

Review

Cullin Family Proteins and Tumorigenesis: Genetic Association and Molecular Mechanisms

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Abstract

Cullin family proteins function as scaffolds to form numerous E3 ubiquitin ligases with RING proteins, adaptor proteins and substrate recognition receptors. These E3 ligases further recognize numerous substrates to participate in a variety of cellular processes, such as DNA damage and repair, cell death and cell cycle progression. Clinically, cullin-associated E3 ligases have been identified to involve numerous human diseases, especially with regard to multiple cancer types. Over the past few years, our understanding of cullin proteins and their functions in genome stability and tumorigenesis has expanded enormously. Herein, this review briefly provides current perspectives on cullin protein functions, and mainly summarizes and discusses molecular mechanisms of cullin proteins in tumorigenesis.

Key words: Cullins, E3 ligase, Tumorigenesis, DNA damage, apoptosis, cell cycle

1. Introduction: overview of cullins and their functions

Cullins are evolutionarily conserved family proteins throughout bacteria, plants and mammals [1]. The human genome encodes six cullin proteins characterized by a cullin homology (CH) domain in the carboxyl (C)-terminal, including CUL1, 2, 3, 4A, 4B and 5 (Figure 1) [2]. Moreover, the human genome also encodes another two atypical cullin proteins (CUL7 and CUL9) that consist of additional homology domains [3], such as DOC1 (destruction of cyclin B) homology domain and IBR (in-between-ring) domain [3]. These cullin proteins potentially assemble RING-box protein 1 (RBX1, also known as ROC1) and/or RBX2 (also known as ROC2 or SAG-sensitive to apoptosis gene), forming Cullin-RING ubiquitin ligases (CRLs) [4].

Generally, CRLs consist of four components: cullins, RINGs, adaptor proteins and substrate recog-

niton receptors [5]. The two RING-box components (RBX1 and RBX2) can bind to two zinc atoms via a $C_3H_2C_3$ motif to form the RING finger domain, which is required for the activity of CRLs [6]. Except for CUL9, all of the other cullin proteins in human can interact with RBX1 or RBX2 through an evolutionarily conserved CH domain at the C-terminus [5]. Moreover, the human genome contains four adaptor proteins, including S-phase kinase-associated protein 1 (Skp1), Elongin B and C, and damaged DNA binding protein 1 (DDB1) [7]. Of them, Skp1 generally interacts with CUL1 and CUL7; Elongin B and C interact with CUL2 and CUL5; and DDB1 interacts with CUL4A and CUL4B (Figure 2) [7]. In addition, BTB (Broad complex, Tramtrack, Bric-a-brac) proteins have been reported to function as adaptors interacting with CUL3 [8]. However, it has not been found adaptor

protein that interacts with CUL9 to date (Figure 2). These CRLs have been identified to harbor more than 400 substrate recognition receptors in human genome, including 78 F-box proteins for CRL1, 80 SOCS (suppressor of cytokine signaling) proteins for CRL2/5, ~200 BTB proteins for CRL3, and 90 DCAF (DDB1 and CUL4-associated factors) proteins for CRL4A/B [5, 9]. These substrate recognition receptors further ubiquitinate and degrade thousands of substrates [5], controlling multiple cellular processes, such as cell cycle regulation, gene transcription, apoptosis, DNA damage and repair, chromatin remodeling, oxidative stress response, and signal transduction [5].

Interestingly, all of these CRLs share a similar core architecture, in which a cullin protein serves as a scaffold that binds to an adaptor protein or a substrate receptor protein at the N-terminus, and interacts with a RING protein at the C-terminus (Figure 2) [5]. The substrate specificity of CRLs is determined by substrate recognition receptors such as F-box proteins and DCAF proteins, whereas the activity of core ligases is controlled by CUL-RBX1 (or RBX2) complex (Figure 2) [2]. Clinically, deregulation of CRLs is linked to uncontrolled proliferative diseases such as cancer [5]. However, only a few components in these hundreds of CRLs are well studied for their involvement in cancer [5]. Generally, cancer-associated CRL components can be categorized into two classes: on-

cogenes and tumor suppressors [5]. The oncogenes such as *Skp2* (S-phase kinase-associated protein 2) and *CUL4A* are frequently overexpressed in cancers [10]. Tumor suppressors such as *FBXW7* (F-box/WD repeat-containing protein 7) and *VHL* (Von Hippel-Lindau) are always mutated and inactivated in human cancers [11].

