E3 Ubiquitin Ligase TRIP12

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The Thyroid hormone Receptor Interacting Protein 12 (TRIP12) protein belongs to the 28-member Homologous to the E6-AP C-Terminus (HECT) E3 ubiquitin ligase family. First described as an interactor of the thyroid hormone receptor, TRIP12's biological importance was revealed by the embryonic lethality of a murine model bearing an inactivating mutation in the TRIP12 gene. Further studies showed the participation of TRIP12 in the regulation of major biological processes such as cell cycle progression, DNA damage repair, chromatin remodeling, and cell differentiation by an ubiquitination-mediated degradation of key protein substrates. Moreover, alterations of TRIP12 expression have been reported in cancers that can serve as predictive markers of therapeutic response. The TRIP12 gene is also referenced as a causative gene associated to intellectual disorders such as Clark–Baraitser syndrome and is clearly implicated in Autism Spectrum Disorder. The aim of the review is to provide an exhaustive and integrated overview of the different aspects of TRIP12 ranging from its regulation, molecular functions and physio-pathological implications.

Keywords: TRIP12, ubiquitination, E3 ubiquitin ligase ; cancers ; intellectual disorders

1. Introduction

Thyroid hormone Receptor Interacting Protein 12 cDNA was cloned from the human myeloid KG-1 cell line and named the unidentified KIAA0045 human gene. The deduced coding sequence of KIAA0045 showed partial identity to that of the *S. cerevisiae* UFD4 protein (Ubiquitin Fusion Degradation 4)[1]. Another study identified Thyroid hormone Receptor Interacting Protein 12 (TRIP12) as a member of the structurally and functionally related E3 ubiquitin ligases based on the identification of a domain homologous to the E6-associated protein carboxyl terminus (HECT domain), which is a protein that induces the ubiquitin-dependent degradation of P53 in the presence of the E6 protein from the papillomavirus[2].

TRIP12 was later characterized as a thyroid hormone receptor-interacting protein by the trap yeast interaction system to isolate proteins that interact with the ligand binding/dimerization/transcriptional activation domain of the rat TR-β1 (Thyroid Receptor-β1)[3]. Fifteen cDNAs encoding Thyroid hormone Receptor Interacting Proteins (TRIPs) were isolated. All the TRIPs interacted with the Thyroid hormone Receptor (TR), but only when thyroid hormone T3 was present (TRIP1 to TRIP11) or only when T3 was absent (TRIP12 to TRIP15). Protein sequences of the fifteen TRIPs are unrelated, but sequences similarities with known proteins and functional motifs were found for nine of them based on blast alignment analyses with no demonstration of functional significance. The TRIP12 sequence was found to show 55% identity with the C-terminus of E6-associated protein, and more than a decade following its identification, TRIP12 was demonstrated to function as an E3 ubiquitin ligase[4]. Evidence of functional roles was later provided for the other TRIPs proteins (Supplemental Table). Among them, TRIP12 is the only TRIP family member bearing an E3 ubiquitin ligase activity.

The biological importance of TRIP12 was revealed by the embryonic lethality of a murine model bearing an inactivating mutation in the *TRIP12* gene[5]. Several molecular functions of TRIP12 in important cellular processes and signaling pathways have been demonstrated in recent years. Accumulating evidence indicate that TRIP12 ubiquitinates key proteins for cell homeostasis, regulates gene expression (See Section 5), and plays important roles in cancers and neurological diseases. Up to now, more than 58 publications related to TRIP12 have been referenced in PubMed, half of them in the last 5 years. We have recently contributed to broadening knowledge on the role of TRIP12 by discovering a new substrate essential for pancreatic acinar cells differentiation [6] and by demonstrating that TRIP12 is a new chromatin-associated protein with several implications in the cell cycle progression and in the maintenance of genome integrity[7]. Due to its implication in several important physiological and pathological processes, TRIP12 now emerges as a protein of interest for molecular mechanisms researches and as a potential new therapeutic target.

2. Functions of TRIP12 in Ubiquitin-Mediated Proteolysis

A detailed description of ubiquitination process, different ubiquitin ligase families, and functions are excellently reviewed elsewhere [8][9][10][11]. In this section, we will provide an overview of the essential information on TRIP12 actions in ubiquitin-dependent proteolytic pathways.

