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Cullin 5-RING E3 ubiquitin ligases, new therapeutic targets?

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Ubiquitylation is a reversible post-translational modification of proteins that controls a myriad of functions and cellular processes. It occurs through the sequential action of three distinct enzymes. E3 ubiquitin ligases (E3s) play the role of conductors of the ubiquitylation pathway making them attractive therapeutic targets. This review is dedicated to the largest family of multimeric E3s, the Cullin-RING E3 (CRL) family and more specifically to cullin 5 based CRLs that remains poorly characterized.

Abbreviations: ASB, ankyrin repeat-containing proteins with a SOCS box; β -Trcp, β -transducin repeat-containing protein; BTB, bric-a-brac; CIS1, cytokine-inducible Src homology 2-containing protein; CRL, Cullin-RING E3 ubiquitin Ligase; CAND1, cullin-associated and neddylation-dissociated 1; CSN, COP9 signalosome complex; DCAF, DDB1- and CUL4-associated factor homolog; DCNL, defective in cullin neddylation protein 1-like proteins; DCN1, defective in cullin neddylation protein 1; DDB1, DNA damage-binding protein 1; DUB, deubiquitylating enzyme; Elo, Elongin B; EloC, Elongin C; E1, E1 ubiquitin-activating enzyme; E2, E2 ubiquitin-conjugating enzyme; E3, E3 ubiquitin ligase; FAK, Focal Adhesion Kinase; Fbw7, F-box WD40 repeat-containing protein 7; HECT, Homologous to E6-associated protein carboxyl terminus; HIV-1, human immunodeficiency virus-1; IRS, Insulin Receptor Substrate; JAK2, Janus Kinase 2; KSHV, Kaposi's sarcoma-associated herpes virus; LANA, latency-associated nuclear antigen; MAL, Myeloid differentiation primary-response gene 88 -Adaptor-Like protein; MLL, Mixed Lineage Leukemia; MuHV-4, murid herpes virus-4; NAE, NEDD8-activating enzyme; Mre11, meiotic recombination 11; NEDD8, Neural precursor cell expressed developmentally down-regulated protein 8; RING, really interesting new gene; RBX, RING box protein; ROC, regulator of cullins; SKP1, S-phase kinase-associated protein 1; Skp2, S-phase kinase-associated protein 2; SOCS, suppressors of cytokine signaling; SPSB, SPRY domain-containing proteins with a SOCS box; TNF-R2, tumor necrosis factor receptor 2; VHL, von Hippel-Lindau; WSB, WD40 repeat-containing protein with a SOCS box; Vif, viral infectivity factor.