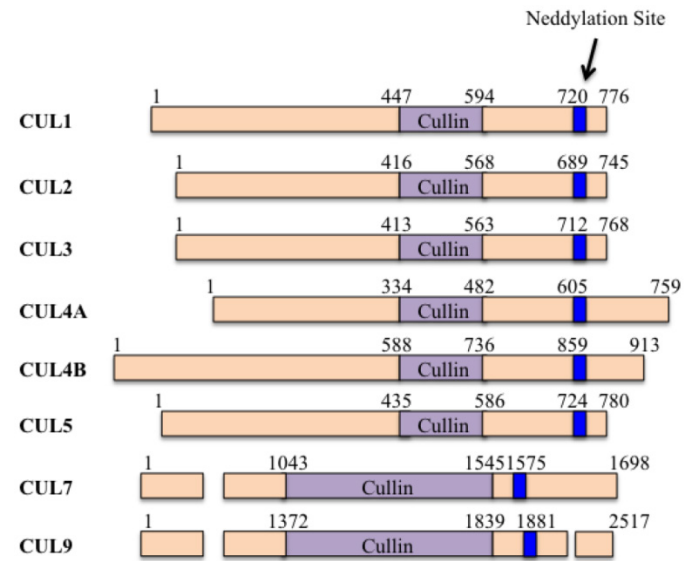


Figure 1. The domain structures of human cullin proteins. Numbers represent the amino acid codons. The blue lines indicate the position of the neddylatation site [5].

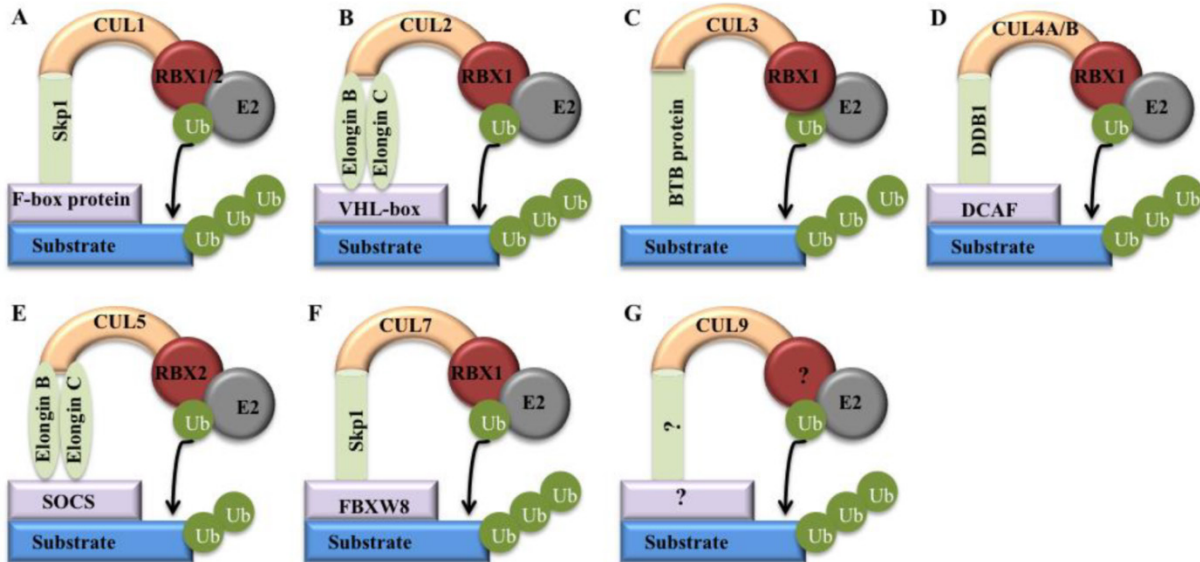


Figure 2. Models of cullin-RING E3 ligases. Cullin proteins recruit adaptor proteins (Skp1 for CUL1 and CUL7, Elongin B/C for CUL2 and CUL5, BTB protein for CUL3, and DDB1 for CUL4A and 4B), receptor proteins (F-box proteins for CUL1, VHL-box for CUL2, DCAFs for CUL4A and 4B, SOCS for CUL5, and FBXW8 for CUL7), and RING protein (RBX1/2) to form CRL E3 ligases that promote ubiquitin to transfer from RBX1/RBX2-bound E2 to a substrates [5]. (A) CRL1; (B) CRL2; (C) CRL3; (D) CRL4; (E) CRL5; (F) CRL7; (G) CRL9.

CRLs are the largest family of E3 ubiquitin ligases and they are responsible for the ubiquitination of about 20% of intracellular proteins through ubiquitin-proteasome system (UPS) [5]. Cancer cells utilize the UPS for their increased growth and decreased apoptotic cell death [12]. The UPS consists of six components: ubiquitin (Ub), the Ub-activating enzyme (E1), a variety of Ub-conjugating enzymes (E2s), numerous Ub ligases (E3s), the proteasome and the deubiquitinases (DUBs) [12]. Protein degradation by the UPS mainly includes two distinct steps: (1) ubiquitination, which adds a mono- or poly-Ub-tag to the targeted proteins, and (2) proteasomal degradation, which degrades the Ub-tagged proteins into oligopeptides [12]. Two major ubiquitin-ligase complexes, including Skp1-CUL1-F-box (SCF) complex and Anaphase Promoter Complex or Cyclosome (APC/C), are responsible for ubiquitin-mediated proteolysis in mammals [13]. Previous studies have revealed that UPS contributes to oncogenesis through a variety of mechanisms, such as controlling cell proliferation and survival, regulating the turnover of key proteins (cyclins, p27 and p53) involved in cell cycle progression, and regulating the NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells) pathway [12]. Moreover, cancer cells can utilize the UPS to achieve aberrant growth and resistance to apoptosis [12].

