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E3 ubiquitin ligases in B-cell malignancies

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Summary

Ubiquitylation is a post-translational modification (PTM) that controls various cellular signaling pathways. It is orchestrated by a three-step enzymatic cascade known as the ubiquitin proteasome system (UPS). E3 ligases dictate the specificity to the substrates, primarily leading to proteasome-dependent degradation. Deregulation of the UPS components by various mechanisms contributes to the pathogenesis of cancer. This review focuses on E3 ligase-substrate pairings that are implicated in B-cell malignancies. Understanding the molecular mechanism of specific E3 ubiquitin ligases will present potential opportunities for the development of targeted therapeutic approaches.

The Ubiquitin Proteasome System

The ubiquitin proteasome system (UPS) plays a significant role in the regulation of cell growth and survival, in addition to maintaining cellular homeostasis. By means of the UPS, cells can precisely and temporally degrade approximately 80% of the entire proteome. However, failure to do so results in numerous diseases, including hematological malignancies and cancer¹⁻³. Protein ubiquitylation is catalyzed by a three-step enzymatic cascade in which the ubiquitin is first activated by an E1 enzyme (ubiquitin-activating enzyme) and subsequently transferred to an E2 enzyme (ubiquitin-conjugating enzyme). Finally, ubiquitin is attached to a specific substrate that is selected by an E3 ubiquitin ligase that governs substrate specificity. One of the principal outputs of protein ubiquitylation is degradation via the proteasome complex. The proteasome comprises of a regulatory 19S cap complex that unfolds the substrates in an ATP-dependent manner and a catalytic 20S core complex that has proteolytic activities¹. Proteins that are tagged by the ubiquitin chains are

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recognized, deubiquitylated, and unfolded by the 19S complex and subsequently fed through the inner channel of the 20S proteasome chamber, which cleaves proteins into peptides⁴.

Ubiquitin contains total eight attachment sites (seven lysine residues and the amino N-terminus) for the formation of polymeric chains⁵. Substrates can be modified at multiple lysine residues with a single ubiquitin molecule (multimono-ubiquitylation), or a single ubiquitin molecule can build a chain using ubiquitin as substrate⁶. Moreover, ubiquitin chains can be homotypic conjugates where they are elongated through the same lysine as in Lys11-, Lys48-, Lys63-linked chains or methionine (M-linked) residue, as in linear chains⁶. Lys-11-linked chains and Lys48-linked chains target proteins for the proteosomal degradation^{7, 8}. On the other hand, Lys-63-linked chains regulate DNA repair, endocytic trafficking, NF- κ B activation, and assembling a signaling complex for mRNA translation⁹⁻¹². M-linked chains or linear ubiquitin chains play an important role in immune, inflammatory and NF- κ B signaling¹³⁻¹⁵. The significance and the roles of Lys6-, Lys27-, Lys29-, Lys33- linked chains are still poorly understood although, recently, they have been implicated in DNA repair, trans-Golgi trafficking, and mitochondria damage^{16,17}.

Ubiquitin ligases

Ubiquitin ligases are categorized into different classes based on their specific structural configuration and the composition of subunits—HECT (homologous to E6-AP1 (E6-associated protein 1) carboxy-terminus)-type, RING (really interesting new gene)-finger-type, U-box-type, or RING-in-between RING (RBR)-type (Fig. 1).

The HECT-type E3 ligases are the only ones that demonstrate intrinsic catalytic activity, as they receive the ubiquitin from an E2 enzyme and transfer it to the substrate¹⁸. HECT-type E3 ligases are the first family of E3 ligases that have been described and consist of ~30 HECT domain E3 ligases in mammals. They play important roles in several biologic areas, including protein trafficking, cell growth and survival, immune regulation, and many others¹⁹. The N-terminus of the HECT-type E3 ligases mediates substrate targeting, while the C-terminus contains the conserved HECT domain, which interacts with the E2 and contains an active cysteine that accepts the ubiquitin-moiety. The topology of the HECT-type E3s with the E2s depends on the status of the ubiquitin transfer of E2 and the non-covalent interaction with all N-terminus, C-terminus, E2 and ubiquitin^{20, 21}.

The RING-finger and the U-box- type E3 ligases act as a scaffold protein to bridge an E2 enzyme and a substrate proximally for ubiquitin conjugation (Fig. 1). The RING-finger-type E3 ligases are generally thought to be the biggest family of ligases and contain a Zn²⁺-coordinating domain with spaced cysteine and histidine residues, facilitating E2-dependent ubiquitylation²². The RING finger ubiquitin ligase family functions either as a monomer, dimer, or a multi-subunit complex. Homodimerization and/or heterodimerization usually occurs through the RING finger domain²³.

Multi-subunit RING-type E3 ligases are exemplified by the CULLIN-RING-ligase (CRL) and the anaphase-promoting complex/cyclosome (APC/C)¹⁹. CRLs constitute the biggest family of other multi-component E3 ligases. These consist of a cullin scaffold protein

(CULLIN 1, 2, 3, 4A, 4B, 5, 7), a substrate receptor, an adaptor, and a RING domain protein for E2 enzyme recruitment. A large body of evidence suggests that CRLs share a similar molecular architecture, where substrates are recruited at the N-terminal regions of the cullins, which comprise of an adaptor protein and a substrate receptor^{24, 25}. For the well-characterized example of CULLIN1, the adaptor protein is SKP1, which recruits numerous substrate receptors (*i.e.*, the F-box proteins). SKP1 can interact with both CULLIN1 and CULLIN7, while CULLIN2 and CULLIN5 utilize the elongin B/C adaptor proteins to recruit the substrate receptors; a family of proteins, which is named suppressor of cytokine signaling/elongin BC (SOCS/BC)-box-protein. CULLIN3 is unique; it utilizes a BTB domain-containing protein that can function both as an adaptor and a substrate receptor. CULLIN4A uses the adaptor protein DNA damage-binding protein-1 (DDB1), which in turns binds to substrate receptor proteins such as the DDB1 and CUL4 associated factors (DCAFs). The biological function of the cullins is involved in various cellular processes; these include cell cycle, signaling transduction, cell proliferation and survival, and DNA damage response²⁴.

