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Functional analysis of Cullin 3 E3 ligases in tumorigenesis

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Abstract

Cullin 3-RING ligases (CRL3) play pivotal roles in the regulation of various physiological and pathological processes, including neoplastic events. The substrate adaptors of CRL3 typically contain a BTB domain that mediates the interaction between Cullin 3 and target substrates to promote their ubiquitination and subsequent degradation. The biological implications of CRL3 adaptor proteins have been well described where they have been found to play a role as either an oncogene, tumor suppressor, or can mediate either of these effects in a context-dependent manner. Among the extensively studied CRL3-based E3 ligases, the role of the adaptor protein SPOP (speckle type BTB/POZ protein) in tumorigenesis appears to be tissue or cellular context dependent. Specifically, SPOP acts as a tumor suppressor via destabilizing downstream oncoproteins in many malignancies, especially in prostate cancer. However, SPOP has largely an oncogenic role in kidney cancer. Keap1, another well-characterized CRL3 adaptor protein, likely serves as a tumor suppressor within diverse malignancies, mainly due to its specific turnover of its downstream oncogenic substrate, NRF2 (nuclear factor erythroid 2-related factor 2). In accordance with the physiological role the various CRL3 adaptors exhibit, several pharmacological agents have been developed to disrupt its E3 ligase activity, therefore blocking its potential oncogenic activity to mitigate tumorigenesis.

Keywords

Cullin 3; Ubiquitin; SPOP; Keap1; Tumorigenesis; Mouse models

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Transparency document

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1. Introduction

1.1. The enzymatic cascade of ubiquitination

Protein ubiquitination and degradation, carried out by the ubiquitin proteasome system (UPS), is an evolutionally conserved post-translational modification among eukaryotic species, and is essential during cell proliferation, transcriptional regulation and apoptosis [1,2]. Apart from its cell regulatory functions, the UPS has also been implicated in pathogenesis of various diseases such as neoplastic and neurodegenerative disorders [3,4]. Biochemically, this intracellular activity is catalyzed by an enzymatic cascade, the components of which are an E1 activating enzyme, an E2 conjugating enzyme, and an E3 ligase that imparts biological selectivity [4].

Ubiquitin, a 76-amino-acid polypeptide synthesized in an inactive form, is activated by the E1 enzyme in an ATP-dependent manner, which chemically forms a thioester bond linking the cysteine residue of the E1 and the glycine residue at the C-terminus of ubiquitin. This activating reaction effectively exposes the carboxyl group of glycine at the C-terminus of ubiquitin, which is the site that is utilized for covalent attachment of ubiquitin to the substrate protein. Subsequently, following a trans-esterification, the active form of ubiquitin is then conjugated to the E2 enzyme through the formation of another thioester bond by the cysteine residue on the E2 enzyme. Finally, the activated ubiquitin is transferred to the E3 ligase in a type-dependent manner, which dictates the specificity as well as having a role in modulating either ubiquitin-substrate attachments or inter-ubiquitin connections during poly-ubiquitin chain elongation [5]. The carboxyl group at the C-terminus of ubiquitin targets generally lysine residues within the substrate or attached ubiquitin. Although lysine is the predominate amino acid for ubiquitin modification, it is not the exclusive site for ubiquitin conjugation. Furthermore, the amino group at its own N-terminus may also serve as a binding site, increasing further the diverse structural orientation of poly-ubiquitin chains [6].

The fate of ubiquitin-modified proteins is largely determined by the pattern of the ubiquitin chain. Generally, mono-ubiquitinated proteins undergo conformational alterations that do not mediate degradation but lead to alterations in subcellular localization, functional activity, or interaction partner binding. Given that there are seven lysine residues located in the ubiquitin polypeptide itself (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48, and Lys63, respectively), a variety of linkages can be formed in the poly-ubiquitin chain. Certain poly-ubiquitination linkages, such as K48 or K11, largely induce the degradation of targeted proteins in most circumstances, with at least four ubiquitin molecules within a chain necessary before the 26S proteasome mediates proteolytic cleavage of the substrate protein. However, other linkages such as K63 or linear (also called N-terminal linkages) often governs the enzymatic activity of the substrate protein. Furthermore, as a result of different categories of inter-ubiquitin chains, multiple attachments of ubiquitin may also alter subcellular localization of the target proteins, govern the DNA damage repair process, or activate certain kinases and signaling pathways [7,8]. However, until recently, the specific functions, or the coding information, associated with the diverse forms of polyubiquitination linkages have not been fully understood, which requires additional in-depth studies.

In the human genome, two E1 enzymes, thirty-eight E2 enzymes, and more than six hundred E3 ligases have been identified, with the total number of E3 ligases being estimated to approach one thousand across mammalian species [9]. The enormous variety of regulatory enzymes, together with the diverse modification patterns, ensure the comprehensiveness and highlights the importance of ubiquitination in regulating cellular homeostasis [10,11].

1.2. Ubiquitin ligases and the Cullin-RING superfamily

Mammals have developed hundreds of ubiquitin ligases (also called as E3 ligases) to confer diversity of substrate specificity, thereby adapting the vast demand of protein processing, which also reflects the core role of E3 ligases on the ubiquitination machinery [10,12]. As described above, ubiquitin ligases account for the final step of transferring an activated ubiquitin to corresponding substrates, which may partly explain why hundreds of distinct E3 ligases share homologous catalytic elements but differ largely in their substrate-recognition subunits. This lessens the requirement on matching counterparts (such as E2 enzymes), while maintaining a relatively large capacity for substrate identification [13].

Based on architectural features and functional mechanisms, E3 ligases have been grouped into two main classes, the RING (really interesting new gene) family and the HECT (homologous to the E6AP carboxyl terminus) family. With respect to the RING family, E3 ligases bind to the ubiquitin-E2 complex and substrate simultaneously, enabling a direct transfer of ubiquitin to substrates without any intermediate. As for HECT subtypes of E3 ligases, this procedure is split into two separate steps such that the ubiquitin moiety first reacts with the cysteine residue in the E3 via forming a thioester bond, and then the E3 ligases specifically recognizes its substrate and facilitates the ubiquitin-substrate conjugation [5,14].

Recently, a new type of E3 ligases have been discovered and termed RING-IBR-RING (RBR) proteins, consisting of two RING domains (RING 1 and RING 2) and an in-between-RINGs (IBR) zinc-binding region [15]. However, while containing RING domains, RBRs prefers a HECT-style modification pathway since it utilizes a two-step transfer of ubiquitin from the E2 to the substrate instead of a direct transfer. Based on structural modeling, its RING2 finger may harbor an ability to form a thioester bond similar to that of the HECT catalytic units [14,16,17]. Therefore, the RBR proteins are regarded as a RING-HECT hybrid and therefore is classified independently from either HECT or RING family of E3 ligases.

Based on different quaternary protein structures necessary for functionality (single polypeptide or multi-subunits complex), the RING ligases have been additionally divided into several subfamilies, including Cullin-RING ligases (CRLs), c-Cbl (Casitas B-lineage lymphoma), heterodimer of BRCA1 (breast cancer 1), BARD1 (BRCA1-associated RING domain 1) and the anaphase-promoting complex (APC), each harboring a RING catalytic domain [14].