The cullin family proteins are also known as substrates of NEDD8 (neural precursor cell expressed, developmentally down-regulated 8), an ubiquitin-like protein [14, 15]. The NEDD8-associated ubiquitination includes several critical steps: (1) NEDD8 is activated by NEDD8-activating enzyme E1 (also known as NAE1); (2) the activated NEDD8 is further transferred to ubiquitin-conjugating enzyme E2 (UBC12 or UBE2F) [15]; and (3) NEDD8-conjugating enzyme E2 collaborates with NEDD8-E3 ligase, conjugating NEDD8 to its target substrates [15]. The NEDD8 pathway is critical in mediating the ubiquitination of numerous CRL substrate proteins, such as DNA replication factor CDT1 (cdc10-dependent transcript 1), the NF- κ B transcription factor inhibitor pIkB α , and the cell cycle regulators cyclin E and p27 [16, 17].

2. Cullins, DNA damage and repair

DNA damage is one of the most pervasive characteristics of tumor cells [18]. Numerous biotic and abiotic stresses can result in DNA damage, such as genotoxic chemicals, ionizing radiation (IR) and ultraviolet (UV) radiation [19, 20]. DNA damage mainly includes DNA double-strand breaks (DSBs), DNA lesions, single-strand breaks, DNA base mismatches, and DNA crosslinks [21, 22, 23]. Organisms have evolved sophisticated mechanisms to repair

damaged DNA and maintain genome integrity. Generally, DSBs are repaired through nonhomologous end joining (NHEJ), alternative NHEJ, or homologous recombination (HR) [19, 24]. UV-induced DNA lesions and other DNA bulks are repaired by nucleotide excision repair (NER) [19, 25]. Small and non-helix-distorting base lesions are repaired by base-excision repair (BER) [19]. Single-strand breaks and DNA base mismatches are corrected by mismatch repair (MMR), while DNA crosslinks are repaired by Fanconi anemia (FA) pathway [19].

DNA damage can induce cascades of posttranslational modifications, such as phosphorylation, ubiquitination and sumoylation [19]. The biochemical and molecular studies have indicated that ubiquitination regulates and coordinates various pathways of DNA damage recognition, signaling and repair [19, 26]. Two cullin proteins (CUL4A and CUL4B) associate with DDB1 and DCAF-type substrate receptors, forming numerous E3 ligases to promote substrate ubiquitination in response to DNA damage [27, 28]. The DDB1-CUL4-DCAFs complexes can ubiquitinate histone H2A, H3 and H4 at sites of UV-induced DNA damage, thereby facilitating their removal from the nucleosome and promoting subsequent DNA repair [29]. In different organisms, a variety of DCAFs have been reported to interact with CUL4-DDB1 in response to DNA damage and repair, such as DDB2 [30], cockayne syndrome A (CSA) [31], two CSA homologues in *Arabidopsis* (CSAat1A and CSAat1B) [32], transducin-like enhancer of split 1 to 3 (TLE1-3) [33], WD40 repeat protein 5 (WDR5) [27], *Drosophila* lethal (2) denticleless protein (L2DTL) (also known as CDT2) and the Polycomb-group protein EED (also known as ESC) [33].

In human, *CUL4A* overexpression has been widely found in multiple cancer types, including breast cancer [34], ovarian cancer [35], hepatocellular carcinomas [36], adrenocortical carcinomas [37], and childhood medulloblastoma [38]. However, the relevance between CUL4B and cancer remains to be determined. Mutations of the *CUL4B* gene are causally associated with human X-linked mental retardation [39]. CRL4 ubiquitin ligase complex recognizes a variety of substrates involved in DNA damage and repair, such as DDB2, XPC (xeroderma pigmentosum complementation group C), H2A, H3 and H4, UNG2 (uracil-N-glycosylase 2) and SMUG1 (single-strand-selective monofunctional uracil-DNA glycosylase 1) [10]. DDB2 acts as a substrate receptor in complex with CUL4A and DDB1 to target another NER damage sensor (XPC) for ubiquitination, which can increase its affinity to DNA [10]. Downregulation of DDB2 and its substrates limits NER activity, whereas enhanced *DDB2* expression or knockout of

CUL4A results in the increase of DNA repair efficiency following genotoxic insults, thereby protecting genome from carcinogenesis [10]. Moreover, *CRL4^{DDB2}* E3 ligase also can target histone H2A, H3 and H4, facilitating the initiation of NER [29, 40]. Both *UNG2* and *SMUG1* function to prevent mutagenesis by eliminating uracil from DNA molecules via cleaving the N-glycosylic bond and initiating the BER pathway [41, 42]. *CRL4* ubiquitin ligase can mediate *UNG2* and *SMUG1* degradation, thereby maintaining genome integrity [43, 44].