A third class of E3 ligases is that of the RBR E3 ligases, which consists of a RING1, an intermediate RING (IBR), and a RING2 domain (Fig. 1). These ligases use a unique mode of catalyzing the ubiquitin transfer by combining both the RING-type and HECT-type E3 ligase mechanisms. Similar to classical RING-type ligases, these recognize the E2 conjugated ubiquitin by the RING1 domain, and form a HECT-like intermediate by accepting the ubiquitin to the cysteine of the RING2 domain. The ubiquitin is finally transferred to the substrate by the RING2²⁶.

Ubiquitin ligases in B-cell lymphoid malignancies

Increasing evidence supports that the B-cell lymphoid malignancies develop from various stages of B-cells, hijacking the mechanisms that drive B-cell differentiation and activation²⁷. For instance, mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (CLL) originate from pre-germinal center (GC) mature B-cells, while most of the non-Hodgkin's lymphomas and multiple myelomas (MMs) originate from the GC B-cells or B-cells that have gone through a germinal center reaction. Each of these B-cell lymphoid malignancies features different genomic alterations, including chromosomal translocations, amplifications, frameshift deletions, and mutations, to ultimately activate the oncogenic signaling programs that promote growth and survival²⁸. Sequencing efforts have identified many E3 ubiquitin ligases that are mutated, amplified, or deleted in B-cell lymphoid malignancies, functioning either as a tumor suppressor or oncogene²⁹. Importantly, the success of a proteasome inhibitor (bortezomib), in both MM and MCL, has further inspired the investigation of the biological significance of protein ubiquitylation and degradation in B-cell cancers³⁰⁻³². Thus, the understanding of how the ubiquitylation of protein is achieved, and of the downstream molecular events, is critical for the development of therapeutic approaches. Targeting specific E3 ubiquitin ligases has garnered attention as it confers a selective advantage over a proteasome inhibitor by preventing any unwanted toxic effects on intracellular proteins.

In this review, we will discuss a wide spectrum of E3 ligases that have been implicated in the pathogenesis of B-cell malignancies (Fig.1 and Table.1).

KLHL6

Kelch-like protein 6 (KLHL6) is a CULLIN3-based E3 ligase. KLHL6 utilizes the BTB domain to interact with CULLIN3 and the Kelch domain to recruit substrates³³. KLHL6 is expressed at all stages of B-cell development and is up-regulated in sheep Peyer's patch, human tonsil B cells, and germinal center (GC) B-cells^{34, 35}. *Klhl6*^{-/-} mice are viable, but they display a smaller spleen, reduced GC formation upon antigen-stimulation, and defects in mature B-cell populations³⁴. Recently, Bertocci et al. have shown that the reduction of mature B cells is due to the inability of the transitional type 1 B cells to survive and segregate to the next stage³⁶. Furthermore, type 1 B cells of *Klhl6*^{-/-} mice display an overexpression of the cell cycle and proliferation genes, whose expression is down-regulated upon BAFF stimulation. Overall, KLHL6 seems to control BAFF-induced B cell differentiation by regulating the cell cycle pathway genes.

It is important to note that point mutations in the KLHL6 locus occur in B-cell malignancies. Most of the mutations hit diffuse large B-cell lymphoma (DLBCL), and more rarely, the chronic lymphocytic leukaemia (CLL), follicular lymphoma (FL), multiple myeloma (MM) and marginal zone lymphoma (MZL)³⁷⁻⁴⁴. Most KLHL6 mutations are missense, and localize in the BTB domain, resulting in a protein that lacks the ability to interact with CULLIN3³³. Although these cancer-associated mutations are equally stratified between the germinal center (GC)- and the activated B cell (ABC)-DLBCL subtypes, they correlate specifically with poorer survival rate in ABC-DLBCL patients. Whether these mutations would have the same loss-of-function phenotype in other B-cell malignancies still remains to be determined.

Roquin2 is the first bona-fide substrate of KLHL6 that was recently identified using proteomic approaches. It is an RNA-binding protein that promotes mRNA decay of tumor suppressors and NF- κ B inhibitor genes, many of which are dependent on B-cell receptor (BCR) signaling. Thus, the loss of Roquin2 degradation in DLBCLs with KLHL6 mutations results in the hyperactivation of the NF- κ B pathway, promoting cancer cell growth and proliferation³³.

Further studies will be required to identify other substrates and to fully understand the contribution of KLHL6 to the pathogenesis of B-cell malignancies.

LUBAC

The linear ubiquitin chain assembly complex (LUBAC) is the only known E3 ligase responsible for the linear polyubiquitin chain formation¹³. LUBAC consists of heme-oxidized iron-responsive element-binding protein 2 (IRP2) ubiquitin ligase-1 [HOIL-1; also known as RanBP-type and C3HC4-type zinc finger-containing protein 1 (RBCK1)], the HOIL-1-interacting protein (HOIP; also known as RNF31) and the SH3 and multiple ankyrin repeat domains protein (SHANK)-associated RBCK1 homology (RH)-domain-interacting protein (SHARPIN). Both HOIL-1 and HOIP are RING-between-RING (RBR) E3 ligases, but HOIP is considered to be the key component for LUBAC catalytic activity.

