The role of Trim25 in development, disease and RNA metabolism

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Abbreviations:

TRIM – TRIpartite Motif protein

ER – Estrogen Receptor

ATBF1 – AT-Binding transcription Factor 1

MDM2 – Mouse Double Minute 2 homolog

TGF-β – Transforming Growth Factor-β

RIG-I – Retinoc acid-Inducible Gene 1

CARD – Caspase Recruitment Domain

RBP – RNA-Binding Proteins

RBD – RNA-Binding Domain

RNP – RNA-Protein

TUT - Terminal Uridylyltransferase

Abstract

Trim25 is a member of the tripartite motif family of E3 ubiquitin ligases. It plays major roles in innate immunity and defence against viral infection, control of cell proliferation and migration of cancer cells. Recent work identified Trim25 as being able to bind to RNA and to regulate Lin28a-mediated uridylation of pre-let-7. Here we review the current knowledge of the role of Trim25 in development, disease and RNA metabolism.

Introduction

The TRIpartite Motif (TRIM) family of E3 ubiquitin ligases consists of more than 70 members, all containing a Really Interesting New Gene (RING) zinc finger, B-Box and coiled-coil domains at the N-terminal end of the protein followed by a variable C-terminal which can help determine substrate specificity [1]. TRIM family proteins perform a variety of roles in humans, including roles in innate immunity and defence against viral infection, control of cell proliferation and migration of cancer cells. Recent work identified several members of this family, Trim25, Trim28, Trim56 and Trim71, as being able to bind to RNA [2-4], adding to a growing pool of RNA-binding E3 ubiquitin ligases [5]. E3 ubiquitin ligases catalyse the addition of ubiquitin moieties to their target proteins, which can have several functions depending on the type of ubiquitin chains. The most well studied of these are K48 and K11-linked polyubiquitin chains which target proteins for degradation via the 26S proteasome, however others such as K63-linked polyubiquitin chains and mono-ubiquitin have been shown to have roles in signalling pathways, protein localisation and modulation of protein-protein interactions [6]. Trim25, for example, can catalyse the addition of K48 and K63-linked polyubiquitin chains and has roles including the targeting of the scaffold protein 14-3-3σ for degradation and as an effector of downstream signalling in the innate immune response to the presence of viral RNA. Our group has recently demonstrated that Trim25 is an RNA-dependent co-factor for the Lin28a/TuT4-mediated uridylation of let-7 precursors [7]. This mini-review explores the various roles of Trim25 and what we can learn from this multi-functional protein in the future.

Roles of Trim25 in Development and Disease

Trim25 in Estrogen Response and Uterine Development

Trim25 is thought to be essential for cell proliferation and organ development in response to estrogen. Trim25 was first identified and designated as Estrogen-responsive Finger Protein (EFP) by the lab of Masami Muramatsu in a screen for regions of human genomic DNA binding to the Estrogen Receptor (ER) and was demonstrated to be upregulated in ER-positive mammary cells [8]. Further work by the same lab identified the mouse homolog of this protein and demonstrated that although mice in which Trim25 was disrupted were viable and fertile, uterine response to estrogen was greatly attenuated and the uteri of the mice were underdeveloped [9, 10]. Further investigation elucidated a possible mechanism for the role of Trim25 in the estrogen response. It was found that in the presence of estrogen Trim25 ubiquitinates ERα, both increasing its transcriptional activity by promoting interaction with transcriptional co-activators such as Tip60 and simultaneously targeting it for degradation (Figure 1) [11]. Trim25 also regulates other proteins involved in the estrogen response. For example, it forms part of an autoregulatory feedback loop controlling levels of the tumour suppressor AT-Binding transcription Factor 1 (ATBF1) (Figure 1). ATBF1 competes with ERα for co-activators and acts as a negative regulator of estrogen-mediated cell proliferation [12]. Trim25, itself upregulated in response to estrogen by ERα, acts as a negative regulator of ATBF1 by

ubiquitinating it and targeting it for degradation. Trim25 performs a similar role regarding the transcription factor and TGF-β co-factor KLF5, targeting it for degradation (Figure 1) [13] .