3. Cullins and cell cycle progression

Cells that progress through the cell cycle unchecked may eventually develop as malignant tumors [45]. Cell cycle progression is controlled by a series of cyclin-dependent kinases (CDKs), proteins interacting with cyclins and CDK inhibitors (CKIs) [46]. The activity of CDKs is regulated by several mechanisms, including association with regulatory subunits, protein modification such as phosphorylation and dephosphorylation, and interaction with CKIs [46, 47]. The levels of cyclins, CKIs and many other cell cycle regulators oscillate during the cell cycle as a result of periodic proteolysis through UPS [48]. Two major ubiquitin ligases, APC/C and SCF, are responsible for the periodic proteolysis of many cell cycle regulators [49]. The APC/C is required for the transition from M phase into G₁, and this process is mediated by the ubiquitination of anaphase inhibitors known as securins and mitotic cyclins, respectively [50]. The SCF complex targets cyclins and CKIs for ubiquitination, functioning in most phases of the cell cycle [48]. The SCF complex can bind to multiple F-box proteins functioning as receptor components and three of these F-box proteins including Skp2, FBXW7, and β -transducin repeat-containing protein (β -TrCP, also known as FBXW1) are well characterized and implicated in cell cycle regulation [51]. Clinical evidence suggests that dysregulation of these F-box proteins contributes to human cancers [15].

Skp2 behaves as an oncogene in cell systems and overexpression of *Skp2* is frequently observed in human cancer progression and metastasis [52]. Multiple cancer types, including lymphomas, prostate cancer, melanoma, nasopharyngeal carcinoma, pancreatic cancer and breast carcinomas, have been reported to associate with *Skp2* overexpression [11]. Biochemically, Skp2 functions as the substrate-recruiting component of SCF complex, and SCF^{Skp2} can recognize and promote the degradation of several negative cell cycle regulators, including p27, p21, p130 and p57 [53]. The expression of *Skp2* is significantly increased in many transformed cells during S and G₂ phases of the cell cycle, but not in G₁ [54]. Additionally, Skp2 mediates

the ubiquitination of the p27, which is degraded via the UPS in G₁ phase [48]. The CDK inhibitor p27^{Kip1} is also involved in cancer, playing roles in inhibiting cyclin E-CDK2 complex [55]. Levels of p27^{Kip1} are regulated by SCF^{Skp2} complex during cell cycle through proteasome system [55].

FBXW7 protein is a well-established cell cycle regulator and functions as tumor suppressor required for substrate recognition in an SCF complex [15]. SCF^{FBXW7} can ubiquitinate several substrates involved in oncogenesis, including cyclin E, c-Myc (myelocytomatosis oncogene), c-Jun (jun proto-oncogene) and Notch, thereby promoting cells to exit from the cell cycle [10]. Interestingly, FBXW7 binds to each of these substrates through a conserved phosphorylated domain known as the CDC4 phosphodegron [56]. The *in vitro* analysis of *FBXW7* gene silencing enhances the expression of *c-Myc* and *cyclin E* genes, and promotes cell proliferation [57]. Thus, FBXW7 is crucial for preventing carcinogenesis as its important roles in cell cycle regulation [56].

β -TrCP is emerging as an important protein in carcinogenesis because of the deregulated proteolysis of its substrates [52]. Previous studies have identified numerous substrates of β -TrCP, and these substrates can be divided into two main groups: cell cycle regulators such as CDC25A (cell division cycle 25 homolog A), CDC25B, WEE1, I κ B α (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha), I κ B β , I κ B ϵ , p100 and p105, and pro-apoptotic regulators such as pro-caspase, p63, STAT1 (signal transducers and activator of transcription 1) [52]. Importantly, β -TrCP plays critical roles in regulating cell cycle checkpoints, and the increased activities of the SCF ^{β -TrCP} complex may promote tumor cell growth and/or genetic instability by accelerating cell cycle events [58]. In response to DNA damage, β -TrCP contributes to CDK1 dysfunction by mediating the degradation of CDC25A in collaboration with Chk1 (checkpoint kinase 1), thereby preventing cell cycle progression before the completion of DNA repair [59].