While HOIP alone is not sufficient to induce linear ubiquitin chains, any combination of the LUBAC components with HOIP can stimulate a catalysis of linear ubiquitin chains^{14,15}. LUBAC function has been implicated in various signaling pathways such as TNF, Toll-like receptors (TLRs), CD40/CD40L, RIG-I, IL-1 β , and the nucleotide-binding oligomerization domain 2 (NOD2)⁴⁵.

Sharpin^{-/-} mice develop chronic proliferative dermatitis with splenomegaly and systemic inflammation, while *Hoip1*^{-/-} mice are embryonic lethal due to the vascular defects in the yolk sac⁴⁶⁻⁴⁸. Remarkably, while *Hoi1-1*^{-/-} mice are viable without any abnormal phenotype⁴⁹, human patients with a loss of function mutations in HOIL-1 develop chronic autoimmune symptoms with impaired TNF- and IL-1 β -induced NF- κ B activation⁵⁰.

LUBAC can be recruited to the CARD11-BCL10- MALT1 (CBM) complex by binding K63-linked chains through its subunit RNF31. The RNF31 domain is also responsible for promoting NEMO (also known as IKK γ) poly-linear ubiquitylation, which controls the NF- κ B pathway^{51,52}. The ubiquitinated NEMO, in turn, brings additional IKK complexes that induce the auto-phosphorylation and activation of the IKK β and increase NF- κ B activation.

A subtype of DLBCL patients, called activated B-Cell (ABC)-DLBCL, carry missense mutations of the LUBAC member RNF31 at the positions Q584H and Q622L⁵³. These mutations increase the ubiquitin ligase activity of LUBAC by enhancing the interaction between HOIL-1 and HOIP, which in turn promotes the NF- κ B activity. In addition to NEMO, LUBAC sustains BCR signaling in the ABC-DLBCL cell lines by promoting MALT1 activity; a member of the CBM complex, which activates the IKK complex. Consistently, the depletion of components of LUBAC is toxic to the ABC-DLBCL cells^{53,54}. Given that LUBAC controls various pathways that engage with NF- κ B, it is conceivable that a small molecule inhibitor of LUBAC could be a potential therapeutic target in the ABC-DLBCLs.

PELI1

The Pellino protein family consists of three members: Pellino1 (also known as PRISM), Pellino2, and Pellino3⁵⁵. Pellinos contain a putative RING-like motif with a conserved pattern of cysteine and histidine residues, suggesting that it belongs to the RING class E3 ubiquitin ligase⁵⁶. All three Pellino members have E3 ubiquitin ligase activity *in vitro*, and utilize various E2 enzymes to promote poly-ubiquitination via K11, K48, and K63-linked chains. The first evidence of Pellino proteins having ubiquitin ligase activity came from the identification of IRAK1 as a bona-fide substrate^{57,58}. Importantly, RING-like motif mutants of Pellino are impaired in the IRAK1 ubiquitylation. The Pellino members also interact with IRAK4 kinase, Toll-like receptors (TLRs), and interleukin-1 (IL-1R) signaling molecules like TRAF6 and TAK1, regulating innate and adaptive immune responses^{57,59,60}.

Pellino1^{-/-} mice are viable without obvious phenotypes. However, they show a diminished response to TLR3 and TLR4 signaling, thus displaying resistance to septic shock caused by TLR engagement⁶¹. Consistently, the mice showed reduced activation of the NF- κ B and decreased expression of pro-inflammatory genes like TNF and IL-6. In contrast, Pellino1 transgenic mice exhibit B-cell lymphoma formation with constitutive activation of B-cell

receptor signaling⁶². In this context, Pellino1 has been shown to interact and promote K63-linked polyubiquitylation of BCL6, resulting in its stabilization and in the development of DLBCL, BL, and plasmablastic lymphoma (PL). Pellino1 is often highly expressed in high-grade B-cell cancers, correlating with the expression of oncoproteins such as BCL2, BCL6, c-MYC, and MUM1⁶³. High Pellino1 levels show poor prognosis and survival rates in DLBCL patients, suggesting Pellino1 as an oncogene and a potential therapeutic target in B-cell lymphoma. Although other two Pellino members play key roles in immune signaling, their contribution to the pathogenesis of B-cell malignancies remains currently unknown.

UBR5

UBR5 is a HECT-type E3 ligase, also known as EDD (E3 identified by differential display)⁶⁴. The HECT domain in UBR5 has a unique feature, where its C-lobe does not have a surface for the binding of ubiquitin⁶⁵. Instead, the UBA domain is used for ubiquitin binding and the MLLE/PABC domain is used for substrate interaction^{66, 67}. UBR5 plays a key role in regulating the cell cycle, DNA damage response, and transcriptional and translational control by targeting a variety of substrates; such as KATANIN, E6AP, CTNNB1, hPXR, RNF168, CDK9, PEPCK1, TOPBP1, PAIP2, and TRIP12⁶⁸⁻⁷⁶. Furthermore, UBR5 is required for CHK2 phosphorylation and effective p53 activation⁷⁷.