The Cullin-RING superfamily dominates the E3 ligases as a representative of multi-subunit complexes (Fig. 1) [18]. They consist of four major components: a Cullin protein, an adaptor protein, a substrate receptor protein, and a RING-finger protein. Cullin serves as a core

scaffold to organize and coordinate the various components of the E3 ligase complex, directly binding to adaptor protein at its N-terminus and a RING-finger protein at its C-terminus. Moreover, the RING domain is attached with an activated ubiquitin-loaded E2 enzyme, while the substrate receptor protein is linked to the adaptor protein during the activation step, which jointly completes the structural basis for ubiquitin-substrate recognition [19,20]. Mammals encode eight Cullin proteins (Cullin 1, Cullin 2, Cullin 3, Cullin 4A, Cullin 4B, Cullin 5, Cullin 7 and Cullin 9, respectively), two RING-finger proteins (RBX1 and RBX2), four adaptor proteins (SKP1, ElonginB, ElonginC and DDB1) and more than four hundred substrate receptor proteins. Overall, there are more than 400 CRLs expressed in human cells, critically processing thousands of downstream targeted proteins and likely accounting for almost 20% of ubiquitin-mediated cellular degradation [21,22].

Furthermore, activation of the Cullin scaffold is essential for its subsequent function on ubiquitin transfer, which is carried out by the Neddylation [attachment of NEDD8 (neural precursor cell expressed, developmentally down-regulated 8)] modification on a lysine residue near the C-terminus of the Cullin protein [23,24]. CRL E3 ligases have been linked to many types of human diseases including tumorigenesis, where several components of CRLs have been identified for their involvement in neoplastic events, such as the oncoprotein SKP2 (S-phase kinase-associated protein 2) [25], tumor suppressor VHL (Von Hippel-Lindau) [26,27] and SPOP (speckle-type POZ protein) which functions in a context-dependent manner [28–30]. These CRLs target substrates regulating cell growth and apoptotic pathways in order to influence cellular proliferation and survival.

1.3. Phenotypes of Cullin knockout mouse models

To investigate the physiological and developmental significance of newly discovered genes, knockout mouse models provide the most robust method to analyze their physiological function (Table 1) [31]. In this regard, functional analysis of many of the Cullin-based SCF complexes have been assessed using mouse models (Fig. 1) with the exception of Cullin 2, in which only pVHL has been characterized thus far as an adaptor protein binding with Elongin B/C to promote the turnover of substrates, including the HIF proteins (Fig. 1B).

Cullin 1 was the first identified scaffold member of the Cullin-RING family that constitutes a heterotrimeric complex of SKP1/Cullin1/F-box (SCF) and remains the most extensively characterized CRL (Fig. 1A) [1]. *Cullin 1* knockout mice exhibit embryonic lethality likely due to the failure of cyclin E turnover, which governs DNA replication during cell cycle and thus is important for early embryonic development [32,33].

The scaffold protein Cullin 3 serves as a bridging structure that connects with BTB adaptors and RBX1 in CRL3 complexes (Fig. 1C) [34]. Phenotypically mimicking *Cul1*-null mice, constitutive deletion of *Cul3* causes embryonic lethality, which also may be due to dysregulation in cyclin E degradation, revealing the global significances of CRL3 in cellular division and differentiation [35]. Similarly, the morphological consequences following conditional *Cul3* deletion in various organs and cell types may also be due to cyclin E, the elevated level of which induces renal fibrosis and functional maintenance of hepatic progenitors, thus verifying the regulatory interplay between CRL3-mediated ubiquitination

and stemness/inflammatory pathways [36,37]. Furthermore, loss of *Cul3* in lymphocytes leads to dysregulated production of NKT (natural killer T cell) and marginal-zone B cells, although the underlying mechanisms are not fully understood yet [38].

Via a DNA damage-binding protein 1 and CUL4-Associated Factor (DCAF), Cul4A/B-based E3 ligases recognize and ubiquitinate their substrates (Fig. 1D). The constitutive ablation of *Cul4a* has given conflicting results in relation to embryonic fate. Li and colleagues reported a *Cul4a* knockout in 2002, which exhibited embryonic lethality [39]. However, the Kopanja group later found that despite the occurrence of male infertility, *Cul4a*-deleted embryos demonstrated normal growth and live-birth rates compared to that of littermates [40]. This inconsistency may be partially explained by the possibility that the Li group may have also unintentionally disrupted an adjacent gene called *Psid2*, while deleting exon 1 of *Cul4a*, and *Psid2* encodes a core element of the 26S proteasome complex [40,41]. Additionally, a subsequent report by the Li lab further suggested that a haplo-insufficiency of *Cul4a* led to live offspring, despite erythroid progenitors exhibiting less pluripotency than wild-type animals [42,43]. Other systemic knockouts models of *Cul4a*, regardless of zygosity, also result in a viable phenotype despite some defects in blood pressure homeostasis and cardiac functionality [44,45]. Taking all these results into consideration, CUL4A appears to be dispensable for embryonic development and may be partially compensated by CUL4B, on basis of their homologous similarity in genetic sequences [46].

Unlike its homologous counterpart *Cul4a*, constitutive deletion of *Cul4b* results in embryonic lethality. This may be due to the involvement of CUL4B in regulating the ubiquitination and degradation of substrates that are not targeted by CUL4A, such as cyclin E, whose degradation is necessary for early embryogenesis [47]. Moreover, conditional deletion of *Cul4b* in different organ or cell types leads to various abnormalities. For instance, targeted deletion of *Cul4b* in the epiblast, germ cells, adipose tissue, and hematopoietic progenitors results in mice carrying phenotypes such as XLMR-relevant symptoms, male infertility, severe obesity, and tumor progression, respectively [48–52]. However, under certain circumstances, loss of *Cul4b* displays beneficial effects such as regulating inflammatory restriction, suggesting that Cullin 4B might be a potential target against inflammatory diseases [52,53].

Cul5 is a recently discovered member of Cullin family where systemic haplo-insufficiency leads to repressed inflammatory response following LPS (lipopolysaccharide) exposure (Fig. 1E) [54]. This may be due to the effect of reduced CUL5 on down regulating the expression of p53, which is an essential inducer of inflammatory and stress-response pathways [21].

Similar to Cullin 1 containing SCF complexes, Cullin 7 forms an SCF-like complex by binding to Skp1 and the FBXW8 (F-box and WD repeat domain containing 8) substrate recognition unit (Fig. 1F). Global knockout of *Cullin 7* results in early embryonic death, suggesting that CUL7 containing SCF complexes regulate distinct substrates from CUL1 containing SCF complexes [55]. Further histological analysis has suggested that vascular system morphogenesis appears dramatically altered and could contribute to the observed prenatal lethality, possibly due to dysregulation of CUL7 targets including IRS-1 (insulin receptor substrate 1) and FAP68 (flagellar associated protein 68) [55,56].

As a close homolog of Cullin 7, Cullin 9 and its corresponding E3 ligase complex CRL9 remain the most undefined member of the Cullin-RING ligase family due to lack of understanding of its adaptors and substrate-binding modules (Fig. 1G). Systemic depletion of *Cul9* in mice is nonlethal and these mice carry no obvious phenotypic malformations, suggesting that CRL9 might not be necessary for core events during embryogenesis [57]. However, loss of *Cul9* in the adult may increase the development of spontaneous tumors and lead to aneuploidy in multiple systems by degrading Survivin or interacting with p53-mediated pathway(s), indicating that CRL9 may be involved in controlling genomic stability and neoplastic events [58,59].

2. Cullin 3 E3 ubiquitin ligases in tumorigenesis

2.1. Cullin 3 E3 ligases

Cullin 3 E3 ligases are typically composed of three primary components including a Cullin 3 scaffold protein, RING-box protein 1 (RBX1), and a Bric-a-brac/Tramtrack/Broad (BTB) protein (Fig. 1C) [60]. Unlike other CRLs, there is no separate subunit acting as an adaptor and substrate recognition subunit within the Cullin 3 complex, where the BTB protein carries out this dual functionality [34].

