which is essential for the E3 ligase activity. The defective TRIM25 fails to catalyze K63-linked ubiquitination of RIG-I, thus blocking type I IFN expression [76]. Similarly, the nucleoprotein of SARS-associated coronavirus (SARS-CoV), MERS-CoV and the severe fever with thrombocytopenia virus (SFTSV) also interact with TRIM25 and prevent TRIM25-RIG-I interaction, thereby inhibiting RIG-I signaling [77]. The Non-coding RNAs of the PR-2b strain of Dengue virus bind to the host TRIM25 and prevents USP15-mediated deubiquitination, which is important for RIG-I activation [78]. Secondly, TRIM6 is the target of two RNA viruses. The matrix protein of Nipah virus induces TRIM6 degradation to suppress TRIM6-mediated IFN response [79] whereas Ebola virus utilizes TRIM6 to ubiquitinate its VP35 protein, thereby promoting VP35 activity and virus replication [80]. Lastly, several RNA viruses utilize TRIM proteins to suppress type I IFN signaling pathway. The NS5 protein of yellow fever virus interacts with TRIM23, which results in K6-linked polyubiquitination of NS5. The polyubiquitination facilitates NS5 interaction with STAT2, thereby inhibiting type I IFN response [81].

- **Targeting TRIM proteins by DNA virus**—Like RNA viruses, DNA viruses also target TRIM protein to subvert immune response by several distinct mechanisms. The HBV X protein suppresses TRIM22 mRNA expression by CpG methylation of the 5'-UTR of TRIM22 gene [82]. Epstein-Barr Virus (EBV) targets TRIM28 for phosphorylation at serine residue 824, which impairs the antiviral function of TRIM28 [83]. In addition, several DNA

viruses target TRIM proteins for ubiquitination. The ICP0 protein of HSV-1 ubiquitinates and degrades TRIM27, resulting in the inhibition of immune response [84]. Similarly, Gammaherpesvirus MHV-68 degrades TRIM19 [85].

PERSPECTIVES

The co-evolution of host and virus has kept shaping the viral survival strategy and host defense system. Although these mechanisms exist for a very long time, many of them await for discovery, including the antiviral mechanism of the TRIM protein family. A recent systematic screening suggests that more TRIM proteins are involved in host immunity and viral restriction [5]. Despite the increasing knowledge of TRIM proteins, there are several questions needed to be addressed or directions may be explored in the future. First, what are the underlying mechanisms for the broad-spectrum antiviral activity and specificity of TRIM proteins? As discussed above, some TRIM proteins, such as TRIM22, TRIM19, and TRIM56, demonstrate broad-spectrum antiviral activity by inhibition of multiple viruses. However, whether there is a common mechanism for these TRIM proteins is not clear. For example, TRIM22 ubiquitinates different viral proteins and restricts the infection of viruses from different families. It will be interesting to know whether the interacting viral proteins share a common motif for TRIM interaction. Conversely, multiple TRIM proteins may attack the same virus. For example, HIV-1 has been shown to be limited by several TRIM proteins. However, detailed biochemical and genetic analyses are further needed to clarify the functional redundancy among TRIM proteins. Secondly, how TRIM proteins are activated and regulated upon viral infection? Most studies focus on the mechanisms by which TRIM proteins modulate host immunity and viral infection. Little is known about the regulatory mechanisms for TRIM proteins. Elucidation of the regulatory mechanism for TRIM proteins will help develop a novel strategy to boost host immunity to viral infection. Thirdly, the *in vivo* role of most TRIM proteins is unknown. Recently, several TRIM protein transgenic and knockout mice have been generated. These mice are the valuable tools to study the *in vivo* role of TRIM proteins. However, most of these mouse models have not been examined for viral infection. Lastly, is there any inherent relevance of the genetic mutations of TRIM proteins to human immune diseases? Genetic mutations in the NHL repeats of TRIM32 cause recessive hereditary muscle disorders, including limb girdle muscular dystrophy 2H (LGMD2H) [86]. Recent genome-wide association (GWA) studies implied a link of a group of TRIM proteins to the susceptibility of neuropsychiatric disorders, developmental diseases, cardiovascular and metabolic diseases [86]. However, whether genetic mutations of TRIM proteins are linked to infectious diseases is not clear. Taken all together, studies in the above directions will open new spectrum in anti-viral research and will provide developing strategy for prevention and control of viral infections.