UBR5 is an essential gene as *Ubr5*^{-/-} mice exhibit embryonic lethality due to defects in the vascular and yolk sac development, suggesting its role in vessel formation⁷⁸. Despite this observation, non-synonymous mutations of UBR5 are detectable at high frequency (~18%) in MCL tumors and display a mutually exclusive distribution pattern with alterations of the major driver of MCL, cyclin D1^{79,80}. Most of the UBR5 mutations, including frameshift, affect the cysteine residue of the HECT domain or tend to occur in the HECT and PABC domains, resulting in a loss of E3 ligase activity. Gene ontology analysis reveals DNA damage, cell cycle, and chromosome biology as the predominant factors affected by such UBR5 mutations. Overall, these evidences suggest that UBR5 is a tumor suppressor in MCL, although the relevant substrate(s) responsible for the pathogenesis of the disease remains in question.

Deltex1

In mammals, the Deltex protein family consists of Deltex 1, 2, 3, and 4. These proteins contain two WWE domains, a proline-rich motif, and a RING-finger domain^{81,82}. Deltex1 is a negative regulator of the Notch signaling pathway, controlling ubiquitination and the endosomal recycling of Notch^{81,83,84}. Deltex is a transcriptional target of the nuclear factor of activated T cells (NFAT) and is well known for its involvement in T-cell tolerance. Mice expressing a Deltex1 deleted of its RING-finger domain, or those lacking the first WWE domain, show normal lymphocyte development and function, suggesting that these domains and the E3 ligase activity may be dispensable in lymphocyte differentiation⁸⁵. Although, it is likely that other Deltex members are functionally redundant and compensate for the loss of Deltex1 function, *Deltex1*^{-/-} mice exhibit a profound increase in T-cell activation, which manifests as cell proliferation and a resistance to anergy⁸⁶. *Deltex1*^{-/-} mice are more susceptible to autoimmune syndromes characterized by inflammation and to the production of auto-antibodies.

Mechanically, Deltex has been shown to stimulate the degradation of many proteins such as MAP kinase kinase kinase 1 (MEKK1), protein kinase C θ , phospholipase C- γ 1, HIF-1 α , and c-FLIP⁸⁷⁻⁹⁰. Deltex1 has also been shown to promote the expression of ILT3, the immunoglobulin-like transcript 3, in CLL. ILT3 is a receptor expressed by myeloid cells, which inhibits the activation of AKT following BCR-stimulation through the recruitment of active SHIP1 (Phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase 1)⁹¹. Though ILT3 up-regulation is a marker of CLL cells, how Deltex1 synergistically acts with other specific CLL factors in the regulatory network remains to be understood.

Furthermore, DLBCL patients with non-synonymous mutations in the WWE1-domain of Deltex1 exhibit significantly worse survival than those with a wild-type Deltex1⁹². The Deltex1 deleterious mutations release their negative regulation on Notch, thus promoting Notch activation in DLBCL. Interestingly, DLBCL tumors with Deltex1 mutations also show significantly lower Deltex1 expression. Pathway enrichment analysis studies demonstrate that the expression signatures IFN- γ , JAK-STAT, and the ubiquitin-proteasome signaling pathways inversely correlate with the Deltex1 expression. Alternately, the genes involved in BCR signaling, MAPK signaling, and chromatin remodeling are positively correlated with the Deltex1 expression. Although the Deltex1 seems to be a tumor-suppressing protein, the exact molecular mechanism is yet to be determined.

cIAP1/2

The cellular inhibitor of apoptosis 1 and 2 (cIAP1 and 2) proteins belong to the inhibitor of the apoptosis protein (IAP) family of proteins that share three baculovirus IAP-repeat (BIR) domains²². In addition to the IAP domains that recruit caspases and other proteins, the cIAP1/2 proteins contain a RING-finger domain suggesting that they might bind an E2 enzyme, and thus have E3 ligase activity. cIAP1/2 are involved in various signaling pathways including TNF- α mediated NF- κ B activation⁹³. These proteins directly interact with TRAF2 and the TNF receptors to promote a poly-ubiquitination of RIP1, utilizing K63-linked ubiquitin chains. This molecular event stimulates interaction with the IKK, LUBAC, and TAK1-TAB2/3 complexes^{52,94-96}. cIAP1/2 have also been described to promote the K48-linked polyubiquitination and the proteasomal-degradation of NIK; the main kinase upstream of the alternative NF- κ B pathway^{97,98}.

ciap1^{-/-} or *ciap2*^{-/-} mice have defects in caspase-1 activation and inflammasome assembly, causing resistance to peritonitis. Deletion of both the *cIAP1/2* genes is not compatible with life as embryos die at E12. 5. Conditional ablation in B-cells leads to an increase of the B-cell numbers, characterized by hyperactivation of the alternative NF- κ B pathway and defects in the germinal center immune response⁹⁹. In keeping with the function of being the positive regulator of NF- κ B, the *cIAP1/2* copy number gains have been detected in ABC-DLBCL tumors⁹⁵. In addition to RIP1, cIAP1/2 further contribute to the NF- κ B activity by poly-ubiquitylating BCL10 with K63-linked chains, which in turn promotes IKK recruitment to the CBM complex.

Chemical inhibition (SMAC mimetics) and the genetic knockdown of cIAP1/2 are toxic to BCR-signaling dependent ABC-DLBCL cell lines, but not to BCR-signaling independent ones. SMAC is a small mitochondrial protein that binds and suppresses the IAP proteins

during apoptosis. Mechanistically, SMAC mimetics promote auto-ubiquitylation and degradation of cIAP1/2, suppressing NF- κ B pathway. Whether these small molecules have clinical impacts for treating ABC-DLBCL patients, as a single agent or with the combination of other BCR pathway inhibitors, still needs further investigation.