Structurally conserved and analogous to other Cullin proteins, a characteristic Cullin homology (CH) domain is found in the C-terminus of Cullin 3 [18,61]. This domain functions as a docking site, interacting with the RING-box protein, whereas three “Cullin repeats” (each contains five α -helix folds) in the N-terminus of Cullin 3 mediate the interaction with the BTB containing protein. Of the RING-box proteins, only RBX1 has been found in CUL3 E3 ligases, where the ubiquitin-loaded E2 enzyme is recruited and the transfer of ubiquitin onto the substrate is carried out.

The BTB domain mediates their binding to the N-terminal region of Cullin 3 [2,18,34]. Another functional domain in BTB proteins is the substrate-interaction region, typically including MATH (meprin and TRAF homology) and Kelch domains, which determines the diversity in substrate specificity of the BTB proteins. The total number of BTB containing proteins is estimated to surpass 200 in the human genome [62]. However, only a few have been characterized to function as substrate recruiting adaptors on the basis of an auxiliary 3-box motif that is comprised of a paired helical structure that mediates the CUL3-BTB connection [63]. Moreover, BTB containing proteins have a capability of dimerization that allows for two Cul3 complexes to be joined together. In light of this dimerization potential, BTB proteins have distinguished themselves from other Cullin adaptors, although the presence of a homologous five α -helix fold is also observed [2].

As described above, Cullin 3 ligases carry out fundamentally essential processes exemplified by the observation that deletion of *Cullin 3* causes early embryonic lethality in mice (Table 2) [35]. Genetic editing techniques have allowed for targeting various components of CRL E3 ligases to assess the involvement of Cullin 3 E3 ligases in various physiologically and pathologically biological processes, including cell cycle control [35], protein trafficking [64], stress responses [65], and apoptosis [66], due to the multitude of downstream ubiquitination substrates (Tables 2, and 3). Furthermore, mutations within Cullin 3 subunits

have been observed in relation to their role in regulating metabolic disturbance [67], muscle atrophy [68], and neurodegeneration [69]. Currently, the neoplastic contribution of Cullin 3 ligases has been well described, indicating possible pharmaceutical targets for clinical application. Two substrate recognition proteins, Keap1 and SPOP, are the two most representative cancer-associated adaptors of Cullin 3, and display dual roles concerning cancer progression [2]. Hence, these biological variations encourage more precise investigation into specific adaptor-substrate interactions to allow for greater understanding of the functional consequences of their mutation or loss.

2.2. Cul 3-based tumor suppressive E3 ligase

2.2.1. Keap1—Keap1 is a Cullin 3 adaptor protein possessing both a Kelch and a BTB domain, and its regulatory functions have been well investigated [70]. Systemic knockout of *Keap1* induces early embryonic lethality, implicating it in regulating prenatal development [71]. However, deletion of *Keap1* attenuates inflammatory injury developed following endogenous and exogenous damage in the airway epithelium [72], restores insulin sensitivity within skeletal muscle and pancreatic β cells [73,74], as well as protects hepatocytes against various liver toxins [75–81]. Each of these phenotypes have the common feature of regulating inflammation, indicating a broad regulatory role of Keap1 in controlling inflammatory networks as its depletion dramatically stabilizes the activity of NRF2, which serves as a powerful anti-oxidative and anti-inflammatory factor [82]. Moreover, by ubiquitinating MCM3, PGAM5 and p62, the Cul3-Keap1 complex is involved in the regulation of cell cycle, apoptosis, and autophagy, respectively, demonstrating a ubiquitous role of Keap1 among multiple key cellular pathways [83–85].

It is well known that oxidative or inflammatory stress is intimately connected with malignant transformation through sharing similar signaling pathways and biological outputs. Therefore, emerging results have illustrated the underlying anti-neoplastic role of Keap1. Loss-of-function mutations in *Keap1* has been detected in certain types of malignancies such as lung carcinoma, hinting that Keap1 might exert a tumor suppressive role against tumorigenesis [86,87]. However, additional studies have identified that Keap1, especially in the Keap1-NRF2 axis, exhibits a context-dependent role towards tumorigenesis. Since the interaction between Keap1 and NRF2 is unidirectional, the role of the Cul3-Keap1 E3 ligase in tumorigenesis depends largely on the function of NRF2 (Fig. 2).

The transcription factor NRF2 has been well characterized to maintain organism integrity following oxidative stress [88]. Normally, NRF2 is sequestered in the cytoplasm and is maintained in relatively low abundance due to constitutive degradation by the Cul3-Keap1 E3 ubiquitin ligase. Upon oxidative stress, the ability of Cul3-Keap1 to target NRF2 is blocked, allowing its nuclear translocation to drive the subsequent activation of ARE (antioxidant response element)-dependent gene expression [89,90]. NRF2-dependent activation of genes such as SOD (superoxide dismutase) and GST (glutathione S-transferase), catalyze the removal of oxidative intermediates to maintain genomic integrity [89,91].

As a key regulator of detoxifying pathways, NRF2 has been associated with a variety of pathological conditions as deletion or mutation of *NRF2* sensitizes rodent models to

oxidative and inflammatory induced stress [92]. Additional studies have implicated up-regulation of NRF2 expression blocking or ameliorating cataract formation, neurodegeneration, osteoarthritis, and metabolic syndrome, suggesting great potential in NRF2 targeted therapy for inflammation-oriented disorders [88,93–95].

Oxidative stress triggered loss-of-function mutations on tumor suppressor genes or gain-of-function mutations on oncogenes act as an important cause of malignant transformation in somatic cells [96,97]. Therefore, upon the timely removal of free radicals by NRF2-dependent upregulation of anti-oxidant genes, cells are protected from oxidative stress and resulting genomic instability potentially impacting oncogenesis. Moreover, genetic ablation of *NRF2* increases susceptibility to cancer formation in rodent models, suggesting an important tumor suppressive role for the NRF2-ARE axis [89,98].

However, NRF2 may play an oncogenic role during the progression stages of malignancies. Similar to its anti-oxidant effect in normal tissues, cancer cells are also capable of harnessing the anti-oxidant role of NRF2 signaling for tumor maintenance [89,99]. NRF2 transcriptionally upregulates the expression of anti-apoptotic genes Bcl-2 and Bcl-xL to facilitate survival of tumor cells [100]. Also, by targeting the promoter of HO-1 or Prx1, NRF2 facilitates the induction of VEGF, thereby promoting angiogenesis within a hypoxic environment, thus contributing to the local expansion of malignant tumors [101,102]. Consistent with this mechanism, gain-of-function mutations of *NRF2* have been identified within dermatological, esophageal, and laryngeal carcinoma, while loss-of-function mutations in Keap1 have been observed in gastric, hepatic, mammary, pulmonary, and ovary malignancies [89]. Furthermore, clinical studies have further demonstrated that the expression level of NRF2 is positively correlated with chemo-resistance and metastatic tendency, and inversely correlated with survival among cancer patients [99,103].

2.2.2. KLHL6: a novel adaptor specifically expressed in lymphoid tissues—

KLHL6, an adaptor protein for Cullin 3, is exclusively expressed in lymphoid tissues in mammals [104]. Knockout of *Klhl6* in mice leads to defects in B cell maturation and impairment of transitional B cell survival and differentiation, primarily due to dysregulation of antigen receptor signaling in B lymphocytes and defects in germinal center formation [105].