Moreover, other cullin proteins such as CUL3, CUL4A and CUL5 also have been demonstrated to contribute to cell cycle progression in cancer. CUL3 forms a complex known as BTB-CUL3-RBX1 (BCR) ubiquitin ligase, targeting cyclin E for ubiquitination and controlling S phase in mammalian cells [60]. Overexpression of *CUL3* in mice increases ubiquitination of cyclin E, but not other cyclins [60]. Conversely, the deletion of *CUL3* gene results in increased accumulation of cyclin E protein, and has cell-type-specific effects on S-phase regulation [60]. *CRL4* interacts with the substrate receptor CDT2, promoting protein ubiquitination in S phase and after DNA damage [61]. The *CRL4^{CDT2}* complex can target a variety of sub-

strates involved in cell cycle regulation, such as CDT1 [62], p21 [63], and Chk1 [64]. The CUL4A-DDB1 E3 complex can target tumor suppressor RASSF1A (RAS association domain family 1, isoform A) for degradation during mitosis [65]. *CUL4A* depletion results in increased levels of RASSF1A protein, whereas overexpression of *CUL4A* and *DDB1* significantly enhances RASSF1A protein ubiquitination and results in reduced RASSF1A protein levels [65]. Overexpression of *CUL4A* antagonizes the ability of RASSF1A to induce M-phase cell cycle arrest [65]. *CUL5* is a target of microRNA-7 (miR-7) [66]. Downregulation of *miR-7* increases the expression of *CUL5* to facilitate G₁/S transition in human hepatocellular carcinoma cells [66].

4. Cullins and p53 pathway

p53 functions as a tumor suppressor through several mechanisms: (1) activating DNA repair proteins when DNA has sustained damage; (2) arresting cell cycle at the G₁/S regulation point on DNA damage recognition; and (3) initiating cancer cell apoptosis [67]. In normal cells, p53 is generally kept at low level by proteasome-mediated degradation, allowing cell cycle progression and cell proliferation [68]. Under stress conditions, p53 is stabilized and activated through posttranslational modifications of itself and its regulatory proteins, eventually inducing the expression of downstream genes that are responsible for cell cycle arrest, DNA repair and apoptosis [69]. E3 ligases are also involved in p53 pathway, and over 20 different E3 ligases have been identified to play roles in the control of stability, oligomerization, nuclear export, translocation of p53 to chromatin or nuclear foci [70]. Interestingly, cullin proteins have been reported to directly interact with p53, or regulate p53 expression and its protein stability [71, 72, 73].

An F-box protein JFK (just one F-box and Kelch domain-containing protein) promotes p53 degradation through the formation of an SCF complex in which JFK serves as a substrate receptor [74]. The *CUL1* knockout mouse is lethal at E6.5 before the onset of gastrulation, associating with increased p53 levels and apoptosis in the embryonic ectoderm [73]. The BZLF1 (BamHI Z fragment leftward open reading frame 1) protein directly functions as an adaptor component of the ECS (Elongin B/C-CUL2/5-SOCS-box protein) E3 ligase complex, targeting the phosphorylated p53 for degradation [75]. Deletion of *CUL3* gene in liver cells causes a persistent and massive expansion phenotype, which is dependent on differentiation and undergoes remarkable DNA damage, thereby leading to cell death [73]. Interestingly, this process appears to be controlled by p53, as simultaneous loss of *CUL3* and p53 in hepatic progenitors

transforms these cells to highly malignant tumor-initiating cells that form largely undifferentiated tumors in nude mice [72, 73]. CDT2 and PCNA (proliferating cell nuclear antigen) interact with p53, and regulate p53 poly-ubiquitination and protein stability through both MDM2 (mouse double minute 2 homolog) and CUL4A-DDB1 complexes in unstressed human cells [76]. Moreover, PCNA and DDB1-CUL4A complex also regulate the proteolysis of MDM2 in response to UV-irradiation [76]. Cells deleting *CUL4A* result in proliferation defects and genome instability, and the decrease of p53 levels [77]. Moreover, expression of *CUL4A* increases the decay-rate of p53 and delays the accumulation of p53 in response to DNA damage [78]. Recently, CUL4B has been reported to directly interact with p53 and induce its poly-ubiquitination [79]. VACM-1 (Vasopressin-activated Ca²⁺-mobilizing 1, also known as CUL5) inhibits cell growth by a mechanism that involves MAPKs (mitogen activated protein kinases) and p53 signaling pathways [80]. Additionally, the degradation of p53 by adenovirus E4orf6 and E1B55K proteins occurs via a novel mechanism involving an ECS E3 ligase [81]. The cytoplasmic localized ubiquitin ligase CRL7 can bind to p53 and promote cell growth by antagonizing p53 function [71]. Biochemically, CUL7 only causes mono- or di-ubiquitination of p53 *in vitro*, whereas MDM2 polyubiquitinates p53 in the same condition [71]. In a proteomic screen for p53 interactors, CUL7 was also identified to efficiently associate with p53 [82]. The functional analysis indicated that induction of *CUL7* by DNA damage attenuated p53 function [82].

5. Cullins and WNT pathways

WNTs and their downstream effectors regulate various cellular processes that are important for carcinogenesis and tumor progression, including tumor initiation, tumor growth, cell senescence, cell death, differentiation and metastasis [83]. Cullin proteins, such as CUL1, CUL3 and CUL4, have been identified to involve WNT pathways [84, 85].