Smurf2

Smurf2 is a HECT-type E3 ubiquitin ligase and was initially considered a negative regulator of TGF- β signaling^{100,101}. Later studies have shown that Smurf2 plays a role in various signaling pathways by targeting Smads, Runx2, Id1, Smurf1, TGF-beta receptor, and RING finger protein (RNF20) for ubiquitylation and degradation^{100,102-105}. Moreover, Smurf2 is also an important regulator of senescence as it can activate p53 and pRB pathways¹⁰⁶.

Smurf2^{-/-} mice develop a wide spectrum of tumors in the liver, blood, lungs, and pituitary glands, but most notably in B-cell lymphoma¹⁰⁷. Smurf deficient cells can bypass senescence and increase splenic B-cell proliferation leading to malignant lymphoma. In the context of lymphoma, a key substrate of Smurf2 is YY1, which is ubiquitylated and degraded. YY1 is a master regulator of the GC transcription program, and Smurf2 deficiency induces YY1-dependent transactivation of c-MYC, which contributes to lymphomagenesis. Human DLBCL, BL, and FL tumors show a decrease in the Smurf2 expression with elevated YY1 expression, which correlates with poorer survival rates only in DLBCL and BL¹⁰⁸. This Smurf2-YY1-MYC axis represents the novel mechanism for lymphomagenesis in GC or post-GC B cells. Recently, Smurf2 has been shown to regulate genomic instability by affecting monoubiquitylation of histone H2B and function as a tumor suppressor¹⁰². Smurf2, thus, has multiple tumor suppressive roles by regulating genomic stability, cell proliferation, senescence, and apoptosis. However, the exact mechanism of how Smurf2 contributes to tumorigenesis in different cancer types remains an open question.

FBXO11

FBXO11 is a member of the F-box proteins family, which includes three different groups based on the substrate recognition domains—the FBXW (subclass with WD40 repeat domains), the FBXL (subclass with leucine-rich repeat domains), and the FBXO (subclass with various uncharacterized domains). In most cases, F-box proteins recognize their substrates through short and specific motifs, which are often called degrons⁵. Often, phosphorylation at the degron is the trigger for the efficient recruitment of the F-box protein and the consequent ubiquitylation and proteasomal degradation.

FBXO11 utilizes the C-terminal carbohydrate-binding proteins and sugar hydrolases (CASH) repeat domain as the substrate-binding domain. FBXO11 controls the cell cycle via the degradation of its substrate CDT2 (also known as DTL)¹⁰⁹. CDT2 is a CRL4 complex which promotes a cell cycle exit, DNA replication, and cell migration through p21 and SEDT8 degradation¹¹⁰. FBXO11 also controls epithelial-mesenchymal transition (EMT), and cancer metastasis by degrading the substrates such as BLMP-1 and SNAIL^{111,112}.

Fbxo11^{-/-} mice exhibit perinatal lethality with facial clefting, while *Fbxo11* haploinsufficient mice display reduced weight and a severe infection of the middle ear, as observed in Jeff (deaf mouse mutant) mice, suggesting a role in the inflammation¹¹³⁻¹¹⁵.

Significantly, the FBXO11 monoallelic deletions and inactivating mutations are observed in DLBCL, Burkitt's lymphoma (BL) cell lines, and primary DLBCL patient samples. The mutations target the CASH domain, leading to a loss of the substrate interaction. In the context of B-cell malignancies, a key FBXO11 substrate is BCL6. BCL6 is a critical transcriptional factor that regulates B-cell development and differentiation and is often overexpressed in DLBCL by chromosomal translocation, hypermutation of its promoter, or deregulated proteolysis. This supports the notion that FBXO11 functions as a haploinsufficient tumor suppressor gene in DLBCL by mediating the degradation of the oncogenic BCL6 protein¹¹⁶. Accordingly, a re-expression of FBXO11 in FBXO11-null DLBCL cells has an antitumor effect by inhibiting cancer cell growth. Mice carrying *Fbxo11* inactivation, specifically in GC B-cells, exhibits abnormal GC expansion, up-regulation of BCL6 protein levels, and B-cell lymphoproliferations¹¹⁷. Further studies will assess whether the additional substrates in germinal center B-cells contribute to the developments of any B-cell malignancies.

FBXO10

FBXO10 is another FBXO protein with the CASH domain⁴³. The primary target of FBXO10 is the anti-apoptotic protein BCL2, although the receptor for advanced glycation end products (RAGE) has recently been identified as another substrate^{118,119}. The BCL2 protein is localized to the outer membrane of the mitochondria and inhibits the function of pro-apoptotic proteins such as Bax and Bak. Overexpression of BCL2 induces cellular transformation, in concomitance with the alteration of oncogenes like c-Myc, causing aggressive B-cell lymphoid malignancies¹²⁰.

BCL2 chromosomal translocation that places its promoter next to the immunoglobulin heavy chain locus leads to abnormally high transcription levels of BCL2 in follicular lymphoma¹²¹. Thus, it is conceivable that the deregulation of BCL2 proteolysis could be another mechanism for BCL2 overexpression and lymphoma development. In DLBCL, the FBXO10 mRNA level is down-regulated and frameshift, missense mutations, or deletions are also detected¹¹⁸. Specifically, the R44H mutation in the F-box domain abolishes FBXO10 interaction to SKP1, inhibiting the formation of the SCF complex. Furthermore, the V762L and R825W mutations in the CASH domain presumably lead to the disruption of substrate interaction. All FBXO10 mutants exhibit an impaired ability to degrade BCL2 and are much less toxic than the wild-type FBXO10 when overexpressed in lymphoma cell lines. Interestingly enough, the FBXO10 mutations detected in DLBCLs are heterozygous; thus, FBXO10 functions as a haploinsufficient tumor suppressor. Similarly, like in DLBCLs, high levels of BCL2 expression and low levels of FBXO10 expression are also observed in MCL¹²².