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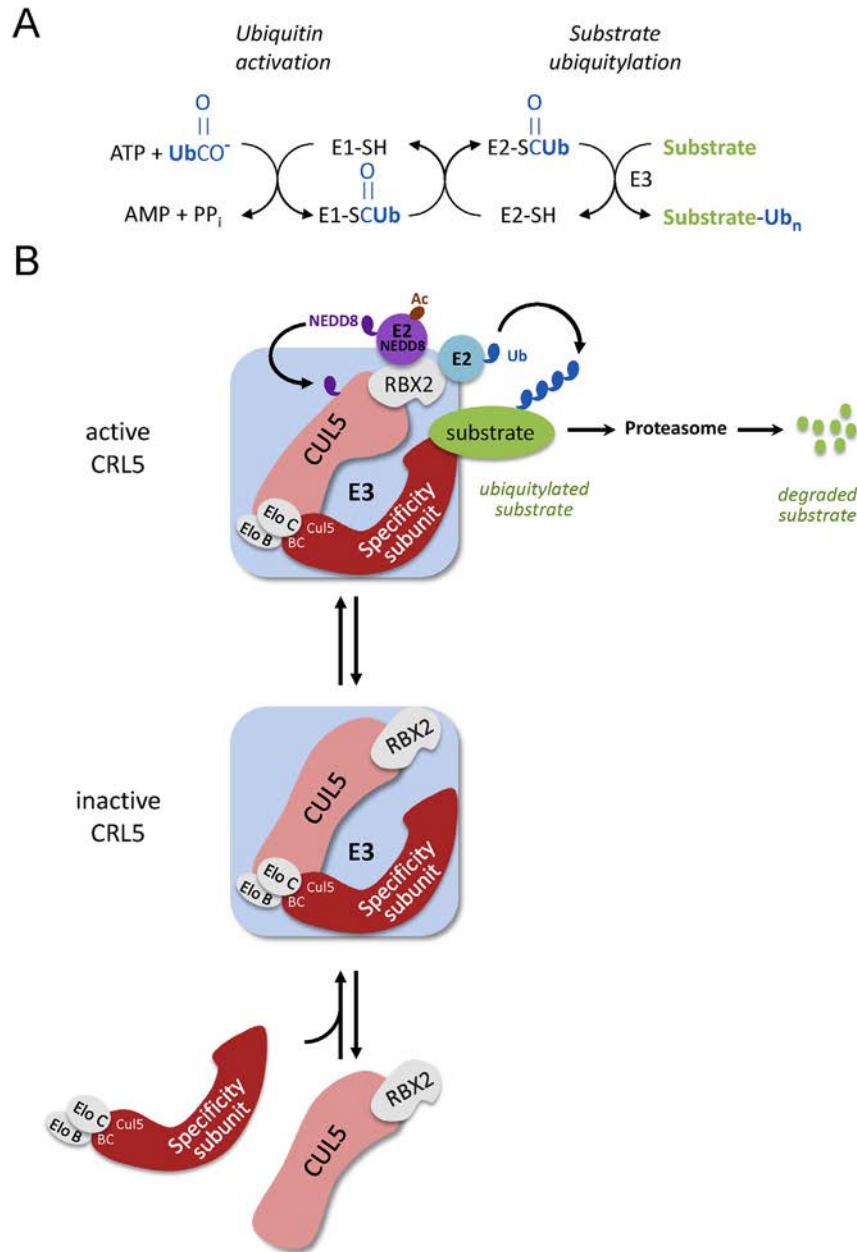


Fig. 1. CRL5 in ubiquitin-dependent protein degradation. (A) Ubiquitin (Ub) is first activated in an ATP-dependent reaction that leads to the formation of a thioester intermediate that involves the carboxy-terminal glycine residue of ubiquitin and the active site cysteine residue on the ubiquitin-activating enzyme E1. Ubiquitin is then transferred to the active site cysteine of a ubiquitin-conjugating enzyme E2. In the case of RING-finger E3s, activated ubiquitin is transferred directly from the E2 to the substrate. After multiple rounds, Lys48-linked ubiquitin chains are formed. These represent the canonical recognition motif for proteasomal degradation of the protein substrate. (B) In the CRL5 family, CUL5 can assemble with the RING subunit RBX2 and with numerous specificity subunits (also called substrate receptor, substrate recognition module or substrate targeting protein) involved in the recruitment of specific targets via adaptors (EloB and EloC). RBX2 is involved in the binding of the E2 enzyme that allows substrate ubiquitylation and is required for the covalent attachment of NEDD8 to a lysine residue of CUL5. NEDD8 ligation to CUL5 is enhanced following N-terminal acetylation (Ac) of the E2 NEDD8-conjugating enzyme. When CUL5 is post-translationally modified by NEDD8 (neddylation), activation of the CRL5 occurs. CUL5 inactivation occurs through NEDD8 removal by deneddylation by the CSN.

Protein ubiquitylation is a reversible post-translational modification that has proteolytic and non-proteolytic roles thereby regulating a wide spectrum of biological functions. As such, defects in the ubiquitin pathway can contribute to disease pathogenesis providing the rationale for drug discovery targeting this pathway.