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Executive Summary

What are TRIM proteins?

- TRIM proteins are a family that shares a characteristic N-terminal tripartite motif consisting of a highly conserved order of three domains: RING, B-box, and coiled coil.
- TRIM proteins are ubiquitin E3 ligases that target substrates for ubiquitination.
- TRIM-mediated ubiquitination regulates protein stability, trafficking, subcellular localization, enzymatic activation, and protein recruitment.

TRIM proteins and intrinsic immunity

- Multiple TRIM proteins target viral protein directly to limit DNA and RNA virus infection.
- TRIM5a and other TRIM proteins restrict HIV-1 infection.
- TRIM32, TRIM41 and TRIM22 limit influenza A virus infection by targeting viral protein for ubiquitination-mediated protein degradation.

TRIM proteins and innate immunity

- TRIM proteins regulate TLR, RLR, NLR and cGAS-mediated innate immune signaling pathways.
- TRIM25 mediates K63-linked ubiquitination of RIG-I, which facilitates RIG-I oligomerization and subsequent activation.
- TRIM56 and TRIM32 mediate K63-linked ubiquitination of STING, which is critical for cytosolic DNA-elicited type I interferon expression.

TRIM proteins and adaptive immunity

- TRIM5 couples innate viral sensing and CD8⁺ T-cell activation to increase species barriers against retrovirus infection.
- TRIM27 inhibits the activation of CD4⁺ cells through K48-linked polyubiquitination of PI3K-C2β.

Targeting TRIM proteins: a newly discovered viral evasion strategy

- Influenza NS1 interacts with TRIM25 and blocks TRIM25-mediated RIG-I activation.
- The ICP0 protein of HSV-1 ubiquitinates and degrades TRIM27, resulting in the inhibition of immune response.

Figure 1. The schematics of the subgroups of TRIM proteins.
Each number in the column of TRIM Family Members is corresponding to the designated TRIM protein number. For example, 1 stands for TRIM1.

Figure 2. The process of ubiquitination.

Ubiquitin is first activated by a ubiquitin-activating enzyme (E1) in an ATP-dependent reaction. The activated ubiquitin is then transferred to a ubiquitin-conjugating enzyme (E2). Subsequently, the E2-E3 enzyme complex conjugates one or more ubiquitin proteins to the substrate.

Figure 3. The role of TRIM proteins in intrinsic immunity.

This figure illustrates that TRIM proteins employ distinct mechanisms to inhibit viral entry, replication or dissemination by interacting with viral proteins.

Figure 4. The role of TRIM proteins in innate immunity.

This figure illustrates that TRIM proteins play both positive and negative roles in TLR, RLR and cGAS signaling pathways.