Trim25 in Cancer

Trim25 has been implicated in the proliferation of many cancer cell types, often in concert with the estrogen response. Trim25 mRNA was detected in MCF7 (breast cancer) cells and activity at its promoter was increased upon estrogen addition [14]. In addition to this, tumour growth in female mice implanted with MCF7 cells was attenuated in a dose-dependent manner upon knockdown of Trim25 with antisense RNA. Further investigation showed that Trim25 was responsible for targeting the negative cell cycle regulator 14-3-3σ for degradation, increasing cell proliferation [15]. Immunohistochemical staining of breast biopsies from breast cancer patients showed that 70% of these tumours were positive for Trim25 expression, 50% of which were increased compared to adjacent normal tissue. Interestingly the same study found that expression of Trim25 did not correlate with the estrogen status of the tumours, indicating that Trim25 may escape from estrogen-mediated control of expression [16]. Moreover, Trim25 immunoreactivity was significantly correlated with poor prognosis of breast cancer patients [17]. Finally, the protein has been found to be overexpressed in several other cancer types including ovarian, gastric and lung cancers while it is downregulated in endometrial carcinomas [18-21].

Trim25 has been found to act through a variety of pathways to promote cancer cell proliferation and migration. For example, two recent papers have suggested a role for Trim25 in the p53/ Mouse double minute 2 homolog (MDM2) circuit. Knockdown of Trim25 by RNAi in lung cancer cells inhibited the proliferation, tumorigenesis and migration of the cells. Trim25 was subsequently shown to interact with p53 in both lung cancer tissues and cells and p53 levels increased upon Trim25 knockdown [18]. In contrast, a separate study found that Trim25 actually increased levels of both p53 and MDM2 by inhibiting the association of MDM2 and p300, a step necessary for the ubiquitination of p53. However, it was also shown that p53 activities in the promotion of apoptosis and DNA damage repair were attenuated in the presence of Trim25, with downregulation of Trim25 resulting in increased acetylation of p53 and increased apoptosis [22]. Trim25 has also been shown to act via the Transforming Growth Factor-β (TGF-β) pathway in gastric cancers where it aids migration and invasion of the cancer cells while having no effect on cell proliferation. Knockdown of Trim25 in these cells decreased levels of migration-associated proteins such as matrix metalloprotease-2 and -9 and adhesion-associated proteins such as β-catenin and fibronectin 1 while levels of another adhesion-associated protein, E-cadherin, were increased. In addition to this levels of TGF-β1 and bone morphogenic protein-4 were decreased upon Trim25 knockdown, while Trim25-dependent increases in cell migration and adhesion activities were abolished when cells were subjected to an inhibitor of TGF-β1 [21]. Taken together, these data indicate that Trim25 can act to stabilise proteins as well as target them for degradation.

Trim25 in Innate Immunity

Numerous proteins from the Trim family, including Trim25, have been shown to have roles in the Retinoc acid-Inducible Gene 1 (RIG-I) mediated interferon response to viral RNAs [23, 24]. RIG-I is a cytosolic Pattern Recognition Receptor (PRR), which recognises viral RNA molecules with a 5'-triphosphate (5'ppp) [25]. Upon detection of 5'ppp-RNA, RIG-I initiates a downstream signalling pathway via MAVS and the protein kinases TBK1 and IKK which results in the phosphorylation-mediated dimerization of the transcription factor IRF3, leading to type I interferon (interferon α and β) expression (Figure 2) [26].