1. The ubiquitylation cascade

The ubiquitin molecule is first activated through an ATP-dependent reaction by forming a high-energy thioester bond between the carboxy-terminal glycine residue of ubiquitin and the catalytic cysteine of an E1. Activated ubiquitin is then transferred

to the active site cysteine of an E2, where a second high-energy thioester linkage is formed. In the last step, an E3 drives the transfer of ubiquitin to a protein substrate, forming an isopeptide bond between the ubiquitin carboxy-terminal glycine residue and substrate lysine residue or forming a peptide bond with the amino-terminus of the substrate (Fig. 1A) [1]. Indeed, E3s can be divided into three main classes: (i) N-end rule E3s, (ii) E3s of the HECT (Homologous to E6-associated protein Carboxyl Terminus) domain family that function as ubiquitin carriers and (iii) E3s of the RING (Really Interesting New Gene) domain family. RING-E3s do not have inherent catalytic activity but use the RING structural motif to recruit and direct a ubiquitin-charged E2 towards specific substrates. RING-domain E3s can be further divided into two subclasses: monomeric E3s, in which a unique protein harbors the RING-finger motif and the substrate binding domain, and multimeric E3s in which RING-finger and substrate binding domains are assembled together from different individual proteins [2]. Some protein substrates can be ubiquitylated with only a single ubiquitin molecule on a single lysine residue (monoubiquitylation) or on multiple lysine residues (multiubiquitylation). After multiple runs, linear and branched polyubiquitin chains can be formed (polyubiquitylation) on one or more of the seven lysines of ubiquitin (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48 and Lys63). Depending on the nature of ubiquitin attachment and the type of linkage of the polyubiquitin chain, the fate of ubiquitylated proteins varies [3]. For example, the Lys48-linked chains represent the canonical recognition motif for degradation of the protein substrate by the proteasome, whereas the K63-linked chains or mono-ubiquitylation have non-degradative functions and alter protein function and subcellular localization.

2. Cullin-RING E3 ubiquitin ligases

CRLs belong to the largest family of E3s and are responsible for as much as 20% of all ubiquitylated proteins within cells [4] controlling a myriad of functions. Cullins (CUL1, CUL2, CUL3, CUL4a, CUL4b, CUL5, CUL7 and CUL9 in mammals) are molecular scaffolds that assemble distinct subunits: (i) a RING finger protein, (ii) a substrate recognition module containing an adaptor and a specificity subunit dedicated to the recruitment of specific target(s). In the case of CRL3 complexes, the adaptor and specificity subunit are within the same polypeptide. More precisely, the carboxy-terminus of cullins can bind to a RING finger protein, RBX1 or RBX2 (RING box protein 1 or 2), also called ROC 1 and 2 (regulator of cullins), respectively. RBX2 uniquely associates with CUL5, whereas RBX1 binds to the other cullins. The RING domain of RBX1/2 recruits a ubiquitin-loaded E2 to mediate ubiquitin conjugation. Cullin amino-terminus binds to distinct adaptor(s) and to a specificity subunit to recruit protein targets. Indeed, many specificity subunits are involved in the recruitment of protein targets to be ubiquitylated: 69 F-box proteins for CRL1 [5], 80 proteins with a SOCS (Suppressors Of Cytokine Signalling)-box or a VHL (von Hippel-Lindau)-box for CRL2/5 [6], 180 BTB (bric-a-brac) proteins for CRL3 [7], and 90 DCAF (DDB1- and CUL4-associated factor homolog) for CRL4A/B [8]. The specificity subunits of CRL1, CRL2, CRL5 and CRL7 use adaptors, a BTB fold protein, the S-phase kinase-associated protein 1 (SKP1) or the elongin B (EloB)-elongin C (EloC) complex, to interact with the cullin. The adaptor subunits for CUL4A/B are composed of DDB1 (DNA Damage-Binding protein 1) and members of the DCAF (DDB1 CUL4 Associated Factor) family.

Although cullins 2 and 5 share identical adaptors (EloB and EloC), they direct the assembly of distinct E3 complexes: CRL2 with the VHL-box proteins and CRL5 with SOCS-box proteins as specificity subunits (Fig. 1B). These proteins can bind to the Elongin BC complex through a conserved EloC-binding sequence motif (S,T,P,A)

LXXX(C,S,A)XXX(L,V,I,A), named BC-box [9–11]. Further studies demonstrated that the BC-box is flanked by a CUL2 box (consensus sequence: Φ PXX Φ XXX Φ) or a CUL5 box (consensus sequence: Φ XXLP Φ PXX Φ XX(Y,F)L) that determine binding specificity for CUL2-RBX1 and CUL5-RBX2 modules, respectively [11,12].