SCF^{β-TrCP} E3 ligase can specifically regulate the stability of β-catenin, thereby having a significant impact on cancer genesis [85]. Recently, FAS-associated factor 1 (FAF1) has been reported to function as a scaffold protein and form a complex with SCF^{β-TrCP} E3 ligase, antagonizing WNT signaling by stimulating β-catenin degradation [86]. Moreover, an F-box/WD40 protein, FWD1, form a complex with SCF, mediating ubiquitin-dependent proteolysis of β-catenin [87].

KLHL12 (Kelch-like protein 12) is a substrate-specific adaptor of a BCR E3 ligase complex [84]. The BCR^{KLHL12} ubiquitin ligase negatively regu-

lates the WNT- β -catenin pathway by targeting and degrading Dishevelled, which is a conserved protein that interprets signals received by Frizzled receptors [84].

The DDB1-CUL4 complex has been demonstrated to be a WNT-induced ubiquitin ligase [88]. The CDK inhibitor p27^{KIP1} is tightly linked with human cancer [88]. Interestingly, the WNT-induced p27^{KIP1} turnover is independent from classical SCF^{Skp2}-mediated degradation in both mouse and human cells [88]. Instead, the turnover requires CUL4A and CUL4B [88]. Additionally, DDB1-CUL4B complex can negatively regulate β -catenin [89]. The knockdown of *CUL4B* in mouse results in an increase in β -catenin levels [89]. Immunoprecipitation experiments suggest that CUL4B and β -catenin form a signal transduction complex in *Drosophila*, mouse and human [89].

6. Cullins regulate the expression or stability of oncogenes

Cullin proteins are also involved in regulating the expression or stability of several oncogenes, such as *c-Myc*, *c-Jun* and *I κ B α* .

c-Myc is a transcription factor that plays important roles in cell cycle progression, apoptosis and cellular transformation [90]. Malfunctions in *c-Myc* have also been found in carcinoma of the cervix, colon, breast, lung and stomach cancer [91]. *c-Myc* enhances the expression of *CUL1* gene, promoting ubiquitin-dependent proteolysis and cell cycle progression through SCF^{Skp2} E3 ligase [92]. TRUSS (tumor necrosis factor receptor-associated ubiquitous scaffolding and signaling protein) is a receptor protein of DDB1-CUL4 complex [93]. The suppression of DDB1-CUL4-TRUSS E3 ligase stabilizes *c-Myc* protein in cancer cells [93]. *CUL7* can function as an oncogene that cooperates with *c-Myc* in transformation by blocking *c-Myc*-induced apoptosis in a p53-dependent manner [94]. The MM-1 (Myc modulator 1) protein binds to *c-Myc* and represses transcriptional activity of *c-Myc* [95]. Further studies indicate that MM-1 facilitates *c-Myc* degradation by recruiting proteasome and the ECS^{Skp2} E3 ligase [95]. Interestingly, Skp2 interacts with *c-Myc* and participates in its ubiquitylation and proteasomal degradation [96]. Surprisingly, Skp2 acts as a positive cofactor for *c-Myc*-regulated transcription [96].

c-Jun is the firstly demonstrated as an oncogenic transcription factor, and it is required at the early stage of tumor development [97]. Deletion of *c-Jun* can significantly suppress tumor formation [98]. The human De-etiolated-1 (hDET1) promotes ubiquitination and degradation of *c-Jun* by assembling DDB1-CUL4A-RBX1 E3 ligase transcription [99]. Ab-

lation of *DDB1*, *CUL4A* or *RBX1* by RNA interference stabilizes *c-Jun* and increases *c-Jun*-activated transcription [99]. Biochemically, RBX2 regulates skin carcinogenesis by targeting *c-Jun/AP1* (activator protein 1) and *I κ B α /NF- κ B* in a stage-dependent manner [100]. RBX2 promotes ubiquitination and degradation of *c-Jun* to inhibit AP1 activity and AP1-induced neoplastic transformation in a mouse epidermal cell model [100]. It appears that RBX2 inhibits tumor formation at the early stage by targeting *c-Jun/AP1* and promotes tumor growth at the later stage by targeting *I κ B α /NF- κ B* [100].

I κ B α functions to inhibit the NF- κ B transcription factor [101]. Biochemically, *I κ B α* can be ubiquitinated by a SCF-like complex containing Skp1, CUL1, β -TrCP1 and β -TrCP2 [102]. All these components are cooperatively recruited to bind to a phosphorylated *I κ B α* (p*I κ B α*) produced by tumor necrosis factor- α (TNF- α) stimulation [102]. *I κ B α* -E3 ligase binds to p*I κ B α* and catalyzes the ubiquitination of p*I κ B α* in the presence of ATP, Ub, and E1-activating and E2-conjugating enzymes [102]. Further studies indicate that NEDD8 modification of CUL1 activates SCF ^{β -TrCP} dependent ubiquitination of *I κ B α* [103].