The neoplastic role of KLHL6 is primarily observed in lymphoid origins. In B cell lymphomas, *KLHL6* loss-of-function mutations contribute to oncogenic transformation of germinal center B cells following overexpression of multiple pro-proliferation genes [105,106]. Further mechanistic analysis has indicated that in a Burkitt's lymphoma cell line, Cul3/KLHL6 targets HBXIP for ubiquitination and degradation to control cell cycle events including cytokinesis. These results suggest that the tumor suppressive role of KLHL6 may be at least partially due to its ubiquitination activity [106]. Nevertheless, in-depth studies are still needed to confirm and expand our understanding of KLHL6 in regulating tumorigenesis and malignant progression.

2.2.3. KCTD10: a BTB adaptor that maintains cardiovascular homeostasis and prevents tumorigenesis—KCTD10, a member of the potassium channel

tetramerization domain containing proteins and an adaptor protein for CRL3, is critical for prenatal development demonstrated by its deletion in mice resulting in early embryonic lethality, while heterozygous knockouts are viable with no effects on fertility [107,108]. In-depth studies have further unveiled that KCTD10 is crucial for cardiovascular homeostasis in both mammalian and non-mammalian species [107,109]. Genetic mutations of *KCTD10* result in dyslipidemia, coronary heart disease, and vessel malformation [110,111].

Tumor suppressive activity of KCTD10 has also been described by multiple studies. Analysis of GIST (gastrointestinal stromal tumor) biopsies, expression levels of KCTD10 has been shown to be restricted in neoplastic tissues compared to adjacent normal tissue, which positively correlates with prolonged survival [112]. Moreover, Notch1, a notorious oncogene contributing to various types of carcinogenesis, can be degraded by Cul3/KCTD10 via the ubiquitination processes [107,113]. Altogether, these results suggest the tumor suppressive potential of KCTD10, although further studies are necessary to assess the role KCTD10 may play in regulation of other tumor types.

2.2.4. KCTD11/REN: a putative tumor suppressor regulating developmental pathways—KCTD11, also named REN, has emerged as an adaptor for Cullin3-RING ligases. This BTB-domain protein was initially identified and found up-regulated in pluripotent stem cells and neural progenitor cells, which promotes homeostatic neuronal differentiation and encephalic expansion [114].

The tumor suppressive function of KCTD11 was initially revealed in medulloblastoma, where its expression was found to be greatly reduced in cancerous tissue compared to normal adjacent tissue [115]. KCTD11 tumor suppressive activity may be attributed to its degradation of HDAC1, which inhibits the Hedgehog signaling [115,116]. Furthermore, reduced expression and prognostic potential of KCTD11 have also been demonstrated in prostate cancer and hepatocellular carcinoma, potentially involving activation of the Hippo pathway [117,118]. These results suggest that KCTD11 might be a crucial tumor suppressor in those cancers that rely largely on the aberrant activation of developmental pathways, including the Hedgehog and Hippo pathways.

2.2.5. RhoBTB2/DBC2: a putative tumor suppressor against broad-spectrum of malignancies—As an atypical member of the Rho GTPase family, RhoBTB2 (also called DBC2) is involved in regulating cell growth, apoptosis, and cytoskeletal trafficking [119], which implicates this BTB-domain Cullin 3 adaptor in neoplastic transformation.

The tumor suppressor function of RhoBTB2 has been validated in multiple settings. First, loss-of-function mutations, epigenetic silencing, or homozygous deletion of *RhoBTB2* are commonly observed in nearly half of breast cancer, lung cancer, and bladder cancer tissues [120,121]. Meanwhile, similar anti-tumor activity of RhoBTB2 has also been detected in other solid malignancies, such as osteosarcoma, thyroid cancer, and gastric cancer, verifying its broad impact on suppressing tumorigenesis [122–124]. However, the molecular mechanisms driving the tumor suppressive function of RhoBTB2 remains unclear, although its regulation of Musashi-2 ubiquitination and degradation may contribute to its tumor suppressive activity in mammary epithelial cells [125].

2.2.6. Other putative tumor suppressor CRL3 adaptors—In addition to the adaptors described above, diminished expression or loss-of-function mutations have also been identified in other CRL3 adaptors including KLHL9, KLHL16, KCTD6, KCTD21, RhoBTB3 and LZTR1 [115,126–130], suggesting their roles as putative tumor suppressors potentially mediated by their function within CRL3 complexes (Table 4). While there is little evidence thus far directly linking these adaptors to tumorigenesis, several adaptors feature putative anti-tumor activities via degrading their oncogenic substrates, such as KLHL25 and KBTBD6 [131,132]. Further analysis of these adaptors and the substrates they target is warranted to decipher the role of Cullin 3 E3 ligases functioning as tumor suppressors.

2.3. Cul3-based oncogenic E3 ligase

2.3.1. KLHL20: an autophagic regulator and possible oncoprotein—KLHL20, a BTB adaptor identified in 2004 and later characterized to contain a Kelch-like domain [133], has been shown to function in regulating the inflammatory response, neurite outgrowth, and protein trafficking (Fig. 3) [66,134,135]. Moreover, global loss of *KLHL20* results in corneal opacification and respiratory failure due to dysregulated angiogenesis, which highlights its crucial participation in vascular homeostasis [136,137]. Thus far, mechanistic understanding of KLHL20 primarily centers on its role in regulating autophagy, where the Cul3/KLHL20 complex mediates the turnover of ULK1 and VPS34 in order to terminate the autophagic process [138]. Given the interplay between autophagy and cancer, the oncogenic role of KLHL20 is therefore widely studied.

Consistent with a possible oncogenic role for KLHL20, increased expression of KLHL20 has been observed in human prostate cancer samples, which positively correlates with malignant grades and cancer progressions [139]. Subsequently, mechanistic studies have further demonstrated that the possible oncogenic activity of KLHL20 may be attributed to its role in regulating the degradation of tumor suppressors including PML, whose inactivation is commonly examined in a variety of malignancies, such as leukemia, prostate cancer, and lung cancer [140–142]. Abrogating Cul3-KLHL20-mediated PML degradation appears to, at least in part, trigger metastasis of colon cancer and proliferation of renal cancer cells [143,144].

2.3.2. Other Cul3-based oncogenic E3 ligases—Contrary to the large proportion of CRL3 adaptors showing tumor suppressive function, fewer Cul3 adaptors feature oncogenic properties (Table 5). Apart from KLHL20, there are additional adaptors possibly playing carcinogenic roles largely based on pathological evidence. KLHL21, an adaptor regulating cell mitosis and migration [145,146], is abnormally overexpressed in hepatocellular carcinoma tissues [147]. Meanwhile, KLHL42 is also believed to regulate mitotic progression and is overexpressed in T-cell lymphoma [148,149]. These results suggest that adaptors controlling mitotic progress may likely be novel oncogenic targets.

Several CRL3 adaptors show oncogenic tendency via interacting with downstream cancer-relevant substrates. Due to inducing activation of Aurora A, Cul3/KLHL18 permits the timely entry of mitosis and could possibly induce tumorigenesis that closely links to Aurora A activation [150], such as breast cancer and pancreatic carcinoma [151,152]. Furthermore,

KBTD2 is a BTB-domain adaptor involved in activation of PI3K signaling and insulin sensitivity, the systemic depletion of which results in diabetes, lipodystrophy, and hepatic steatosis. Its PI3K-activating effect and putatively oncogenic activity may be due to the degradation of p85 α , which functions as an inhibitor of multiple malignancies [153,154]. Furthermore, by counteracting the tumor suppressive function of its substrate PDCD4 [155], the Cul3/IBTK α E3 ligase enhances cellular proliferation to diverse tumor types, including skin cancer and lymphoma [156].