From an evolutionary perspective, yeast genomes encode three cullins (CUL1, CUL3 and CUL8 in *Saccharomyces cerevisiae* and CUL1, CUL3 and CUL4 in *Schizosaccharomyces pombe*). Five cullins are expressed in *Drosophila* (CUL1 to CUL5), six in *Caenorhabditis elegans* (CUL1-CUL6) and seven in mammals, indicating that cullins are ancient genes [13].

3. Regulation of cullin-RING E3 ubiquitin ligases

CRLs are subject to regulation by several mechanisms. Activation of CRLs occurs when cullins are post-translationally modified by the ubiquitin-like protein NEDD8 (Neural precursor cell Expressed Developmentally Down-regulated protein 8) [14,15]. Conversely, inactivation occurs through NEDD8 removal by deneddylation by the COP9 signalosome complex (CSN) [16,17]. Hence, unneddylated cullins are in an inactive state while neddylated cullins are in an active state. NEDD8 is a protein that is covalently linked to a lysine residue in a C-terminal winged-helix motif in cullins (Lys 724 in CUL5) through an E1 NEDD8-activating enzyme (NAE) – E2 NEDD8-conjugating enzyme cascade. NEDD8 E3 ligases catalyze the transfer of NEDD8 to the protein substrate. Among them, RBX1 and RBX2 are involved in the neddylation of cullins [18,19] resulting in the activation of ubiquitin transfer activity by favoring alternative conformations of the cullin C-terminal domain which place the RING domain of RBX1 or RBX2 in a different orientation [20,21]. This is likely to promote the sequential transfer of ubiquitin molecules to form polyubiquitin chains on substrates by multiple catalytic cycles [22]. Full activation of CRL5 requires acetylation of one of the two E2 NEDD8-conjugating enzymes (Ubc12 or UBE2F) at its N-terminus [23]. Indeed, Ubc12/UBE2F acetylation is important for stimulating neddylation of the CUL5-RBX2 module [19]. Cullin neddylation is enhanced by DCNLs (defective in cullin neddylation protein 1-like proteins) such as DCN1 [24]. In fact, DCN1 increases the recruitment of Ubc12 to the CUL1-RBX1 module and favors the orientation of the RBX1-Ubc12-NEDD8 complex for optimal NEDD8 transfer [25,26]. Although the CSN catalyzes the reaction that removes NEDD8 from cullin, the CSN contributes to maintaining CRL function [17,27]. The CSN is a multi-protein complex whose organization resembles that of the 26S proteasome lid [28]. The subunit 5 [CSN5; also known as c-Jun activation domain-binding protein-1 (Jab1)] harbors a regulated zinc-dependent isopeptidase activity [17,29]. In fact, CRLs undergo cycles of neddylation and deneddylation that are required for the CRLs to exert their activity (Fig. 1B).

CRLs are also regulated at the level of their assembly. As mentioned above, many specificity subunits can assemble with the same adaptors and Cullin-RBX1/2 modules. Importantly, the abundance of a specificity subunit dictates the assembly of a given CRL [30]. In this context, it is not surprising that some of them accumulate at particular cellular or developmental stages such as specific phases of the cell cycle (e.g. Skp2 for S-phase kinase-associated protein 2, Fbw7 for F-box WD40 repeat-containing protein 7, and β -Trcp for β -transducin repeat-containing protein [31]) or differentiation commitment (e.g. ASB2 α (Ankyrin repeat-containing proteins with a SOCS-Box 2 α) and ASB2 β proteins [32–34]). Assembly of CRLs is also dependent upon CAND1 (cullin-associated and neddylation-dissociated 1) that acts as a specificity subunit exchange factor [35–37]. Although this has been well-studied for CRL1, CUL4B and CUL5 were also found to associate with CAND1 [30]. This mechanism of regulation of CRL assembly