The BCL2 inhibitor, ABT-199, BTK inhibitor, and ibrutinib, have synergistic anti-tumor effects in both the BCR-dependent and BCR-independent MCL tumor growth. It will be important to investigate whether this inverse correlation between FBXO10 and BCL2 is a general regulation mechanism, which applies to other B-cell malignancies.

FBXO9

FBXO9 has been reported to control the proliferation of MM. Specifically, its mRNA level is elevated in MM cell lines and MM patients, correlating with copy number gains. Expression of FBXO9 correlates with a higher progression-free survival (PFS) and better response rates in MM patients treated with the proteasome inhibitor bortezomib^{123,124}. FBXO9 promotes ubiquitylation and degradation of the mTORC1-bound telomere length regulation protein 2 (TEL2) and Telo2-interacting protein 1 (TTI1), upon growth factor withdrawal. This leads to the attenuation of mTORC1 signaling to restrain cell growth, but activates the PI3K/TORC2/AKT pathway, disengaging the inhibitory feedback and sustaining cell survival. Correspondingly, MM cells with FBXO9 overexpression exhibit low levels of S6K1 phosphorylation and high levels of AKT phosphorylation. Furthermore, it has been shown that the CK2 kinase, one that is frequently overexpressed in many different cancers, primes the FBXO9-dependent degradation of TEL2 and TTI1¹²⁵. Thus, pharmacological inhibition of CK2 in MM cell lines could potentially be of a therapeutic advantage by promoting the stabilization of TEL2 and TTI1. Recently, the FBXO9 has been shown to promote proteasomal degradation of peroxisome proliferator-activated receptor gamma (PPAR γ), playing a role in adipocyte differentiation and adipogenesis¹²⁶. Further investigation to understand other cellular pathways regulated by FBXO9 as well as the assessment of the therapeutic windows might be required to target the FBXO9-TEL2 pathway.

FBXW7

FBXW7 is a member of the FBXW class, featuring eight WD40 repeats that assemble in a β -propeller binding pocket, interacting with phosphorylated substrates. *Fbxw7*^{-/-} mice exhibit embryonic lethality due to defects in their vascular development, probably because of the stabilization of its substrates—NOTCH1 and NOTCH4¹²⁷. In the bone marrow, conditional FBXW7 deletion promotes the p53 dependent-cell cycle entry and cellular apoptosis, leading to a premature decrease in hematopoietic stem cells^{128,129}.

FBXW7 functions as a tumor suppressor by targeting multiple oncogenic proteins such as cyclin E, c-MYC, c-Jun, Aurora B, and MCL1¹³⁰⁻¹³⁴. Recently, FBXW7 has been shown to target STAT3 and pSTAT3 (Y705) regulating cellular apoptosis. The low expression of FBXW7 correlates with the high expression of STAT3, resulting in a poor prognosis in ABC-DLBCL patients¹³⁵. Correspondingly, loss of function mutations of FBXW7 with frequent deletions and inactivating mutations are identified in many cancers¹³⁶. Most missense mutations are heterozygous and occur within the WD40 domain; these mutations directly interfere with the recruitment of phosphorylated substrates, its localization, or translation. Some FBXW7 substrates such as c-MYC and NOTCH show degron mutations in cancer. For instance, in Burkitt's lymphoma, mutations were identified in Threonine 58 of c-MYC that undergoes phosphorylation by GSK3 β , causing c-MYC stabilization¹³⁷.

However, the alteration of FBXW7 is infrequent in some B-cell malignancies, and evidences that FBXW7 serves as a pro-survival factor in MM¹²⁴ and CML¹³⁸ have been shown. In MM, FBXW7 is responsible for the degradation of p100, an inhibitor of the non-canonical NF- κ B signaling pathway. MM cell lines are addicted to NF- κ B activation. Thus,

constitutive degradation of p100 is a requirement for MM growth and survival. As nuclear p100 is phosphorylated by glycogen synthase kinase (GSK3), it is conceivable that GSK3 inhibitors can be a potential therapeutic approach for MM patients. Although FBXW7 mostly functions as a tumor suppressor by degrading oncogenic proteins, it can also exert a pro-survival effect, supporting its role as both context- and substrate-dependent.

Concluding remarks

It has become apparent that the E3 ubiquitin ligases play a critical role in many biological processes through the regulation of substrate ubiquitylation. The precisely controlled and context-specific turnover of proteins in a timely manner is critical for normal cellular homeostasis. It is also evident that the deregulation of E3 ligases or their substrate degradation contributes to the pathogenesis of human diseases, including lymphoid malignancies.

The biology of different types of lymphoid cancers depends on the stages of B-cell differentiation from which they are derived. Moreover, different lymphoid malignancies are driven by various oncogenic signaling pathways, which are regulated by protein ubiquitin system at multiple levels.