Table 1

Summary of the role of TRIM proteins in intrinsic immunity

TRIM protein	Mode of action	Cell line	Method	Reference
TRIM5α	SPRY domain of TRIM5α interacts with the capsid of HIV and restricts the viral uncoating and replication.	HT1080 CELLS	overexpression	13
TRIM11	TRIM11 restricts HIV-1 reverse transcription by accelerating premature viral uncoating	HEK293, THP-1	overexpression, RNAi	14
TRIM14	RINGless TRIM14 interacts with HCV NS5A protein through SPRY domain and mediates its k48 polyubiquitination and degradation.	JFH, THP-1, HEK293, A549, Huh-7	overexpression, RNAi, CRISPR	32
TRIM19	TRIM19/PML blocks HIV viral replication at two distinct steps. First is at early post entry stage and it also causes silencing of transcription	HEK293, MEF and CRFK cells	overexpression, RNAi, CRISPR	17
TRIM22	TRIM22 blocks the intracellular trafficking of the HIV viral structural protein Gag to the surface of the cell through its E3 ligase activity	HOS-CD4/CXCR4 cells, HeLa, HEK293	overexpression, RNAi	16
TRIM22	TRIM22 interacts with class II interactor and sp1, thus inhibits HIV viral gene transcription initiation and elongation	HEK293T, COS-1, HeLa, U937 and macrophages	overexpression, RNAi	18
TRIM22	TRIM22 targets Influenza nucleoprotein and mediates its k48 polyubiquitination and degradation	HEK293, A549, MDCK	overexpression, RNAi	20
TRIM22	TRIM22 inhibits the activity of HBV core promoter (CP) by interacting through C-terminal SPRY domain and acting as a E3 ligase.	HeLa, HepG2 Cells	overexpression	23
TRIM22	TRIM22 interacts with HCV NS5A protein through SPRY domain and mediates its k48 polyubiquitination and degradation	HEK293T, Huh-7	overexpression, RNAi	24
TRIM22	TRIM22 interacts with the viral 3C protease (3C(PRO)) of EMCV and mediates its ubiquitination and degradation	HEK293, HeLa	overexpression	25
TRIM25	TRIM25 inhibits viral RNA synthesis results from its binding to viral ribonucleoproteins (vRNPs) of Influenza.	CRFK, A549	Overexpression, CRISPR	22
TRIM28	TRIM28 binds to acetylated integrase and induced deacetylation of it which restricts integration of HIV viral cDNA into the host genome	HEK293, HeLa	overexpression, RNAi	15
TRIM32	TRIM32 interacts with the PB1 of the Influenza RNA polymerase complex and mediates its ubiquitination and proteasomal degradation	HEK293, A549, MEF	overexpression, RNAi, CRISPR	7
TRIM41	TRIM41 targets Influenza nucleoprotein through its SPRY domain and mediates its k48 polyubiquitination and degradation	HEK293, A549	overexpression, RNAi, CRISPR	19
TRIM52	TRIM52 act against Japanese Encephalitis Virus infection by targeting and degrading viral NS2A through ubiquitination	BHK21, HEK293T	overexpression	33
TRIM56	TRIM56 inhibits BVDV replication by targeting an intracellular viral RNA replication	HEK293, Huh-7, THP-1,	overexpression, RNAi	27
TRIM56	TRIM56 restricts YFV and DENV2 through E3 ligase activity and c-terminal domain interaction	HEK293, HeLa, mosquito C6/36	overexpression, RNAi	28
TRIM56	TRIM56 specifically impede intracellular influenza virus RNA synthesis without acting as a E3 ligase.	HEK293, MDBK	overexpression, RNAi	21