7. Cullins and apoptosis

Studies have revealed a high frequency of apoptosis in spontaneously regressing tumors and in tumors treated with cytotoxic anticancer agents [104]. The cullin proteins such as CUL1, CUL3 and CUL4 are also involved in apoptosis.

CDK-associated CUL1 can promote cell proliferation and inhibit cisplatin-induced apoptosis in the AGS gastric cancer cell line via modulating the expression of *p53*, *BCL2* (B-cell lymphoma 2) and *BAX* (BCL2 associated X protein) [105]. CUL3-based poly-ubiquitination and p62-dependent aggregation of caspase-8 can mediate extrinsic apoptosis signaling [106]. Caspase-8 is a critical initiator of the extrinsic apoptosis pathway, and the stimulation of death receptors 4 and 5 (DR4 and 5) induces CUL3/RBX1-dependent poly-ubiquitination of caspase-8 [106]. Further, the Ub-binding protein p62 promotes caspase-8 aggregation, augmenting the activation and full processing of the enzyme and thereby leading to robust stimulation of effector caspases and apoptosis [106]. CUL4-DDB1^{CDT2} and CUL4-DDB1^{DDB2} E3 ligase complexes have been implicated in the proteolysis of cell cycle inhibitor p21, which can inhibit apoptosis following DNA damage [107]. Moreover, cells lacking *CDC7* expression undergo apoptosis after mild DNA damage, where CUL4-DDB1^{CDT2} E3 ligase induces Tob (Transducer of ErbB-2) ubiquitination and its subsequent degradation [108]. However, *CDC7* phosphorylates and in-

teracts with Tob to inhibit the CUL4-DDB1^{CDT2}-dependent Tob degradation in normal cells [108]. Additionally, CUL4B promotes proliferation and inhibits apoptosis of human osteosarcoma cells [109]. In the osteosarcoma SAOS-2 cells harboring decreased *CUL4B* gene expression by RNA interference (RNAi), cell proliferation rate and clonability are significantly inhibited, whereas the apoptosis rate is greatly increased [109].

8. Cullins and Hypoxia-inducible factor (HIF-1) pathway

HIF-1 is a basic helix-loop-helix-PAS domain transcription factor, and upregulation of *HIF-1* is generally associated with tumor cell growth and progression [110]. Some cullin proteins, including CUL2, CUL3 and CLU5, have been demonstrated to involve HIF-1 pathway.

Previous studies have indicated that neddylation plays a major role in modulating the HIF pathway through CUL2 [111]. The von Hippel-Lindau tumor suppressor protein (pVHL) assembles with CUL2 and Elongin B/C, forming a protein complex, namely, CBC^{VHL} [111]. Remarkably, cells lacking pVHL induce the expression of hypoxia-inducible genes, including *VEGF* (vascular endothelial growth factor), *GLUT1* (glucose transporter 1), and *PDGF-β* (platelet derived growth factor-β) [111], indicating the important roles of CBC^{VHL} in HIF-1 pathway. Recently, inhibition of CUL2 neddylation has been demonstrated to stabilize HIF-1 in mucosal inflammatory responses [112].

A CUL3 substrate adaptor, namely, KLHL20 (kelch-like family member 20), can be induced by HIF-1 [113]. The CUL3-KLHL20 ubiquitin ligase-dependent pathway targets PML (promyelocytic leukemia) to potentiate HIF-1 signaling and prostate cancer progression [113]. Additionally, the PML destruction pathway participates in a feedback mechanism to maximize HIF-1α induction, thereby potentiating multiple tumor hypoxia responses, including metabolic reprogramming, epithelial-mesenchymal transition, migration, tumor growth, angiogenesis, and chemoresistance [113].

RBX2 also can function as a HIF-1 target gene, and it can form a VCB E3 ligase with VHL, CUL5 and Elongin B/C *in vivo*, promoting VHL-mediated HIF-1α ubiquitination and degradation [114]. Interestingly, the endogenous HIF-1α at both basal and hypoxia-induced levels is significantly increased upon *RBX2* gene silencing [114].

9. Cullins and other pathways involved in carcinogenesis

Cullin proteins are also reported to involve some

other pathways, such as oxidative stress and chromatin remodeling.

Oxidative stress affects the stability or expression of numerous key signaling proteins, such as nuclear factor erythroid 2-related factor 2 (Nrf2), kelch-like protein 1 (Keap1), Ras, Raf, MAPK, ERK1/2, MEK, p38α, c-Jun N-terminal kinase (JNK), c-Myc and p53 [115]. Of them, Nrf2 and Keap1 can function together with CUL3 and their molecular mechanisms have been well studied [116]. Nrf2 is rapidly degraded through a Keap1-CUL3-RBX1 E3 ligase-dependent mechanism [116]. Keap1 functions as a substrate adaptor, and is responsible for bringing Nrf2 into the E3 complex, in which it utilizes its DGR domain to bind the N-terminal region of CUL3 [116]. RBX1 recruits the catalytic function of E2 by binding to the C-terminal region of CUL3 [116]. E2 catalyzes poly-ubiquitination of Nrf2 protein on the lysine residues of Neh2 domain [116]. Additionally, *CUL3* overexpression depletes *Nrf2* in breast cancer, which is associated with increased sensitivity to carcinogen, oxidative stress and chemotherapy [117].