Specificity subunits	Substrate(s)	Pathways	Links or potential links to diseases
<i>SH2 domain-containing proteins with a SOCS-box (SOCS)</i>			
CIS	erythropoietin receptor	cell signalling	
SOCS1	p65/RelA FAK IRS1, IRS2 JAK2 MAL TEL-JAK2 Vav	transcription cell signalling cell signalling cell signalling cell signalling cell signalling	insulin resistance syndromes, diabetes leukemia
SOCS3	CD33 receptor	cell signalling	
SOCS6	cKit receptor	cell signalling	
<i>Ankyrin repeat-containing proteins with a SOCS-box (ASB)</i>			
ASB2 α	FLNa, FLNb, FLNc MLL	cell motility, cell differentiation epigenetic regulation	leukemia
ASB2 β	FLNb	cell differentiation	
ASB3	TNF-R2	cell signalling	
ASB4	IRS4 ID2	cell signalling, transcription, cell differentiation	preeclampsia
ASB9	creatine kinase B	metabolism	
ASB11	Delta A	cell signalling	
<i>SPRY domain-containing proteins with a SOCS-box (SPSB)</i>			
SPSB1	iNOS TGF- β type II receptor	innate host response cell signalling	chronic and persistent infections
SPSB2	iNOS	innate host response	chronic and persistent infections
SPSB4	iNOS	innate host response	chronic and persistent infections
<i>WD40 repeat-containing proteins with a SOCS-box (WSB)</i>			
WSB1	HIPK2 D2	cell signalling metabolism	
<i>Other SOCS-box proteins</i>			
Elongin A	Rpb1	transcription	
<i>Viral proteins with a SOCS-box</i>			
HIV-1 Vif	APOBEC3F APOBEC3G	antiviral activity antiviral activity	viral infections
E4orf6/E1B55K	p53 Mre1 DNA ligase IV Integrin α 3 Rep52	cell cycle, apoptosis DNA repair, recombination DNA repair, cell signalling cell motility viral DNA replication	viral infections
GAM1	SAE1	sumoylation	viral infections
BLZF1	p53	cell cycle, apoptosis	viral infections
LANA	p53 VHL	cell cycle, apoptosis ubiquitylation	viral infections
MuHV-4 ORF73	p65/RelA	transcription	viral infections

Fig. 2. CRL5 and corresponding substrates.

may be important when the levels of a specificity subunit and/or its substrates changed drastically during specific biological processes to build new CRLs.

4. CRL5 and SOCS-box proteins: targeting proteins for polyubiquitylation and subsequent degradation by the proteasome

The SOCS-box protein family comprises several members that are defined by the domain associated with the SOCS-box and which

is involved in substrate recognition: SH2 domain for SOCS proteins (SOCS1-7, CIS1 for Cytokine-Inducible Src homology 2-containing protein), SPRY domain for SPSB (SPRY domain-containing proteins with a SOCS-Box) proteins (SPSB-4), WD40 domain for WSD (WD40 repeat-containing protein with a SOCS-Box) proteins (WSB1 and 2), Ras domain for Rab-40A, Rab-40B and Rab-40C, and ankyrin domains for ASB proteins (ASB1 to 18) [38]. As mentioned above, SOCS-box proteins interact with the Elongin BC complex and the CUL5/RBX2 module to form an E3 ubiquitin ligase complex. It is now well-established that viral proteins such as the human

immunodeficiency virus-1 (HIV-1) viral infectivity factor (Vif) [39], Adenovirus proteins E4orf6 and E1B55K [40], Latency-Associated Nuclear Antigen (LANA) from Kaposi's sarcoma-associated herpes virus (KSHV) [41], can hijack CRL5 complexes to trigger degradation of host proteins. Surprisingly, despite the number of SOCS-box proteins, their substrates remain poorly identified. This is probably due to the weak affinity of substrates for their requisite specificity subunit and because of the labile nature of the substrate complexed with the specificity subunit [42]. Furthermore, it should be mentioned that most experiments have been carried out by overexpression of the SOCS-box protein and/or its substrate candidate, often in non-physiological models. Only CRL5 with known substrates are described below (Fig. 2).