Table 2

Summary of the role of TRIM proteins in innate immunity

TRIM protein	Mode of action	Cell line	Method
TRIM4	TRIM4 induces the Lys 63-linked ubiquitination of CARD domain RIG-I, which is crucial for the cytosolic RIG-I signalling pathway	HEK293, THP-1, U937	Overexpression, RNAi
TRIM5	TRIM5 couples innate viral sensing and CD8+ T-cell activation to increase species barriers against retrovirus infection.	U937, HLA-B*2705-restricted CD8+ T	Overexpression
TRIM6	TRIM6 synthesizes unanchored K48-linked poly-ubiquitin chains, which activate IKKε for subsequent STAT1 phosphorylation.	HEK293T, A549, Human DCs	Overexpression, RNAi
TRIM11	TRIM11 inhibits RIG-I-mediated IFNβ production by targeting the TBK1 signaling complex. Interaction is dependent on CC domain of TRIM11	HEK293, HeLa	Overexpression, RNAi
TRIM13	TRIM13 enhances RIG-I activation and positively regulate signaling pathway	HEK293, A549	overexpression
TRIM13	TRIM13 regulates the type I IFN response through inhibition of MDA5 activity	HEK293, MEF, L929, BMDM	Knockout, overexpression
TRIM14	TRIM14 interacts with MAVS through its SPRY domain and undergoes k63 linked autoubiquitination and recruits NEMO to the MAVS complex.	HEK293, A549, HeLa	Overexpression, RNAi
TRIM21	The IRF association domain of IRF5 interacts with TRIM21 via its PRY/SPRY domain and mediates its K48 and k63 ubiquitination & regulation.	HEK293T, HEK293-TLR7, TBH-1	Overexpression
TRIM21	TRIM21 interacts with IRF3 through its SPRY domain and inhibits IRF3 ubiquitination and positively regulates it.	HEK293T, HEK293	Overexpression, RNAi
TRIM21	TRIM21 acts as a E3 ligase and mediates Monoubiquitination of IKKβ Down-regulates NF-κB Signalling	HEK293T, HEK293, HeLa	Overexpression
TRIM21	TRIM21 interacts with Fc fragment of swine immunoglobulin G (sFc) fused VP1 of FMDV and thereby causing its K48-linked degradation.	PK-15, BHK21, HEK293	Overexpression
TRIM23	TRIM23 through its ARF domain Interacts with NEMO resulting in its K27-linked ubiquitination and positively regulate downstream pathway	HEK293, HeLa, MEF	Overexpression, RNAi
TRIM24	T-Cell-Intrinsic TRIM24 Is Required for IL-1β-Mediated Activation of TH2 Cells	CD4+ T cells	Knockout, overexpression
TRIM25	TRIM25 induces the Lys 63-linked ubiquitination of CARD domain RIG-I, which is crucial for the cytosolic RIG-I signalling pathway	HEK293T, MEF and HeLa	Overexpression, RNAi
TRIM25	Short, Unanchored, K63-Linked Ubiquitin Chains mediated by TRIM25 Activate RIG-I	HEK293T	overexpression
TRIM25	TRIM25 mediates k48 linked polyubiquitination of MAVS and positively regulates IRF3 pathway through regulating oligomerization	HEK293, MEF, HeLa	Overexpression, RNAi
TRIM26	Autoubiquitination of TRIM26 helps TBK1-NEMO interaction, and recruits TBK1 to the MAVS signalsome.	HEK293, THP-1, HeLa, MEF	Overexpression, RNAi
TRIM27	TRIM27 negatively regulates signaling involved in the antiviral response and inflammation by targeting the IKKs	HEK293	Overexpression, RNAi
TRIM27	TRIM27 mediates K-48 polyubiquitination of PI3KC2β, leading to a decrease in PI3K activity and negatively regulate CD4 T cells	Jurkat-KCa3.1 T, human CD4 T	overexpression, knockout mice
TRIM27	TRIM27 through its SPRY domain interacts with NDB domain of NOD2 and targets it for K48-linked ubiquitination proteasomal degradation	HEK293, HeLa, SW480	Overexpression, RNAi
TRIM28	TRIM28 acts as a SUMO E3 ligase and negatively regulates IRF7 by SUMOylation	HEK293, HEK293T, A549	Overexpression, RNAi