All of the human genome is packaged into chromatin. Chromatin remodeling proteins are gatekeepers and function as major determinants of accessory factors to nucleosome DNA, thereby allowing a wide repertoire of biological functions [118]. Thus, aberrant expression or epigenetic modulation of remodeling proteins confers a unique ability to cancer cells, reprogramming its genome for the maintenance of oncogenic phenotypes [118]. The CUL4-DDB1-RBX1 E3 ligase can target histones for ubiquitination, and importantly, ubiquitination of histones may facilitate cell in response to DNA damage [119]. Therefore, histone ubiquitination by CUL4 E3 ligase constitutes a novel mechanism through which CUL4 regulates chromatin function and maintains genomic integrity [119]. Knockdown of *CUL4A* or *CUL4B* in HeLa cells significantly reduces the levels of H3 and H4 ubiquitination, but has little effects on H2A and H2B ubiquitination [119].

10. Conclusion

Collectively, cullin proteins play critical roles in cancer through a variety of mechanisms, including DNA damage and repair, cell cycle control, apoptosis, oxidative stresses, chromatin remodeling, regulation of oncogenes, signal transduction involved in p53 and WNT (Figure 3). In which, cullins proteins mainly function as scaffolds to form numerous E3 ligases, and these E3 ligases further recognize different substrates to participate in different cellular processes. Complete inactivation of CRLs causes tumor cell growth suppression, indicating that the major role of CRLs in cancer cells is to target and degrade tumor suppressor

proteins in order to sustain an unlimited proliferation [5]. Thus, this unique feature of cancer cells confers them much more vulnerable to be targeted against CRLs in therapy [5]. It is clear that further studies to explore CRL functions and screen substrates of CRLs

in different cancer types. To develop potential anti-cancer agents, it is important to focus on the development of CRL inhibitors by targeting cullin neddylation and CRL-mediated ubiquitination in the future.

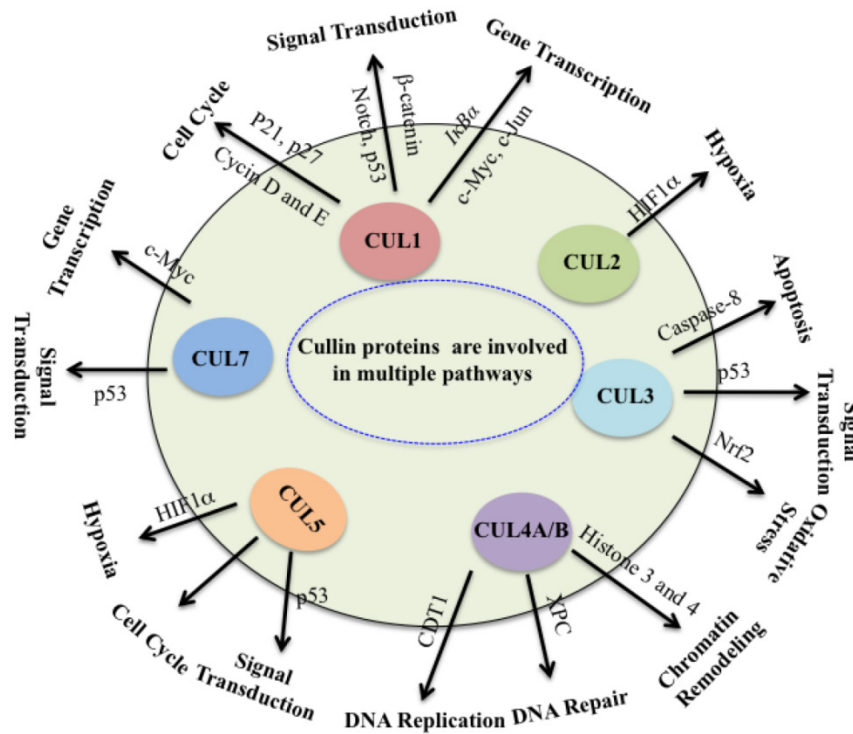


Figure 3. Cullin proteins affect multiple pathways involved in cancer. Cullin proteins play critical roles in cancer through a variety of mechanisms, including DNA replication and repair, cell cycle control, apoptosis, oxidative stresses, chromatin remodeling, hypoxia, regulation of oncogenes, and signal transduction involved in p53 and WNT. Proteins participate in these processes are also indicated.

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Competing Interests

The authors have declared that no competing interest exists.

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