E3 Ubiquitin Ligases – Structure, Function, Disease Implications, and Therapeutic Options

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ABSTRACT

E3 ubiquitin ligases are essential mediators in the ubiquitination cascade, governing protein degradation, DNA repair, and immune responses. This review examines the three primary classes of E3 ligases—HECT, RING, and RBR—detailing their unique structural architectures and catalytic mechanisms. We explore how structural motifs, such as the RING finger and the HECT domain, facilitate the transfer of ubiquitin to substrates. Furthermore, we discuss the clinical implications of E3 ligase dysregulation in cancer and neurodegenerative disorders, alongside emerging therapeutic strategies like PROTACs. By analyzing specific ligases such as WWP2, TRIM31, and TRIM71, this paper highlights the potential of targeting these enzymes to address complex human diseases.

INTRODUCTION

E3 ubiquitin ligases are proteins that aid in the transfer of ubiquitin from E2 ubiquitin-conjugating enzymes to a protein substrate. This enables the last step of ubiquitination (also known as ubiquitylation), the process in which one or more ubiquitin monomers covalently attach to the ε-amino group of the substrate's lysine residue, to commence. Ubiquitination is crucial for protein degradation, which enables various cellular processes, including apoptosis, DNA repair, and immune response.

There are three main classes of ubiquitin ligases: HECT (Homologous to E6AP C-Terminus), RING (Really Interesting New Gene), and RBR (RING-between-RING).[1] RING E3 ligases aid in the transfer of ubiquitin directly to the substrate from the E2 enzyme. RING E3 ligases play pivotal roles in DNA repair, and mutations in those could result in adverse effects in the body, such as a heightened risk of cancer development.[2]

Different Classes of Ubiquitin Ligases

HECT E3 ubiquitin ligase

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These ligases have a conserved C-terminal region, which is essential for their unique function.[3] The HECT E3 ubiquitin ligases form a direct covalent bond with ubiquitin before transferring it to the target protein. E1 ubiquitin-activating enzyme activates ubiquitin in an ATP-dependent manner, which is then transferred to an E2 ubiquitin-conjugation enzyme. The HECT E3 ubiquitin ligase forms a thioester bond with ubiquitin before transferring it to a lysine residue on the substrate protein, typically leading to its degradation via the proteasome. HECT E3 ligases are involved in various essential cellular processes, including Protein Degradation, DNA Repair, Cell Cycle Regulation, and Signal Transduction. Dysregulation of HECT E3 ligases has been implicated in several diseases, including Cancer, Neurodegenerative Disorders like Parkinson's, Alzheimer's, and ALS, and Infectious Diseases.

RING (Really Interesting New Gene) E3 ubiquitin Ligase

RING (Really Interesting New Gene) E3 ubiquitin ligases are a major class of enzymes that play a pivotal role in the ubiquitination process, a fundamental mechanism for regulating protein stability, function, and localization. These ligases contribute to diverse cellular processes, including protein degradation, signal transduction, DNA repair, and immune response. Given their critical role in maintaining cellular homeostasis, dysregulation of RING E3 ligases has been linked to various diseases, including cancer, neurodegenerative disorders, and autoimmune conditions.[4]

RING E3 ubiquitin ligases are characterized by the presence of a RING domain, a zinc-finger-like structure that enables them to mediate ubiquitin transfer. Unlike HECT E3 ligases, which form a covalent thioester intermediate with ubiquitin, RING E3 ligases act as scaffolds, bringing the E2 ubiquitin-conjugating enzyme and the substrate into proximity to facilitate direct ubiquitin transfer.[4]

The ubiquitination process follows a three-step enzymatic cascade: Activation, Conjugation, and Ligation. An E1 ubiquitin-activating enzyme activates ubiquitin in an ATP-dependent manner, which is then transferred to an E2 ubiquitin-conjugating enzyme via conjugation. Later, the RING E3 ligase facilitates the direct transfer of ubiquitin from the E2 enzyme to the substrate without forming a covalent intermediate.

Types of RINGS E3 Ligases

RING E3 ligases can function as monomeric proteins or multisubunit complexes, providing versatility in substrate recognition and regulation.[5] Examples of monomeric RING Ligases include MDM2, which regulates the degradation of the p53 tumor suppressor. Multisubunit RING Ligases include Cullin-RING Ligases (CRLs), such as SCF (Skp1-Cullin-F-box) and APC/C (Anaphase-Promoting Complex/Cyclosome), which regulate cell cycle progression and mitosis.

Biological Roles of RING E3 Ligases

RING E3 ligases play key roles in Protein Degradation, involving targeting proteins for degradation via the ubiquitin-proteasome system to maintain proteostasis, cell cycle regulation, which controls cell division by regulating cyclins, CDK inhibitors, mitotic regulators, and DNA Damage Response, which involves modulating DNA repair proteins to maintain genomic integrity, Immune Regulation influencing immune signaling pathways through the ubiquitination of key regulators.