TRIM protein	Mode of action	Cell line	Method
TRIM29	TRIM29 induces NEMO ubiquitination through K-48 linkage which results in proteasomal degradation and suppression of downstream pathway	MH-S, HEK293 alveolar macrophages	Knockout, overexpression, RNAi
TRIM29	TRIM29 targets STING for K48 ubiquitination and degradation and negatively regulates STING dependent pathway	HEK293, BMDM, MEF, BMDM	Knockout, overexpression, RNAi
TRIM30a	TRIM30a interacts with TAK1-TAB2-TAB3 and promotes the k48 linked ubiquitination of degradation of TAB2 and TAB3 thus suppressing signaling	J774, HEK293T, HeLa, BMDM	Knockout, overexpression, RNAi
TRIM30a	TRIM30a prevents TRAF6 autoubiquitination, diminishes I κ B α phosphorylation and negatively regulates the NF- κ B pathway	J774, HEK293T, HeLa, BMDM	Knockout, overexpression, RNAi
TRIM30a	TRIM30a promotes the degradation of STING via K48-linked ubiquitination and negatively regulates STING dependent pathway	HEK293, MEF, L929, BMDM, D2SC	CRISPR, overexpression, RNAi
TRIM30a	TRIM30 negatively regulates NLRP3 inflammasome activation by modulating and inhibiting ROS production	J774, HEK 293T, BMDM	Knockout, overexpression, RNAi
TRIM31	TRIM31 interacted with MAVS and catalyzed the Lys63 (K63)-linked polyubiquitination and regulates downstream pathway positively	HEK293, MEF	Knockout, overexpression, RNAi
TRIM31	TRIM31 directly binds to NLRP3, promotes K48-linked polyubiquitination and proteasomal degradation of NLRP3.	HEK293T, THP-1	Knockout, overexpression, RNAi
TRIM32	TRIM32 targets STING for K63-linked ubiquitination through its E3 ligase activity, which promoted the interaction of STING with TBK1.	HEK293, THP-1	Overexpression, RNAi
TRIM33	TRIM33 targets DHX33 for K63 linked ubiquitination which activates the formation of the DHX33-NLRP3 inflammasome complex.	HEK293T, THP-1	Overexpression, RNAi
TRIM38	TRIM38 interacts with and mediates K48 linked ubiquitination and degradation of TRIF and negatively regulates TLR $\frac{3}{4}$ dependent pathway	BMDM, BMDM	Knockout, overexpression
TRIM38	TRIM38 activates MDAS- and RIG-I through their SUMOylation which enhances dephosphorylation and activation and inhibits its degradation	HEK293, MEF, BMDM	Knockout, overexpression, RNAi
TRIM38	TRIM38 targets NAP1 for ubiquitination and subsequent proteasome-mediated degradation and suppresses IFN β response.	HEK293, HEK293 -TLR $\frac{3}{4}$, RAW cell	Overexpression, RNAi
TRIM38	TRIM38 Interacts with TAB2/3 Through Its PRY-SPRY Domain and mediates k48-linked ubiquitination, degradation and translocation	HEK293, HeLa	CRISPR, overexpression, RNAi
TRIM38	TRIM38 sumoylates STING during the early phase of viral infection, promoting both STING activation and protein stability	HEK293, HEK293T, MEF	Knockout, overexpression
TRIM38	TRIM38 binds to TRAF6 and promotes its K48-linked polyubiquitination and proteasomal degradation thus suppresses downstream pathway	HEK293, RAW cells	Overexpression, RNAi
TRIM44	TRIM44 acts as a deubiquitinase enzyme and prevents k48 linked degradation of MAVS positively regulate MAVS dependent pathway	HEK293, HEK293T, L929, HeLa	Overexpression, RNAi
TRIM56	TRIM56 interacts with TRIF through its C-terminal domain and enhances TLR3 pathway in a E3 ligase independent manner.	HEK293, HEK293-TLR3, Vero, Huh-7.5	Overexpression, RNAi
TRIM56	TRIM56 interacts with STING and targets it for K63-linked ubiquitination which initiates dimerization and facilitates STING-TBK1 interaction	HEK293, HeLa, human lung fibroblast	Overexpression, RNAi
TRIM56	TRIM56 induces the Lys335 monoubiquitination of cGAS which increases of its dimerization, DNA-binding activity, and cGAMP production.	HEK293T, L929, and Vero	Knockout, overexpression

TRIM protein	Mode of action	Cell line	Method
TRIM65	TRIM65 interacts and promotes K63-linked ubiquitination of MDA5 at lysine 743, which is critical for MDA5 oligomerization and activation.	HEK293, BMDM, MEF HeLa	Overexpression, RNAi
TRIM9s	TRIM9s undergoes Lys-63-linked auto-polyubiquitination and serves as a platform to bridge GSK3 β to TBK1, activation of IRF3 signaling.	HEK293, A549, THP-1	Overexpression, RNAi