2.4. Cul3-based context-dependent E3 ligases

KCTD2 is a CRL3 substrate recognition adaptor that functions in the nervous system, dysregulation of which contributes to neurodegeneration, sleep disorders, and synaptic malfunction [157,158]. Decreased expression of KCTD2 is frequently detected in malignant glioma and associates with unfavorable prognosis, which is mechanistically due to the increase in the KCTD2 substrate c-Myc [75]. However, up-regulated KCTD2 was observed within colon cancer samples, suggesting a possible oncogenic function in the colon cancer setting [159]. These conflicting results suggest that the function of KCTD2 in tumorigenesis is likely to be in a tissue context-dependent manner. Similarly, studies have also demonstrated that contributions of TNFAIP1 and PLZF to tumorigenesis may be either oncogenic or tumor suppressive depending on the type of human cancers being investigated [160–163]. These findings indicate that some Cul3 E3 ligases function in a tissue or cellular context-dependent manner (Table 6). Furthermore, as one of the most investigated Cul3-based E3 ligase, SPOP also displays a context-dependent function depending on the growing substrates, which will be further discussed in the next section (Table 7).

3. The physiological role of SPOP in tumorigenesis

3.1. Exploring SPOP: history, structure, and mechanisms

SPOP (also known as PCIF1), initially discovered by Nagai and colleagues, is a 374-amino-acid protein was named due to its characteristically speckled nuclear appearance as well as harboring a POZ domain [164]. In 2005, Hernandez and colleagues further categorized SPOP as an adaptor for the Cul3 scaffold protein that recognized and eventually ubiquitinated its interacting substrates MacroH2A and BMI1 [165]. Moreover, SPOP was first linked with tumorigenesis due to its suppression of breast cancer through targeting SRC-3 for degradation. Since then the role of SPOP in tumorigenesis has been extensively investigated, implicating SPOP as a possible target for cancer therapeutic strategies [166].

Emerging as a representative BTB adaptor for CRL3s E3 ligases, SPOP is comprised of four domains, a MATH domain, BTB/POZ domain, 3-box domain, and an NLS (nuclear localization sequences) [30]. The MATH domain locates within the N-terminal region and includes specific substrate binding sites spatially formed by its Y87, F102, Y123, W131 and F133 amino acid residues. Correspondingly, a recognition region within substrate proteins are defined as SBC (SPOP-binding consensus) motifs, the phosphorylation of which could abrogate their binding with the SPOP MATH domain [63]. The BTB domain, a motif common among all Cul3 adaptors, acts as a hydrophobic region that interacts with Cullin 3 [167]. Particularly, an II-x-E motif (consisting of M233, E234 and E235 residues) inside the

10-amino-acid α 3- β 4 loop structure of the BTB domain essentially accounts for the connection with Cullin 3 [63]. Additionally, this domain is also found to be responsible for SPOP dimerization largely through its L186, L190, L193 and I217 residues, which form a hydrophobic interface utilized for dimerization [30]. The 3-box region, a paired helical structure in the C-terminal region of SPOP contributes to modulating the adaptor-scaffold interaction [34]. As its name suggests, the nuclear localization sequence provides nuclear translocation capability of SPOP.

Generally, Cul3/SPOP works in a similar mechanism as other Cullin-RING E3 ligases as described above. However, functionality of these motifs in regulating SPOP-based CRL3, especially referring to its dimerization or polymerization, still remains elusive. Along with allowing conformational variation, an oligomeric macromolecule with at least two catalytic and two MATH domains expands the binding flexibility of substrates under distinct contexts, where interference with the dimerization could consequently impair the ubiquitination avidity on substrates in spite of its normal affinity to Cul3 scaffolds [63,168,169].

3.2. Physiological and pathological functions of SPOP: a regulator of development

The CRL3 ligase adaptor SPOP has been implicated in multiple cellular events (Fig. 4). Notably, SPOP influences two fundamental activities namely cytoplasmic signaling and genomic modifications, either physiologically or pathologically [30]. In terms of cytoplasmic transduction, phosphoinositide, apoptotic, and hedgehog signaling are major pathways involving Cul3/SPOP-mediated ubiquitination [170–172]. Furthermore, SPOP is involved in alternative splicing and DNA damage response as well as sustainable inactivation of the X chromosome in females [165,173,174].

A constitutive knockout mouse model (*Spop*^{-/-}) has revealed a role for SPOP in embryonic dorsoventral patterning, potentially through its interplay with downstream hedgehog signaling [175]. In the cardio-vascular system, loss of *SPOP* stabilizes its substrate DAXX in umbilical vein endothelial cells, which disrupts the expression of VEGFR2 (vascular endothelial cell growth factor receptor 2) influencing endothelial homeostasis [176]. Moreover, the Cul3/SPOP/Gli3 and Cul3/SPOP/PDX1 axes promote proper skeletal development and insulin signaling by pancreatic β cells [177,178]. The stability of SPOP is essential to normalize decidualization of endometrial stromal cells in mice, however little is known as to the molecular mechanism [179]. Taken together, these results highlight key molecular pathways regulated by SPOP-mediated ubiquitination.

3.3. SPOP regulation of tumorigenesis: evidences from animal models, cell line, and human specimens

Owing to the far-reaching involvement of Cul3-SPOP ligase towards development, cellular signaling, and genomic integrity, questions have been raised as to whether SPOP regulates malignant transformations. Over the past decade, researches on multiple fronts have elucidated a role for SPOP in regulating carcinogenesis. However, this role is likely tumor type-dependent, although its tumor suppression appears to be the primary functionality. Therefore, further discussion will be separated into two parts according to its specific tumorigenic roles.

3.3.1. Tumor suppressive role of SPOP: a dominant function in the majority of malignancies

3.3.1.1. Prostate cancer.: Prostate cancer is the clearest example demonstrating the tumor suppressive nature of SPOP [180]. Spontaneous development of prostatic intraepithelial neoplasia occurs in mice with conditional *SPOP* knockout in the prostate epithelium [181]. As one of the most frequently mutated genes in prostate cancer, with mutation rates up to 25.0% of human cancers, irrespective of hereditary history, histological grade, or ethnicity group [182–186]. Furthermore, the expression level of SPOP negatively correlates with patient survival, emerging as a potential prognostic indicator [187]. Restoration of SPOP in malignant cells protects against proliferation, invasion, and therapeutic refractoriness of prostate cancer, covering nearly every aspect of prostatic carcinogenesis from tumor initiation.

Although numerous studies have observed lower expression of SPOP in neoplasms in contrast to adjacent normal tissues, currently there is only one direct report referring to the influential impact of SPOP in prostate cancer initiation, suggesting that the elevated spontaneity of tumorigenesis resulting from SPOP ablation may be in part due to dysregulation in the turnover of the oncoprotein c-Myc. Further silencing of c-Myc successfully reverses the susceptibility of prostate cancer transformation due to loss of function of SPOP [181].