4.1. SH2 domain-containing proteins with a SOCS-box (SOCS)

Cytokine-inducible SH2 domain containing protein (CIS) mediates proteasomal degradation of the erythropoietin receptor [43]. SOCS1 is involved in the ubiquitylation and subsequent degradation of Vav [44], the NF-kappaB family member p65/RelA [45], Focal Adhesion Kinase FAK [46] and Myeloid differentiation primary-response gene 88 -Adaptor-Like protein MAL [47]. The Janus Kinase 2 (JAK2) and the TEL-JAK2 oncofusion protein are also substrates of SOCS1 [48–50]. Importantly, inhibitory effect of SOCS1 on TEL-JAK2 transformation of primary mouse hematopoietic cells *in vivo* was demonstrated indicating that modulation of SOCS1 E3 activity may be therapeutically useful for treatment of hematologic malignancies induced by tyrosine kinase fusions [49]. Insulin Receptor Substrates IRS1 and IRS2 are also targeted for degradation by SOCS1 [51]. Therefore, compounds that would protect IRS1 and IRS2 from SOCS1-mediated degradation might have value for the treatment of the insulin resistance syndromes and diabetes. SOCS3 is involved in the degradation of the CD33 receptor [52]. SOCS6 ubiquitylates the c-KIT receptor and the Src kinase Lck leading to their degradation [53,54].

4.2. Ankyrin repeat-containing proteins with a SOCS-box (ASB)

We have largely contributed to the understanding of the mechanisms of action of the largest family of SOCS-box containing proteins since the demonstration that ASB2 α is the specificity subunit of a CRL5 complex [55]. We further showed that ASB2 α drives polyubiquitylation and degradation by the proteasome of the actin-binding protein filamins A, B and C [56–58]. In contrast, filamin B but not filamins A and C, is a substrate of the second ASB2 isoform, ASB2 β [33,59]. Although the levels of JAK2 and JAK3 were shown to be affected by ASB2 α in overexpression experiments [60,61], neither endogenous nor exogenously expressed ASB2 α induces degradation of JAK proteins in hematopoietic cells [62]. The MLL (Mixed Lineage Leukemia) protein was also shown to be degraded by ASB2 α [63]. Importantly, the MLL-AF9 fusion protein resulting from the translocation between the *MLL* and *AF9* genes escape from ASB2 α -mediated degradation suggesting that ASB2 α mis-function may participate in MLL fusion mediated leukemia development [63]. The *ASB2* gene is also dysregulated at the transcriptional level in human leukemia. Indeed, *ASB2* is a transcriptional target of three acute myeloid leukemia oncoproteins, PML-RAR α , PLZF-RAR α and AML1-ETO, that act as transcriptional repressors, suggesting that *ASB2* repression may participate in the leukemogenesis process [32,64–66]. ASB3 was shown to trigger ubiquitylation of the tumour necrosis factor receptor 2 (TNF-R2) leading to its proteasomal degradation [67]. ASB4 mediates ubiquitylation and degradation of the adaptor molecule IRS4 [68]. ASB4 regulates trophoblast cell differentiation into placental vasculature through the degradation of the transcriptional regulator ID2

(Inhibitor of DNA binding 2) and loss of ASB4 in pregnant mice resulted in vascular dysfunction of the placenta, phenocopying women with preeclampsia [69]. ASB9 regulates creatine kinase B turnover [70]. ASB11 is able to specifically ubiquitylate and degrade Delta A thereby regulating the canonical Delta-Notch signaling pathway [71,72].

4.3. SPRY domain-containing proteins with a SOCS-box (SPSB)

SPSB1 enhances polyubiquitylation and degradation of the TGF- β type II receptor [73]. Inducible nitric oxide synthase (iNOS) is a substrate of SPSB1, SPSB2 and SPSB4 proteins [74,75]. *SPSB2* knockout macrophages showed prolonged iNOS expression, resulting in enhanced killing of *Leishmania major* parasites. Therefore, disruption of the SPSB–iNOS interaction may be beneficial in chronic and persistent infections such as leishmaniasis [74]. Expression of SPSB1 is also linked to cancer. Indeed, SPSB1 expression correlated with c-MET activity in human breast cancers and with increased risk of relapse in breast cancer patients [76].