One of key events in lymphoma arising from a post-GC B cells is the constitutive activation of NF- κ B pathway that can promote cancer cell survival and proliferation. In this review, KLHL6, LUBAC, and cIAP1/2 are the prime examples of E3 ligases that contribute to stimulation of NF- κ B transcriptional activity. All three ligases engage chronic active BCR signaling to promote NF- κ B signaling, which is central to the pathogenesis of ABC-DLBCLs. Although they generate different polyubiquitin-linked chains (Lys-48, Lys-63, and linear) of distinct structures, they are directly affected by malignant lesions and deregulated by multiple mechanisms. Both aberrant proteolytic and non-proteolytic protein ubiquitination ultimately activate the survival NF- κ B pathway.

BCL6, BCL2, and c-MYC are dominant oncogenes deregulated by chromosomal rearrangement and missense mutations in lymphoma arising from germinal center B-cells such as BL, FL, or GCB-DLBCL. Protein ubiquitylation adds another layer to the biology of GC lymphoma as discussed in this review. Specifically, BCL6 is overexpressed and stabilized by inactivation of FBXO11 and Pellino1-induced Lys63-polyubiquitylation. MYC is up-regulated by Smurf2-mediated activation of YY1 or by mutations of its FBXW7 degenon. In addition, the substrate interaction domain of FBXO10 is disrupted to impair the proteosomal degradation of BCL2. Thus, different lymphoma subtypes are characterized by inappropriate turnover of key cellular players for that particular stage of B-cell differentiation (Fig.2).

Although our knowledge of the biochemical and biological functions of E3 ubiquitin ligases has increased in B-cell malignancies, a deeper characterization of cell-context dependent substrate regulation and biological relevance is needed. The recent approval of a general proteasome inhibitor, VELCADE or bortezomib, has demonstrated a great efficacy for the treatment of MM and MCL³⁰⁻³². However, toxicity and the relevant side effects, which

include anemia, neuropathy, and thrombocytopenia, in addition to bortezomib resistance, make this drug imperfect¹³⁹⁻¹⁴¹. Recent discoveries have shown that thalidomide-based drugs target Cereblon (CRBN)¹⁴², a member of the CULLIN4 E3 ubiquitin ligase complex, to promote proteasomal degradation of specific targets^{143,144}. This promising area of research has shown the achievement of higher drug potency, resulting in the rapid destabilization of targets via ubiquitin-dependent proteasomal degradation.

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Highlights

- The Proteasome ubiquitylation System is deregulated in a variety of B-cell cancers
- E3 ligase and substrate pairings in malignant B-cell cancer contribute to the initiation and maintenance of tumorigenesis
- Understanding the mechanism of specific E3 ligases will help the development of potential therapeutic targets

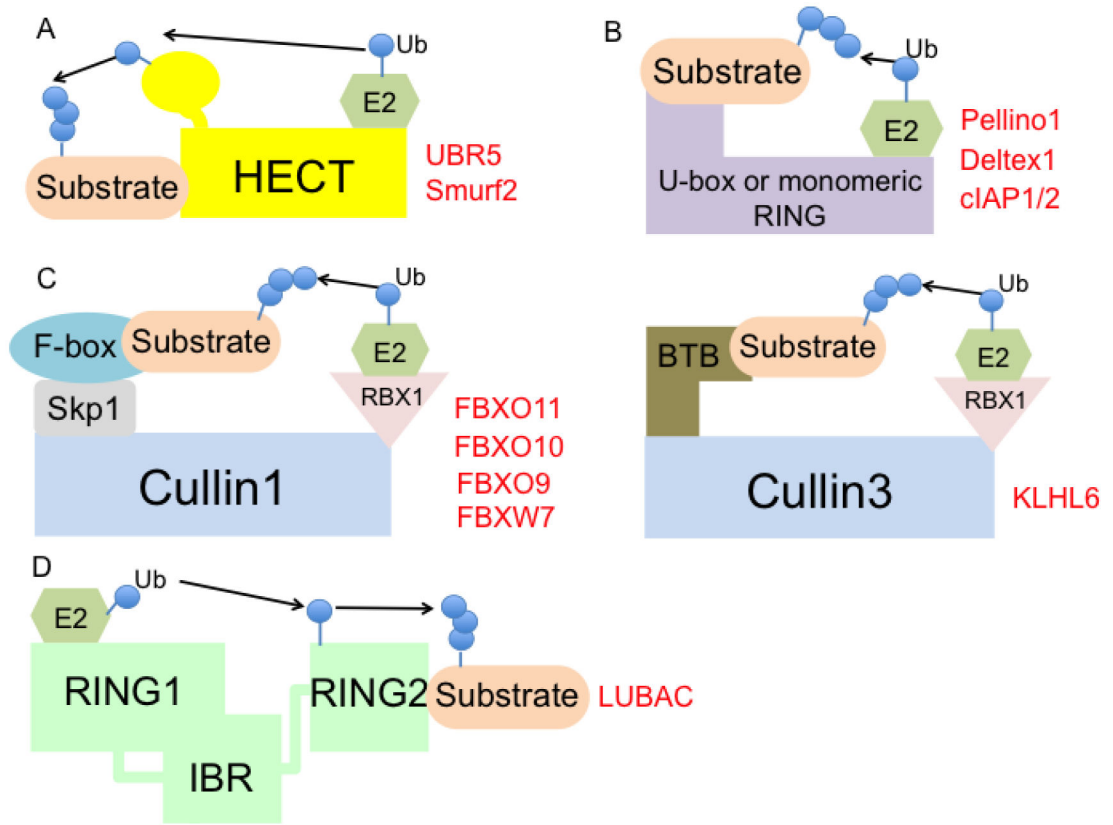


Fig.1. Different types of E3 ubiquitin ligases

Ubiquitin ligases are classified into different groups—HECT-type, RING-type or U-box type, Multi-subunit RING-type, and RING-Between-RING (RBR) type.