2.1. Ubiquitination

Ubiquitination is an essential ATP-requiring process leading to protein modification by the covalent conjugation of ubiquitin, which is a highly conserved 76 aa polypeptide (≈8.5 kDa)[9]. Although best known for targeting proteins to degradation by the 26S proteasome, ubiquitination is potentially linked to all cellular processes. Canonical ubiquitin conjugation involves the attachment of the C-terminal glycine 76 (G76) of ubiquitin (Ub) to the ε-amino group of a lysine on the protein substrate. Substrates can be either modified by a single ubiquitin on one (monoubiquitination) or more sites (multi-mono-ubiquitination) or by chains of ubiquitin[11]. In the case of poly (or multi) Ub chains, the C-terminal G76 of one Ub molecule is linked to the ε-NH_2 group of one of the seven lysines (K6, K11, K27, K29, K33, K48, or K63) of the preceding ubiquitin. The poly-Ub chains can have different lengths and topologies resulting in different consequences on the fate of the modified protein substrate. For instance, K48-linked polyubiquitination primes proteins for proteolytic destruction by the proteasome, whereas non-K48 linkages target proteins for various cellular functions and responses[10][11].

Ubiquitination is ensured by a three step-concerted action of three enzymes. An Ub-activating enzyme (E1) catalyzes the formation of a thiol-ester linkage between the C-terminal lysine G76 of Ub and the active cysteine (C) residue of E1; the Ub molecule is transferred to the cysteine of the Ub-conjugating enzyme (E2). E3 ligases are classified into three major classes, the RING (Really Interesting New Gene), the RBR (RING-between RING-RING), and the HECT ligases. For HECT and RBR E3 ligases, the transfer of Ub to the substrate lysine-amino group involves a thioester intermediate with a conserved cysteine in the catalytic site of the HECT domain or the Rcat domain, respectively[8]. The RING E3 ligases catalyze the direct transfer of ubiquitin from a thioester-linked E2-ubiquitin conjugate to a substrate[8]. In some cases, the cooperation of an additional E4 ubiquitin ligase, which may encode for independent ligase activity or enhance the processivity of the E3, is required. To act as catalysts, E3s recruit their substrates for degradation by recognizing a specific portion in the protein sequence or degron. Ubiquitin conjugation can be reversed by deubiquitinating enzymes (DUBs)[12].

2.2. Known TRIP12 Substrates

Based on the literature, TRIP12 regulates the stability and promotes the proteolysis of nine protein substrates: ASXL1 (Additional Sex Combs Like 1)[13], USP7 (Ubiquitin-Specific Peptidase 7)[14], PTF1a (Pancreas Transcription Factor 1a)[6], SOX6 (SRY-Box Transcription Factor 6)[15], RNF168 (Ring Finger Protein 168)[16], BAF57 (BRG1-Associated Factor 57) also known as SMARCE1 (SWI/SNF Related, Matrix Associated, Actin-Dependent Regulator Of Chromatin, Subfamily E, Member 1)[17], P14/ARF (P14/Alternate Reading Frame) also known as CDKN2A (Cyclin Dependent Kinase Inhibitor 2A), for which TRIP12 was named ULF (Ubiquitin Ligase for ARF)[18], APP-BP1 (Amyloid Protein-Binding Protein 1) also known as NAE1 (NEDD8-Activating Enzyme E1 Subunit 1)[4] and PARP1 (Poly-(ADP ribose) polymerase 1)[19]. These proteins exert important functions in biological and/or pathological processes that will be outlined in Section 5.

The TRIP12 HECT domain (detailed in Section 3.4) is essential for the degradation of these proteins, but the role of TRIP12 as direct interacting E3 ligase is still questionable for ASXL1[13] and RNF168[16] for which experimental approaches that prove ubiquitination by TRIP12 are lacking. TRIP12 polyubiquitinates its substrates with the exception of APP-BP1, which is monoubiquitinated[4]. APP-BP1 polyubiquitination requires an additional E4 activity [4]. TRIP12 mediated-K48-linked polyubiquitination was demonstrated only for USP7[14] and PTF1a[6]. While the interaction between the E3 ligase and its substrates was validated, the interaction domains of TRIP12 have been mapped only in few cases. Although these studies used different isoforms of TRIP12, they all emphasize the role of consensus protein/protein interaction domains of TRIP12. A region (aa 611–1259 of isoform c) that includes the WWE domain but not ARM repeats is responsible for the interaction with APP-BP1[4]. A sequence of 577 aa (aa 234–810 of isoform a) preceding the WWE domains interacts with USP7[14]. In addition, TRIP12 interacts with parylated PARP1 via its WWE (aa 797–911 of isoform a) domain. TRIP12 is freshly considered as an identified PAR-targeted ubiquitin ligase (PTUbL) controlling PARP1 turnover[19] Finally, we demonstrated that PTF1a binds a region containing ARM repeats and the WWE domain (aa 446–1552 of isoform c)[6].