4.4. WD40 repeat-containing proteins with a SOCS-box (WSB)

WSB1 regulates ubiquitylation and degradation of the HIPK2 kinase [77] and of the thyroid-hormone-activating type 2 iodothyronine deiodinase (D2) [78].

4.5. Other SOCS-box proteins

Elongin A can assemble in a CRL5 complex to drive ubiquitylation and degradation of Rpb1 following UV irradiation [79].

4.6. Viral proteins with a SOCS-box

The Vif protein of HIV-1 is the specificity subunit of a CRL5 [80] that polyubiquitylates the cellular intrinsic restriction factors APOBEC3F [81] and APOBEC3G [39]. The E4orf6 and E1B55K proteins from human adenovirus type 5 can assemble in an active CRL5 complex [82–84] that recognize different proteins to be degraded: p53 [83,84], meiotic recombination 11 (Mre11) [85], DNA ligase IV [86], integrin α 3 [87] and adeno-associated virus type 5 Rep52 and capsid proteins [88]. The Gam1 protein of the avian adenovirus CELO (chicken embryo lethal orphan) promotes ubiquitylation and degradation of the SUMO E1 subunit SAE1 [89]. Unexpectedly, Gam1 can assemble in a CRL2 or a CRL5 complex [89]. The transcriptional transactivator of the Epstein–Barr virus BZLF1 can bind to CUL2 and CUL5 leading to p53 degradation [90,91]. The LANA protein from KSHV induces the degradation of VHL and p53 [41]. The ORF73 protein encoded by the Murid Herpes Virus-4 (MuHV-4) targets p65/RelA to proteasomal degradation [92]. Through polyubiquitylation and proteasomal degradation of host proteins, all these viral proteins create a more amenable cellular environment for viral propagation.

5. Targeting CRL5 for therapeutic interventions

Proteasome inhibition has proved to be of therapeutic utility [93]. A small molecule called CC0651 is a specific inhibitor of the human E2 enzyme Cdc34A that inhibits the proliferation of human cancer cell lines [94]. Indeed, CC0651 stabilizes the normally low-affinity interaction between the E2 and ubiquitin at the donor site thereby inhibiting E2 catalytic activity [95]. The E2 enzymatic step in the ubiquitylation cascade was therefore proposed to be susceptible to small-molecule intervention. While these approaches affected steps common to many E3s, the strategy of modulating the activity of E3 ubiquitin ligases appears to be more

specific. Given the role of CRLs in many biological pathways, CRL dysfunction is likely to be involved in the development of diseases. Because CRL5s are hijacked by viruses, the inhibition of their activity holds also promise as an antiviral therapeutic.

MLN4924 is a small molecule that binds covalently to NAE thereby blocking its enzymatic activity and inhibiting cullin neddylation [4,96]. MLN4924 is currently in clinical trials and showed clinical activity in some solid tumor and hematologic malignancies. Indeed, this compound inhibits tumor cell growth by inducing apoptosis in diffuse large B-cell lymphoma models [97], in chronic lymphocytic leukemia B cells [98], in acute myeloid leukemia [99], in multiple myeloma [100], in Ewing sarcoma [101], or by inducing irreversible senescence [102]. MLN4924 is also cytotoxic to KSHV-infected lymphoma cells showing that inhibition of neddylation represents a novel approach for the treatment of KSHV-associated malignancies [103]. Importantly, MLN4924 induces autophagy in several human cancer lines [104,105]. This constitutes an overall survival signal for tumor cells. Consequently, abrogation of autophagy enhanced MLN4924-induced apoptosis and growth suppression suggesting that combination of MLN4924 with autophagy inhibitors may enhance therapeutic efficacy of MLN4924 [105]. Furthermore, MLN4924 induces autophagy and apoptosis to suppress liver cancer cell growth in a xenograft mouse model indicating that MLN4924 exhibits an antitumor activity *in vivo* [104]. Interestingly, MLN4924 was shown to be an effective radiosensitizing agent in breast cancer cell lines [106] and in pancreatic cancer both *in vitro* and *in vivo* [107]. A synergic cytotoxic effect between cisplatin and MLN4924 in both platinum-sensitive and platinum-resistant ovarian cancer cells was also reported [108,109]. This has been furthered to other DNA-damaging agents such as mitomycin C, cytarabine, SN-38 and gemcitabine [110,111]. Altogether, these studies highlight the therapeutic potential of MLN4924 in preclinical cancer models and provide a strong rationale for clinical investigations of antitumor agent and MLN4924 combinations in cancer therapy. However, not surprisingly, heterozygous mutations in the NAE β (UBA3) subunit of NAE in cell lines and xenograft models of cancer can be observed following selection pressure with MLN4924 [112,113]. Therefore, second-generation NAE inhibitors may be developed to overcome resistance to MLN4924 mediated by mutations in NAE β .