Uncontrollable growth and metastasis are the most prominent properties of solid malignancies, which accounts for almost 90% of cancer death [96,188]. Correspondingly, the tumor suppressive role of SPOP against prostate cancer progression attracts large interest. At least eight substrates are believed to be key to SPOP tumor suppressor activity, including INF2 (Inverted Formin 2), EglN2 (Egl nine homolog 2), TRIM24 (Tripartite motif protein 24), SENP7 (Sentrin-specific protease 7), ERG (Ets-related protein), DEK, AR (Androgen receptor), SRC-3, and BRD4 [28,29,180,189–193]. A majority of these substrates function largely as oncoproteins either activating oncogenic cascades or serving as transcriptional factors to boost the expression of downstream oncogenes. It has been well established that androgen signaling is the key pathway that promotes the development, expansion and spread of prostate cancer [194]. SPOP directs its tumor-preventive role by directly degrading AR or its co-activators SRC-3 and/or TRIM24. Mutant SPOP fails to promote the ubiquitination of substrates, such as AR, thus allowing propagation of prostate cells even in the setting of low androgen [180,192,193]. Furthermore, there are additional pathways regulated by SPOP, including inhibition of oxygen-sensing cascades (EglN2) [29], repression of mitochondrial fission (INF2) [189], induction of senescence (SENP7) [191] and suppression of oncogenic transcription (ERG) [28], which contribute to the tumor suppressive role of SPOP in prostate cancer.

For patients bearing advanced-stage prostate carcinoma, therapeutics seem more suitable over surgical options. An anti-androgen regimen has become the classical first-line strategy due to the close association between aberrant androgen pathway activation and prostate carcinogenesis [195]. Nonetheless, resistance to anti-androgen therapy usually hinders the efficacy and overall survival. SPOP mutations that abrogate the degradation of AR or AR

activators promote the formation of castration-resistant prostate cancer and anti-androgen refractoriness [180,192,193]. *SPOP* mutations are also connected to resistance of targeted therapy. Recent findings have clarified that *SPOP* mutations confer resistance to BET inhibitors by reducing BRD2/3/4 degradation [196,197]. Moreover, therapeutic resistance to other targeted drugs such as a CDC20 inhibitor is also reported to rely on the presence of *SPOP* mutations [198].

3.3.1.2. Gynecological malignancies.: Gynecological malignancy involves a spectrum of solid cancers that originate exclusively in female genital system, typically consisting of ovarian, endometrial and cervical carcinoma. Due to some etiological similarities, we will also discuss breast cancer in this section [199]. From a mechanistic perspective, these cancers nearly always exhibit connections with hormonal dysregulation and are readily mitigated secondary to steroid stimulation [200]. Results have emerged showing SPOP as a considerable tumor suppressor against ovarian, mammary, and endometrial carcinoma. Protein analysis by Hu et al. found that more than 50% of ovarian cancer samples carried loss-of-function mutations of *SPOP* compared to 0% in adjacent tissues. Meanwhile, an inverse correlation exists between the tumor clinic pathological grade and the positivity of SPOP [201]. With regard to breast cancer, its proliferative and migratory potentials may be restricted due to SPOP-induced degradation on SRC-3 and PR (progesterone receptor) in vitro and in vivo, which usually amplifies the estrogen/progesterone-mediated expansion and dispersion of breast cancer cells [166,202,203]. However, according to genomic analysis in cBioPortal, *Spop* is often amplified in human breast cancer samples, suggesting an alternative functionality of SPOP in breast carcinogenesis. Whether this disparity in breast cancer is subtype dependent or due to post-transcriptional silencing requires further clarification. Additionally, endometrial cancer is another area of investigation for SPOP. Genetically, the overall mutation incidence of *Spop* in endometrial cancer patients ranges from 5.7% to 18% [204,205]. Dysregulation of ER α turnover by mutant SPOP appears to promote endometrial tumorigenesis, consistent with the classical association between estrogen dysregulation and onset of endometrial cancer [205]. In summary, these outcomes suggest a potential of SPOP as a therapeutic target for various cancer types.

3.3.1.3. Digestive malignancies.: Tumorigenesis occurring in digestive organs is responsible for the largest amount of malignant fatalities of any cancer type [206]. Several canonical pathways including hedgehog and ubiquitin cascades are found to contribute to the progression of digestive tumors, hence it is worthwhile to explore whether SPOP is involved in digestive neoplastic tumorigenesis [11,207,208].

So far, compelling evidence has described the inhibitory activity of SPOP on gastric, liver, and colorectal cancers. Immunohistochemistry studies have detected reduced expression of *SPOP* in 30% of gastric cancer samples compared to normal controls [209]. In-depth analysis suggests that Gli2, a transcriptional component within the hedgehog signaling pathway, functions as a substrate of SPOP in gastric cancer [210]. In terms of liver cancer, a reduction in SPOP expression inversely correlates with clinical severity and triggers proliferation and metastasis among hepatic cancer cells. Subsequent evidence has shown that the decreased expression of the transcriptional repressor ZEB2 may partially contribute to

the impact of SPOP [211]. Similarly, 20%–62% of colorectal cancer tissues harbor lowered expression of SPOP [209,212]. Both Gli2 and HDAC6 have been demonstrated to be SPOP substrates in colorectal cancer models, the turnover of which disrupts the activation of hedgehog signaling and transcriptional suppression on tumor-suppressor genes by histone deacetylase, respectively [206,213].

Distinct from prostate cancer, few somatic mutations have been observed among digestive malignancies [186]. These unexpected findings may actually reveal a probability of upstream mechanisms down-regulating SPOP activity rather than its own loss-of-function mutations that eventually contributes to pro-tumorigenic transformations. Recently, this assumption has been further demonstrated by Zhi et al. that hyper-methylation of the SPOP promoter region epigenetically silences SPOP [213]. However, the specific mechanisms in digestive cancer cells that initiate and sustain epigenetic silencing remains unclear.

3.3.1.4. Other malignancies.: Glioma is one of the common malignancies of the central nervous system [214]. Down-regulated expression of SPOP is observed in 62.2% of glioma samples compared with adjacent normal tissue. The level of reduced SPOP expression correlates with survival prognosis. In addition, SPOP up-regulation has the ability to suppress glioma metastasis in cellular experiments [215]. Nevertheless, few mechanistic details has been observed concerning the role of SPOP on glioma development, where hedgehog signaling may play a causative role in glioma tumorigenesis and Gli2/Gli3 are substrates of SPOP [216].

Non-small cell lung cancer (NSCLC) is another tumor type that involves loss of SPOP during malignant expansion. A report from Luo and colleagues has suggested that 6 out of 7 NSCLC specimens feature reduced expression of SPOP. Also, SPOP targets the NAD⁺-dependent deacetylase SIRT2 for degradation in order to inhibit the proliferative properties among NSCLC cell lines [217].

3.3.2. Tumor promoting role of SPOP: the less explored aspect of SPOP in carcinogenesis—Contrary to the tumor suppressive nature of SPOP in vast majority of cancer types, the tumor promoting role of SPOP has only been observed in clear cell renal cell carcinoma (ccRCC) [218], which dominates the renal cell carcinoma family as the most prevalent subtype (75% occupancy) [219]. Overexpression of SPOP, transcriptionally mediated by hypoxia-induced factors (HIF), is immunochemically traced in almost every ccRCC sample [220]. Overexpression of SPOP is an unfavorable prognostic indicator among ccRCC patients according to survival regression estimates [221]. Depletion or specific pharmaceutical inhibition of SPOP induces apoptosis and ultimately leads to apoptosis of ccRCC cells, demonstrating a great potential for SPOP-targeted therapeutics in kidney cancer [222,223].