Clinical Relevance and Disease Implications

Dysregulation of RING E3 ligases is implicated in several diseases, like cancer.[2] Overactive RING ligases, such as MDM2, promote the degradation of tumor suppressors like p53, contributing to uncontrolled cell proliferation.[6] It is also implicated in many neurodegenerative disorders. Impaired ubiquitination can lead to the accumulation of toxic proteins, as seen in Alzheimer's and Parkinson's disease. It is also seen to affect autoimmune diseases, as aberrant ubiquitination of immune regulators can result in excessive or deficient immune responses.

Therapeutic Potential and Research Directions

RING E3 ligases are emerging as promising drug targets, with research focused on inhibitors of overactive RING ligases, such as MDM2 inhibitors for cancer therapy.[2] Targeted protein degradation strategies, like PROTACs (Proteolysis-Targeting Chimeras), which leverage RING ligases to selectively degrade disease-causing proteins, and gene therapy and CRISPR-based interventions to modulate RING ligase function in genetic disorders.

Protein Structures and Functions of E3 Ubiquitin Ligases

WWP2

The protein WWP2 is an HECT E3 ubiquitin ligase that plays a crucial role in DNA repair and gene expression, thereby helping the body maintain homeostasis. Because it can target specific substrate proteins, it plays an important role in disease formation. Due to this property, there is a huge therapeutic potential for targeting WWP2.[1]

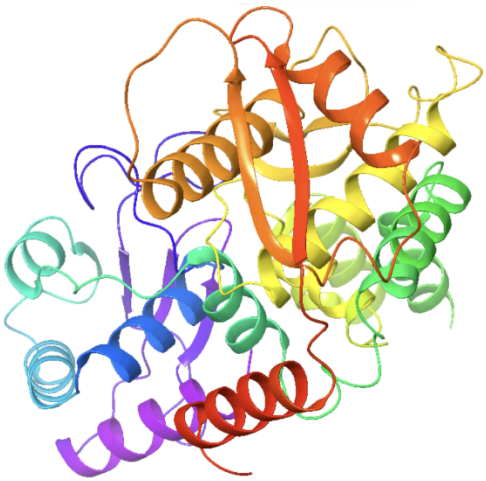

Figure 1: Crystal Structure of WWP2 (PDB: 4Y07)

Crystal structure studies of WWP2 (Figure 1) and other HECT-type E3 ligases, such as NEDD4, show that the HECT domain is composed of two lobes: an N-terminal lobe that binds to the E2 enzyme and a C-terminal lobe that contains the catalytic cysteine residue (Cys838 in WWP2). This cysteine forms a thioester bond with ubiquitin before it is transferred to the substrate protein. Other conserved amino acids, such as tyrosine, aspartate, and histidine, help stabilize the active site and correctly position ubiquitin during the transfer process. The flexibility between the two lobes allows the enzyme to move into the proper position to complete ubiquitination efficiently. These structural features are essential for WWP2's activity and are important to consider when exploring WWP2 as a therapeutic target.

TRIM31

TRIM31 is a RING E3 ubiquitin ligase, a part of the TRIM family, which is involved in transcriptional regulation, intracellular and immune signal transduction, and apoptosis. TRIM31 promotes the ubiquitination of the p53 protein, a tumor suppressor. This makes it a promising therapeutic target.[7]

Like other RING-type E3 ligases, TRIM31 does not form a covalent intermediate with ubiquitin. Instead, it facilitates the direct transfer of ubiquitin from the E2 conjugating enzyme to the substrate. The RING domain in TRIM31 contains conserved cysteine and histidine residues, such as Cys15, His17, and Cys28, that coordinate two zinc ions, which are necessary to stabilize the domain's structure. Structural data from similar TRIM proteins (e.g., TRIM25) show that these zinc-binding motifs maintain the conformation required for E2 interaction. In addition, hydrophobic residues on the surface of the RING domain are involved in positioning the E2-ubiquitin complex. This structural arrangement enables TRIM31 to regulate the degradation of p53 effectively, potentially contributing to its role in cancer therapy.

The structure available in the PDB data bank is only the ring domain, not the full-length trim 31 protein, which has 425 residues, as opposed to what we see in the NMR structure, which has 56 residues.