Since E3s determine the specificity of protein substrates they represent an important class of potential drug targets for pharmaceutical intervention. Through the degradation of several substrates, CRL1 control a multitude of processes including cell cycle progression and their dysregulation is implicated in many malignancies. Most studies have focused on the development of anticancer therapeutics by targeting CRL1 and some compound are currently at the preclinical stage of development [114,115]. Indeed, effective modulation of the activity of CRL5 for therapeutic means will benefit from our understanding of the structural architecture of CRL5 complexes and of the molecular mechanisms of substrate binding to specificity subunits. Indeed, crystal structures of components of CRL5 have been solved: SOCS2-EloB-EloC [116], SOCS4-EloB-EloC [117], SOCS2-EloB-EloC-CUL5 [118], ASB9-EloB-EloC-Cul5 [119,120] and HIV-1 Vif-CBF β -CUL5-EloB-EloC [121]. Using the crystallographic structures of the SOCS2-EloB-EloC [116] and CUL1-RBX1-SKP1-SKP2 [122] complexes, we modeled the ASB2 α -EloB-EloC-CUL5-RBX2 E3 complex bound to an E2 enzyme [62]. Small molecule inhibitors of E3s often act by disrupting the interface between the specificity subunit and its substrate. Because CRLs may have multiple specific substrates, identification of the substrate whose degradation drives the biological functions of the CRL is crucial for targeting approaches. In this context, it is crucial to determine whether the ubiquitin ligase activity of the E3 drives polyubiquitylation and degradation of the substrate candidate in

physiological relevant settings. Indeed, selectivity of an E3 for a given substrate may be cell-type specific, dependent upon subcellular localization, timely regulated (e.g. cell cycle progression, differentiation, developmental stages, embryogenesis vs adult life, ageing ...) or regulated by extracellular signals from the microenvironment. Furthermore, deciphering the structural determinants for recognition of the substrates by the specificity subunit is also key step for the identification of targetable interfaces and for the design and/or optimization of inhibitors.

Despite the large number of CRL5, their specific substrates remain poorly identified (Fig. 2) and many remain with no established substrates. This has somehow limited the development of compounds targeting CRL5. One of them, the small molecule RN-18 antagonizes HIV-1 Vif function and inhibits viral replication only in the presence of APOBEC3G without affecting general ubiquitin-mediated protein degradation [123]. The HIV-1 Vif-APOBEC3G axis can also be modulated using the small inhibitor VEC-5 through inhibition of the binding of EloC to Vif [124]. Through inhibition of the degradation of the host antiviral factor APOBEC3G, these compounds made the proof-of-principle that blocking the activity of hijacked E3s could lead to the development of novel antiviral therapies.

6. Conclusion

Thanks to their high specificity for substrates, CRL5 represent promising drug targets. The understanding of the biological roles of CRL, the identification of their specific substrates and mechanism of recruitment, as well as the knowledge of CRL assembly and regulation will undoubtedly contribute to additional new drug targets.

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