The oncogenic mechanism of SPOP in ccRCC may be attributed to its specific destabilization of substrates PTEN (phosphatase and tensin homolog) and DUSP7 (dual specificity phosphatase 7), both of which are repressors of carcinogenic transcription and proliferation [218]. Moreover, the loss of SPOP activity enables the activation of the β -catenin/TCF4/ZEB1 axis, consequently enhancing the transcriptional activities of epithelial-

mesenchymal transition (EMT)-related genes that contribute to the dissemination of ccRCC [221]. These results have implicated the crosstalk between SPOP and cancer pathways in ccRCC, and the role of SPOP in regulating tumorigenesis may depend largely on tumor type.

4. Discussion and perspective

4.1. Clinical implications

Due to the extensive involvement of Cul3 E3 ubiquitin ligases in multiple biological processes, therapeutic options that target these adaptors have gained much attention recently.

Although SPOP seems to be the most extensively studied adaptor in recent years, and a small molecule, Compound 6b, functions as its inhibitor against the neoplastic properties of ccRCC (Fig. 5A) [223], the most developed category of adaptor-targeted drugs still concentrate on Keap1/NRF2 axis, a core mediator for anti-oxidant response and oncogenesis. Therefore, we use it as an example for the mechanistic strategies on pharmacological design.

Since the tumor suppressive mechanisms of NRF2 are well defined, current approaches have primarily focused on Keap1 inhibition in order to boost NRF2 levels in cancer cells. The adaptor proteins of Cullin 3 have two correlative functions, specific substrate recognition and ubiquitin moiety transfer between the E2 enzyme and the substrate. Hence, strategies may either aim to disrupt the ubiquitin transfer or block the interaction between Keap1 and NRF2 [224]. Regarding ubiquitination blockage, three major drugs have been developed, including CDDO-Me, DMP and MMP, all of which electrophilically disable the cysteine residue within the Keap1 peptide, interfering with the temporary formation of the thioester bond and thus hinder the transfer of the ubiquitin moiety (Fig. 5B, Table 8) [224]. Tecfidera, a DMP produced by Biogen, demonstrates great therapeutic activities towards multiple sclerosis and solid cancers [225,226]. Similarly, both CDDO-Me and MMP serve as applicable agents against diverse malignancies, chronic kidney disease, and neurodegeneration [224,227–229].

There are NRF2 activators, which disrupt the protein-protein interaction between Keap1 and NRF2 to promote NRF2 protein abundance, including Compound 1 to Compound 16 [224,230]. Compared to inhibitors on Keap1 cysteine residue, these PPI inhibitors could lead to more specific effects against disorders characterized with specific NRF2 contributions. Nevertheless, due to the pharmacological limitations, there is still much work to do before drugs targeting this pathway are developed for clinical use. Taken together, targeted therapy on specific Cullin 3 adaptors seems promising in cancer treatment, thus more effort is needed for its broader clinical applications.

4.2. Summary

Cullin 3 adaptors and their ligase complexes account for multiple dysfunctions through specific interaction (either degradation or non-degradation) with substrates, especially in tumorigenesis (Table 7). As one of the most representative adaptor of CRL3, SPOP primarily demonstrates anti-tumor activity against a variety of malignancies especially

prostate cancer, while occasionally playing an oncogenic role in certain types of cancer such as ccRCC. The entire spectrum of adaptors mediates distinct regulatory mechanisms towards neoplastic formation (oncogenic, tumor suppressive or context-dependent), displaying a versatile role of Cul3 adaptors and emphasizing the clinical value of treatments targeting the adaptor protein functions.

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Fig. 1. Structural components of the Cullin-RING ligase family.

A. Cullin1-RING ligase complex; B. Cullin2-RING ligase complex; C. Cullin3-RING ligase complex; D. Cullin4A/4B-RING ligase complex; E. Cullin5-RING ligase complex; F. Cullin7-RING ligase complex; G. Cullin9-RING ligase complex; Ub: ubiquitin; NA: not available.

Fig. 2.
Functional illustrations of Keap1 and its specific substrates in tumorigenesis.

Fig. 3.
Functional illustrations of KLHL20 and its specific substrates in tumorigenesis.

Fig. 4. Functional illustrations of SPOP and its specific substrates in tumorigenesis.

As indicated, SPOP plays tumor suppressor functions by targeting oncoproteins (ER, PR) in breast cancers and (SEN7, DAXX, ERG, SRC-3, DEK, TRIM24, AR, BRD2/3/4 and Gli2/3) in prostate cancer, while SPOP displays oncogenic functions by targeting tumor suppressor proteins (PTEN and DUSP7) in ccRCC.

Fig. 5. Modular demonstrations of major Cul3 adaptors and therapeutically targeted sites.
A. Key functional modules inside human SPOP sequence and the targeted region of Compound 6b; B. Key functional modules inside human Keap1 sequence and the targeted region of CDDO-Me, DMF and MMF.

Table 1

Phenotypic summary for knockout mouse models of various Cullin scaffolding proteins.

Scaffold type	Knockout mode	Tissue specificity	Phenotype	Impacts on relevant substrates	Reference
CUL1	Systemic (<i>Cul1^{-/-}</i>)	Systemic	Early stagnation on embryonic development	Degradation of cyclin E (Important cell cycle regulator)	[32,33]
CUL3	Systemic (<i>Cul3^{-/-}</i>)	Systemic	Embryonic lethal	Degradation of cyclin E	[35]
	Conditional (<i>Cul3^{-/-}</i>)	Lymphocyte	Dysregulated production of NKT cells and marginal-zone B cells	-	[38]
	Conditional (<i>Cul3^{-/-}</i>)	Kidney	Inflammation, fibrosis, diabetes and electrolyte disturbance	Degradation of cyclin E	[37]
	Conditional (<i>Cul3^{-/-}</i>)	Liver	Quantitative expansion and sterness maintenance of hepatic progenitor cells	Degradation of cyclin E	[36]
CUL4A	Systemic (<i>Cul4a^{-/-}</i>)	Systemic	Embryonic lethal	-	[39]
	Systemic (<i>Cul4a^{-/-}</i>)	Systemic	Male infertility (smaller testes, less sperm count and worse motility)	Degradation of CDT-1 (a key licensing factor that prevents DNA re-replication)	[40,231]
	Systemic (<i>Cul4a^{-/-}</i>)	Systemic	Decreased hepatocytic proliferation after exposure to liver toxicants	Degradation of p53 (suppressor of proliferative that inhibits abnormal cellular expansion)	[41]
	Systemic (<i>Cul4a^{-/-}</i>)	Systemic	Systolic hypertension and cardiac dysfunction in male mice	Degradation of GRK2 (a member of GRKs that positively correlates with cardiac insufficiency)	[44,45]
	Systemic (<i>Cul4a^{-/-}</i>)	Systemic	Reduced number of erythroid progenitors and aberrant terminal differentiation into red blood cells	Degradation of p27 (a vital kinase inhibitor that restricts the transition towards synthetic phase during cell cycle)	[42]
	Systemic (<i>Cul4a^{-/-}</i>)	Systemic	Impaired engraftment and self-renewal among hematopoietic stem cells	-	[43]
CUL4B	Conditional (<i>Cul4b^{-/-}</i>)	Skin	Resistant to UV-induced tumorigenesis	Degradation of DDB2	[232]
	Systemic (<i>Cul4b^{-/-}</i>)	Systemic	Embryonic lethality	Degradation of cyclin E	[47]
	Conditional (<i>Cul4b^{-/-}</i>)	Germ cell	Male infertility (aberrant motility of sperms despite of normal testicular shape)	Degradation of INSL6 (a member of insulin family) to induce mitochondrial dysfunction and ATP depletion	[50,51]
	Conditional (<i>Cul4b^{-/-}</i>)	Hematopoietic system	Tumor progression, blocked differentiation and enhanced accumulation of MDSCs	-	[49]
	Conditional (<i>Cul4b^{-/-}</i>)	Adipose tissue	Increased severity of obesity however featuring better metabolic parameters (lowered inflammatory cytokines and insulin resistance)	Degradation of PPAR γ (an inevitable mediator during lipid and glucose metabolism)	[52]
	Conditional (<i>Cul4b^{-/-}</i>)	Nervous system	Abundance of GFAP ⁺ cells in diversified brain regions	-	[233]
	Conditional (<i>Cul4b^{-/-}</i>)	Macrophage	Strengthened proliferation and reduced production of pro-inflammatory cytokines by macrophage	-	[53]
	Conditional (<i>Cul4b^{-/-}</i>)	Epiblast	XLMR-relevant phenotypes, such as learning and memory impairment, as well as enhanced epileptic susceptibility	Degradation of p21 (a member of cyclin-dependent kinase inhibitor relevant to cell cycle arrest)	[48]