TRIM-NHL

The TRIM-NHL sub-family is part of the TRIM family, which can bind to RNA, causing them to be classified as RNA-binding ubiquitin Ligases (RBULs). Due to this property, they play significant roles in many cellular processes, and their dysregulation can lead to pathological conditions.[8]

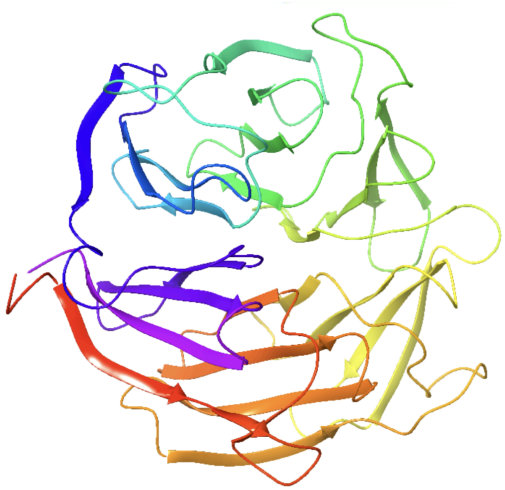

Figure 2: Crystal Structure of TRIM-NHL (PDB: 7QRV)

TRIM-NHL proteins are unique in that they combine E3 ligase activity with RNA-binding ability. In addition to the typical RING domain found in TRIM family members, they contain a C-terminal NHL domain, which adopts a six-bladed β-propeller structure. This domain enables RNA binding through conserved aromatic amino acids such as tryptophan and tyrosine (e.g., Trp575 and Tyr527 in TRIM71), which engage in π-stacking interactions with RNA bases. Meanwhile, the RING domain contains zinc-coordinating cysteine and histidine residues that maintain the structural integrity required for ubiquitination. This combination of RNA-binding and ubiquitin ligase activity allows TRIM-NHL proteins to regulate gene expression at the post-transcriptional level. Disruption in their normal function has been linked to developmental disorders and other diseases, making them important targets for further study.

TRIM71

TRIM71 is an RING E3 ubiquitin ligase that belongs to the TRIM family, which is crucial for maintaining homeostasis and plays a critical role in the immune response. TRIM71 helps regulate miRNA activity, stem cell behavior, and cell reprogramming.[9] TRIM71 binds to target

mRNAs, thereby triggering mRNA degradation. Consequently, it plays a role in neural differentiation and embryogenesis.

When mutated, the TRIM71 protein can cause a multitude of detrimental effects on the human body. Namely, it is linked to diseases like Congenital Hydrocephalus (CH) and even some cancers. Since the target mRNAs that the protein binds are different, it can cause errors in the translation process, leading to mutated genes, which can further develop into malignancies.

The TRIM71 protein, being part of the TRIM family, has an N-terminal RING finger, a B-box, and a coiled-coil domain (Figure 3). The NHL domains of TRIM71 adopt a conserved six-bladed β-propeller architecture. A large amount of diversity is present in its C-termini, which contain many protein interaction modules, of which the properties of many remain unknown.[10]

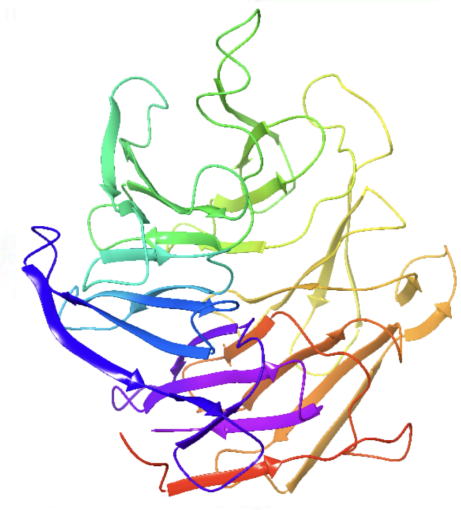

Figure 3: Crystal Structure of TRIM71 (PDB: 7QRX)

TRIM71 Domains

TRIM71 has a RING Finger domain located at the N-terminus. Since it is a type of zinc finger, it enables TRIM71 to tag target proteins for ubiquitination. Following that, it has B-box domains, which contain one or two B-box zinc finger motifs. They contribute to the protein-protein interactions and add to structural integrity. The coiled-coil domain facilitates the formation of homo- or heterodimers, which help set up the functional assembly of TRIM71 and its interactions with other proteins. The NHL domains help the protein bind with mRNA. Current research to find potential inhibitors for TRIM71 is ongoing.[11]

CONCLUSION

Based on the above findings, mutations to E3 ubiquitin ligases can cause numerous adverse health effects, namely CH, non-Hodgkin lymphoma, and other diseases. Research is ongoing to find inhibitors for such mutated ligases, though none have been discovered yet.

Current research is primarily focused on understanding behaviors and mechanisms of E3 ubiquitin ligases. If found, such breakthroughs would be beneficial in the treatment and prevention of the aforementioned diseases and provide insights into finding inhibitors to other ubiquitin ligases.

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