Trim25 is responsible for the K63-linked ubiquitination of one of the two CAspase Recruitment Domains (CARDs) of RIG-I, which is required for efficient recruitment of its downstream partner MAVS and therefore efficient interferon response [27]. Upon detection of 5'ppp-RNA, RIG-I undergoes a conformational change, which exposes the first CARD, allowing interaction with Trim25, which can subsequently ubiquitinate the second CARD. A mutant CARD, which does not associate with Trim25 is not ubiquitinated and RIG-I loses its ability for downstream signalling [28]. RIG-I can also be activated by CARD-dependent binding of short, unanchored polyubiquitin chains generated by Trim25 [29]. The ubiquitination of RIG-I by Trim25 is a target for suppression of the interferon response in more than one case. For example, the influenza A protein NS1 blocks the ubiquitination of the RIG-I CARD by Trim25, allowing the virus to avoid triggering interferon response (Figure 2) [30]. A mutant NS1, which cannot block ubiquitination fails to prevent RIG-I signalling and leads to the loss of virulence for influenza A in mice. This inhibitory activity is also greatly attenuated in an NS1 mutant which does not contain an RNA-binding domain, raising questions about a possible link between this and the RNA-binding activity of Trim25 [31]. Another mechanism for inhibition of RIG-I ubiquitination is dependent on the linear ubiquitin assembly complex (LUBAC), containing the RING-IBR-RING (RBR) E3 ligases HOIP and HOIL-1L. LUBAC acts as a negative feedback modulator to switch off RIG-I signalling by simultaneously competing with Trim25 for RIG-I binding and targeting it for degradation via the proteasome (Figure 2) [32].

Trim25 is an RNA-binding protein

Early experiments identified around 400 mammalian RNA Binding Proteins (RBPs) [33]. Recently however, the development of high through put methods such as interactome RNA-Protein (RNP) capture [3] allowed identification of hundreds of new RNA binding proteins, many without classical RNA Binding Domains (RBDs). They identified 860 RBPs in HeLa cells, half of which do not have canonical RBDs. This included 315 novel RNA-binding proteins that not only were not known to bind RNA, but have no known functional relation to RNA. Similarly Baltz et al. identified 797 RBPs in human embryonic kidney HEK293 cells, 245 of which were novel RBPs and 112 of these without a link to RNA [34]. In addition to this, Beckmann et al. identified 729 RBPs in human hepatocytic HuH-7 cells and, merging their results with the two previous in HeLa and HEK293 cells, concluded that between them they had identified 1217 human RBPs, 326 of which lack known RBDs or any functional relation to RNA [4]. At the moment we do not understand what most of these novel proteins do in relation to regulation of RNA metabolism.

Trim25 was one of the proteins identified as a novel RBP in both Hela and HEK293 cells [3, 4] and in mouse Embryonic Stem Cells (mESCs) [2]. In addition, other TRIM family members were also identified as binding to RNA; Trim28 [34], Trim56 [3, 4, 34], and Trim71 [2, 4]. Trim25 and Trim71, were validated to bind RNA using different RBDs, with Trim71 using NHL repeats while Trim25 utilises its middle section, containing the coiled coil domain. Both proteins are expressed abundantly in mouse ES cells and their expression pattern follows that of other stem cell specific factors (Lin28 and Oct4) by reducing during differentiation [2].

The importance of this was accentuated when we found that Trim25 is a co-factor in Lin28a-mediated uridylation of pre-let-7a-1 [7]. Lin28a is a conserved protein across many species, and it was first described in *C. elegans*, in which mutations of the protein cause defects in developmental timing and accelerate the differentiation of several types of cells [35]. In metazoans Lin28a is abundantly expressed in the early stages of embryonic development, during which it inhibits the biogenesis of miRNAs from the let-7 family. Lin28a expression is gradually restricted with development, which allows de-repression of let-7 production in more developed and differentiated cells [36-40]. The main