Interacting domains or ubiquitinated residues were identified for certain proteins regulated by TRIP12. A sequence of 80 aa within and flanking the central coiled-coil region of BAF57 was found to be critical for BAF57 stabilization and to contain lysine residues essential for ubiquitination[20]. The N-terminal part of Tumor necrosis factor-receptor associated

factor (TRAF), also known as the Meprin And TRAF-C Homology (MATH) domain of USP7, is responsible for its interaction with TRIP12[14]. We found that the lysine 312 of PTF1a is essential for its TRIP12-mediated degradation[6]. It was previously reported that the tumor suppressor ARF is a natural lysine-less protein that undergoes N-terminal polyubiquitination[21].

Intriguingly, TRIP12 targets several proteins that are unbound to their physiological binding partners. This is the case for BAF57, which is a subunit of the SWI/SNF (SWItch/Sucrose Non-Fermentable) chromatin remodeling complex (detailed in Section 5.4.)[17]. Most if not all SWI/SNF subunits are assembled into a complex for which maintenance of the stoichiometry is essential and no free subunits exist in the cell; otherwise, they are degraded. The stabilization and protection of BAF57 from proteasome-mediated degradation operates via protein–protein interaction with the BAF155 subunit [22]. TRIP12 was demonstrated to compete with BAF155 for BAF57 and to degrade only an unbound form of BAF57[20]. The same type of interaction was found for the ubiquitination of the neddylation E1 enzyme subunit APP-BP1[4]. In fact, TRIP12 does not interact with APP-BP1 in the heterodimeric form bound to Uba3 (Ubiquitin-Like Modifier-Activating Enzyme 3), which is the specific active E1 of the NEDD8 pathway. A similar scheme is described for ARF, which is stabilized and protected from TRIP12 ubiquitination when sequestered by nucleophosmin and TRADD (NFR1-Associated Death Domain Protein) in the nucleolus (detailed in Section 5.2.)[18][23]. Indirect evidence suggests a similar mode of action for the degradation of RNF168[16]. Indeed, the destabilization of RNF168 induced by a silencing of its binding partner HERC2 (HECT and RLD Domain Containing E3 Ubiquitin Protein Ligase 2) could be mitigated by a depletion of TRIP12. The TRIP12-ubiquitinated lysine 312 of PTF1a is located near the domain interacting with RBP (mammalian suppressor of Hairless) essential for the transcriptional activity of PTF1a. One may hypothesize that free PTF1a could be ubiquitinated, while binding to RBP could prevent TRIP12-mediated PTF1a degradation. Therefore, TRIP12 appears as a regulator of the balance between bound and unbound forms of its protein substrates to maintain them in their complexed forms.

2.3. Role of TRIP12 in Ubiquitin Fusion Degradation and N-Degron Pathways

TRIP12 is sequelogous to the UFD4 HECT E3 ligase, which catalyzes the ubiquitination of Ubiquitin Fusion Degradation (UFD) substrates in yeast[24]. These substrates are artificial fusion proteins consisting of an N-terminal uncleavable ubiquitin moiety that acts as a degron. The UFD pathway consists in the poly-ubiquitination of the uncleavable N-terminal ubiquitin. TRIP12 was shown to mediate the degradation of the aberrant form of ubiquitin named UBB^{+1}[25]. UBB^{+1} is a physiological human UFD substrate resulting from a dinucleotide deletion in the mRNA of the ubiquitin B gene. UBB^{+1} accumulates in the brain in neurodegenerative disorders[26]. The HECT E3 HUWE1 (HECT, UBA, and WWE domain containing E3 ubiquitin protein ligase 1) was reported to cooperate with TRIP12 for the degradation of UBB^{+1}[27]. Although the conservation of the UFD pathway from yeast to human attests its importance for proteolysis in cells, except for UBB^{+1}, no other native substrates have been identified. Most studies use artificial substrates for specific applications. In particular, enhanced immune response after UFD ubiquitination of antigens opens up interesting perspectives in the development of tumor vaccines[28].

The N-degron pathway (formerly N-end rule pathway) is another ubiquitin-dependent proteolytic pathway that regulates the half-life of proteins according to N-terminal degradation signals or N-degrons. UBR1, UBR2, UBR4, and UBR5 (ubiquitin protein ligase E3 component N-recognin) are the four E3s (or N-recognins) encoded by the mammalian genomes that can recognize Arg/N-degrons[29]. Interestingly, a study demonstrated that UBR1 and UFD4 E3s interact and cooperate to enhance substrate ubiquitination in both pathways in yeast[30]. This mutually cooperative physical interaction between E3s operating in parallel pathways are likely to be relevant to all eukaryotes and might apply to TRIP12 and UBR5 control of RNF168 accumulation[16].

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