(A) The HECT-type E3 ligase directly accepts ubiquitin molecules from the E2 enzyme and transfers it to the target substrate. This includes Ubr5 and Smurf2.

(B) The RING-type ligase uses the RING-domain binding the E2 enzyme and the other end interacting with the substrate, which bridges in close proximity to transfer the ubiquitin from the E2 to the target. This includes Pellino1, Deltex1, cIAP1/2.

(C) Multi-subunit RING-type E3 ligase utilizes different CULLIN scaffolds, a substrate receptor, an adaptor, and a RING-domain protein for E2 enzyme recruitment. CULLIN1 uses SKP1 (adaptor), different F-box proteins (substrate receptor), and RBX1 (binding E2). This includes FBXO11, FBXO10, FBXO9, FBXW7. In contrast, CULLIN3 uses BTB protein (adaptor and substrate receptor) and RBX1. This includes KLHL6.

(D) RBR-type E3 ligases use combination mechanisms of the HECT-type and RING-type. This includes LUBAC.

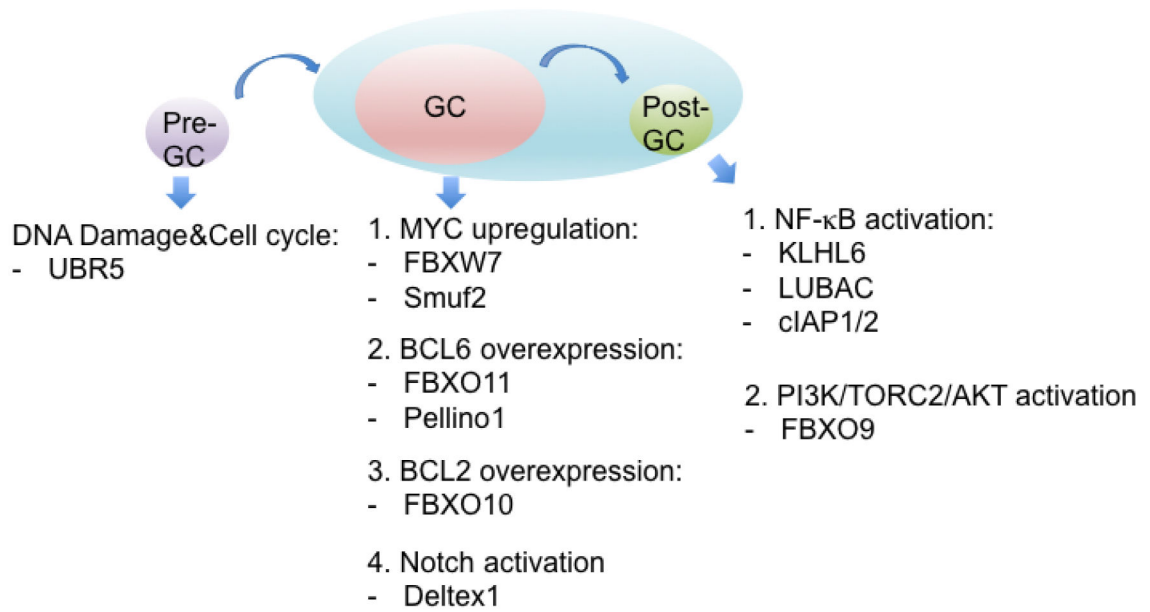


Fig.2. Different E3 ubiquitin ligases altered at particular stages of B-cell differentiation
The relevant ubiquitin ligases that are genetically altered through overexpression, deletion, and mutation along with the subsequent pathways or substrates that are deregulated are shown at a pre-GC, GC, or post-GC stage.

Table. 1

Summary of E3 ligases implicated in B-cell malignancies

E3 ligases	Targets	Pathway associated in B-cell malignancies	B-cell malignancies associated
KLHL6	Roquin2	BCR signaling, NF- κ B pathway	ABC-DLBCL, CLL, FL, MM, MZL
LUBAC	NEMO/IKK γ	BCR signaling, NF- κ B pathway	ABC-DLBCL
Pellino1	BCL6	BCR signaling, BCL6 stabilization	GCB-DLBCL, BL, PL
UBR5	Katanin, E6AP, CTNNB1, hPXR, RNF168, CDK9, PEPCK1, TOPBP1, PAIP2, TRIP12	DNA damage, cell cycle, chromosome regulation	MCL
Deltex1	MEKK1, PKC θ , PLC- γ 1, HIF-1 α , c-FLIP	Notch activation	CLL, DLBCL
cIAP1/2	RIP1, NIK, BCL10	BCR signaling, NF- κ B pathway	ABC-DLBCL
Smurf2	Smads, Runx2, Id1, Smurf1, RNF20, YY1	Transactivation of c-Myc	GCB-DLBCL, BL, FL
FBXO11	CDT2, BCL6, BLMP-1, SNAIL	GC expansion, BCL6 up-regulation	GCB-DLBCL, BL
FBXO10	BCL2, RAGE	Overexpression of BCL2	GCB-DLBCL, MCL
FBXO9	TEL2, TTI1, PPAR γ	PI3K/TORC2/AKT pathway	MM
FBXW7	NOTCH1, NOTCH4, cyclin E, c-Myc, c-Jun, Aurora B, MCL1, STAT3, p-STAT3 (Y705)	c-Myc, NOTCH, STAT3 stabilization, alternative NF- κ B pathway	ABC-DLBCL, BL, MM, CML