mechanism by which Lin28a inhibits let-7 biogenesis is based on its interaction with the conserved terminal loop [41] of pre-let-7 [42-44]. This creates a platform for terminal uridylyltransferase 4 (TUT4) and other members from the TUT family, which catalyze the reaction by adding a poly(U) tail to pre-let-7 [45]. Poly-uridylation ultimately results in pre-let-7 destabilization and a decrease of mature let-7 [45, 46]. The degradation of poly(U) pre-let-7 is performed by 3'-5' Dis3l2 Pearlman syndrome exoribonuclease [47, 48], which has a preference for unstructured and poly-U-rich RNAs [47]. Lin28a is known to bind many miRNAs precursors, however only some are efficiently uridylated by the terminal uridyl transferase TUT4, leading to degradation by the exonuclease Dis3L2 (Figure 3) [49]. We demonstrated that binding of Trim25 to the conserved terminal loop of a pre-let-7a-1 leads to more efficient uridylation in the presence of Lin28 (Figure 3). This shows that the RNA binding ability of Trim25 can have a functional effect on the processing and stability of that RNA. Further studies are needed to determine the mechanism behind this effect, and whether the ubiquitination activity of Trim25 is required for this. It is possible that Trim25 ubiquitinates and stabilises a protein that is needed for Lin28a/TUT4-mediated uridylation, perhaps bridging between Lin28 and TUT4, or aiding the formation of the RNP complex. It is also possible that Trim25 negatively regulates a protein that is blocking uridylation by ubiquitinating it and targeting it for degradation via the proteasome.

To further understand what role Trim25 has as an RBP it will be important to identify what RNAs Trim25 binds to and what RNP complexes it interacts with, especially in the context of RIG-I activation by viral RNAs. It will be also crucial to understand why Trim25 has multiple roles and targets. We hypothesise that binding of Trim25 to RNA can lead to ubiquitination of neighbouring RBPs. Our working model is that when an RBPM enzyme binds RNA in the vicinity of a canonical RNP complex, it will modify it, thereby altering or acting to fine-tune its molecular function. Importantly, we hypothesise that in the context of Trim25 role in RIG-I signalling, RNA can bring Trim25 into closer proximity with RIG-I, allowing it to ubiquitinate RIG-I and activate downstream signalling pathway. Alternatively, Trim25 binding to RNA might hinder its ability to activate RIG-I signalling by sequestering it away from the RIG-I. In this light, it will be also interesting to verify if Trim25's RNA-binding ability is important in its regulation of cell cycle or the estrogen response.

Additionally, Trim25 is only one example of an RNA binding enzyme. Among the novel RBPs are several protein-modifying enzymes, such as kinases (MARK2, SRPK2, PKM1/2, ADK, FASTKD1 and PKN2), phosphatases (PTPN1 and TNS1) and E3 ubiquitin ligases (Trim25, Trim56, Makorin and Roquin) [3]. This shows that the idea that Trim25 is using the RNA as a scaffold to get close to and modify RNP complexes might not limited to Trim proteins. This was also demonstrated by the E3 ligases Dzip3 and Mex3b which bind the long non-coding RNA HOTAIR, which acts as an RNA scaffold bringing them closer to their respective substrates, Ataxin-1 and Snurportin-1, leading to their ubiquitination [50].

Identifying the RNAs and proteins that these new RNA-binding interact with will be pivotal in understanding their roles in development, disease and RNA metabolism. A recent genome-wide bioinformatic screen of the human proteome identified more than 2600 proteins with RBP signatures [51]. Around 30% of these are enzymes. We hypothesize that RNA-binding protein-modifying enzymes, such as Trim25, provide an additional layer of control over canonical RNA-binding proteins and can dictate and define their function. This opens the possibility for a new layer of regulation of RNA metabolism.

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Figure Legends

Figure 1. Trim25 has a role in estrogen response and protein degradation. In the presence of estrogen Trim25 ubiquitinates ERα, increasing its transcriptional activity by promoting interaction with transcriptional co-activators such as Tip60 and simultaneously targeting it for degradation. Trim25 also targets ATBF1 and KLF5 for K48-mediated proteasome degradation.

Figure 2. Trim25 is involved in the RIG-I/interferon pathway. Upon binding to viral 5'ppp-RNAs, RIG-I undergoes a conformational change that allows Trim25 to bind to one of its tandem caspase recruitment domains (2CARDs). Trim25 subsequently ubiquitinates the remaining CARD, leading to RIG-I activation and recruitment of the downstream effectors which in turn lead to expression of type I interferons and other antiviral proteins. Influenza A protein NS1 and endogenous linear ubiquitin assembly complex (LUBAC), containing the HOIP/HOIL-1L proteins inhibit Trim25 ubiquitination of RIG-I.

Figure 3. Trim25 is involved in RNA metabolism. Trim25 is an RNA-dependent co-factor for the Lin28a/TuT4-mediated uridylation and degradation of let-7 precursors (pre-let-7). Lin28a binding to microRNA precursors such as pre-miR-302 does not lead to uridylation due to lack of Trim25 binding. The ubiquitination substrates in Lin28a-mediated uridylation of pre-let-7 are not yet identified.

Figure 1

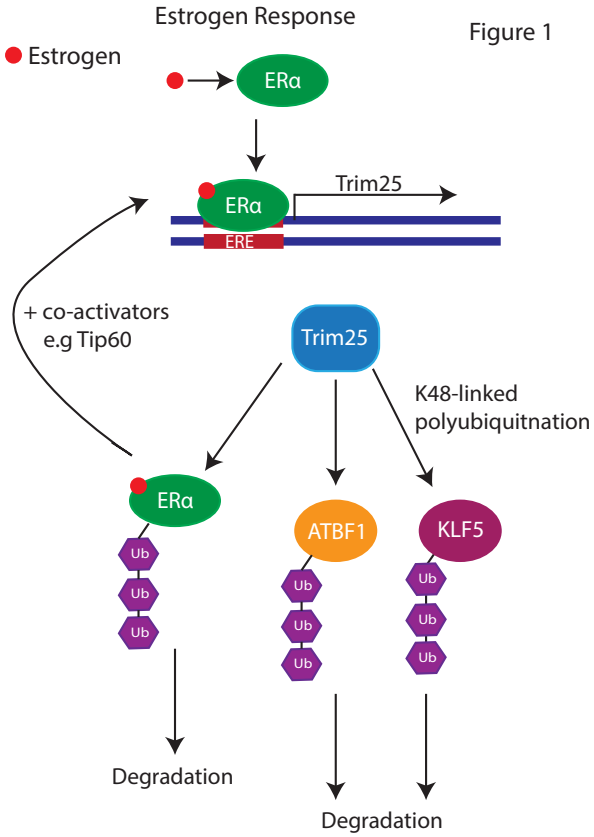

Figure 2

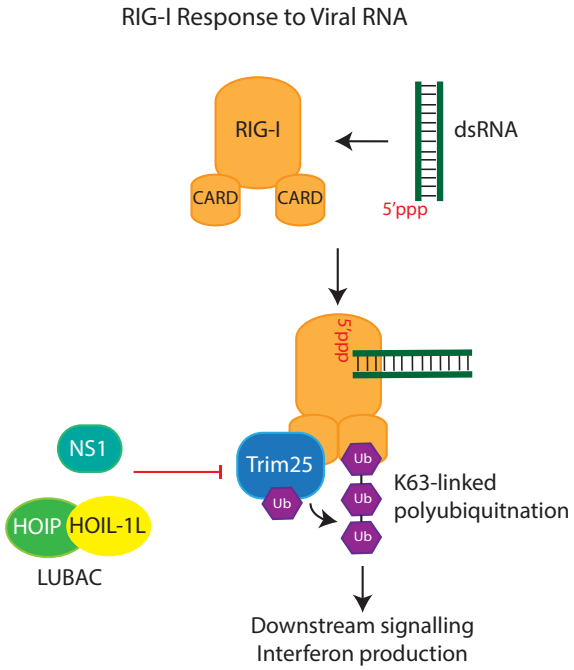

Figure 3