Scaffold type	Knockout mode	Tissue specificity	Phenotype	Impacts on relevant substrates	Reference
CUL5	Systemic (<i>Cul5^{-/-}</i>)	Systemic	Decreased inflammation and elongated survival after LPS exposure	-	[54]
CUL7	Systemic (<i>Cul7^{-/-}</i>)	Systemic	Retarded growth and embryonic lethality due to aberrant vasculargenesis	-	[55]
CUL9	Systemic (<i>Cul9^{-/-}</i>)	Systemic	Nuclear malformation and aneuploidy in multiple organs	Degradation of Survivin (an inhibitory protein of apoptotic pathway)	[59]
	Systemic (<i>Cul9^{-/-}</i>)	Systemic	Spontaneous tumorigenesis in various systems	-	[58]
	Systemic (<i>Cul9^{-/-}</i>)	Systemic	Healthy at birth and no phenotypic abnormalities	-	[57]

Abbreviations: CUL1: Cullin 1, CUL3: Cullin 3, CUL4A: Cullin 4A, CUL4B: Cullin 4B, CUL5: Cullin 5, CUL7: Cullin 7, CUL9: Cullin 9, LPS: lipopolysaccharides, MDSCs: myeloid-derived suppressor cells, INSL6: insulin-like peptide 6, PPAR γ : peroxisome proliferator-activated receptor γ , GFAP: glial fibrillary acidic protein, KLHL3: kelch-like family member 3, XLMR: X-linked mental retardation, UV: ultraviolet, DDB2: DNA damage binding protein 2, DNA: deoxyribonucleic acid, NKT: natural killer T, GRK2: G-protein-coupled receptor kinase 2, GRKs: G-protein-coupled receptor kinases, CDT-1: chromatin licensing and DNA replication factor

Phenotypic overview of knockout mouse models on Cullin 3-based E3 ligase substrate adaptor proteins.

Table 2

Adaptor	Knockout model	Tissue specificity	Phenotype	Reference
SPOP	Systemic (Spop ^{-/-})	Systemic	Neonatal lethality	[177]
	Systemic (Spop ^{+/-})	Systemic	Elevated mass of prostate	[181]
	Systemic (Spop ^{+/-})	Systemic	Quantitative restoration of pancreatic cells	[178]
	Conditional (Spop ^{-/-})	Prostate	Spontaneous development of prostatic intraepithelial neoplasia	[181]
	Conditional (Spop ^{-/-})	Limb	Brachydactyly and osteopenia	[177]
KLHL2	Systemic (Klhl2 ^{-/-})	Systemic	Without PHAI-like symptoms	[234]
KLHL3	Systemic (Klhl3 ^{-/-})	Systemic	PHAI-like symptoms	[235]
	Systemic (Klhl3 ^{+/-})	Systemic	Without PHAI-like symptoms	[235]
KLHL6	Systemic (Klhl6 ^{-/-})	Systemic	Impaired B cell survival and differentiation	[106]
GAN (KLHL16)	Systemic (Gan ^{-/-})	Systemic	Hind limb muscle atrophy	[236]
Keap1 (KLHL19)	Systemic (Keap1 ^{-/-})	Systemic	Early postnatal lethality	[71]
	Conditional (Keap1 ^{-/-})	Lung	Attenuated inflammatory injuries by acute cigarette smoking	[72]
	Conditional (Keap1 ^{-/-})	Pancreatic β cell	Functional and quantitative restoration of β cells	[74]
	Conditional (Keap1 ^{-/-})	Skeletal muscle	Reduced body weight and ameliorated insulin sensitivity	[73]
	Conditional (Keap1 ^{-/-})	Liver	Better blood glucose level; Reduced liver steatosis; Less hepatic injury after phalloidin, acetaminophen or microcystin exposure; Diminished PVBL or ischemia induced damage	[75–81]
KLHL20	Systemic (Klhl20 ^{-/-})	Systemic	Corneal opacification and stromal neovascularization	[136]
	Systemic (Klhl20 ^{-/-})	Systemic	Early postnatal death due to respiratory failure	[137]
	Conditional (Klhl20 ^{-/-})	Muscle	More susceptible to streptozotocin-induced muscle atrophy	[138]
KCTD10	Systemic (Kctd10 ^{-/-})	Systemic	Embryonic lethality due to growth retardation	[107]
	Systemic (Kctd10 ^{+/-})	Systemic	Viable and fertile	[107]
KBTBD2	Systemic (Kbtbd2 ^{-/-})	Systemic	Diabetes, lipodystrophy, and hepatic Steatosis	[153]
ZBTB28 (BAZF)	Systemic (Bazf ^{-/-})	Systemic	Retarded angiogenesis in developing retina and skin wound healing; Reduced number of cycling hematopoietic progenitor cells	[237,238]

Abbreviations: PHAI: pseudohypoadosteronism type II; PVBL: portal vein branch ligation.

Table 3

Major downstream ubiquitin substrates of Cullin 3-based E3 ligases.

Adaptor	Substrate	Modification	Biological impact*	Reference
SPOP	EgIN2	Degradation	Suppression of prostate tumorigenesis	[29]
	BRD2	Degradation	Enhanced sensitivity to BET inhibitors in prostate cancer cells	[196,197]
	BRD3	Degradation	Enhanced sensitivity to BET inhibitors in prostate cancer cells	[196,197]
	BRD4	Degradation	Enhanced sensitivity to BET inhibitors in prostate cancer cells	[196,197]
	DAXX	Degradation	Reversal of transcriptional repression and induction of apoptosis; Essential for endothelial homeostasis; Progression of kidney cancer	[172,176,218]
	SRC-3	Degradation	Inhibited oncogenic signaling in breast cancer; Less malignant traits of prostate cancer cells	[166,180]
	Gli2	Degradation	Reduced activation of hedgehog pathway; Induction of apoptosis in colorectal cancer cells;	[170,210,213,218]
			Decreased tumorigenesis in gastric cancer; Progression of kidney cancer	
	Gli3	Degradation	Reduced activation of hedgehog pathway	[170,177]
	BMI1	Altered protein activity	Epigenetic silencing	[165]
	MacroH2A	Altered protein activity	Epigenetic silencing	[165]
	PIPKI β	Altered protein activity	Phosphoinositide transduction	[171]
	HDAC6	Degradation	Suppressed proliferative and migratory capability in colon cancer cells	[239]
	INF2	Subcellular re-localization	Restriction of mitochondria fission and the consequential cell invasiveness of prostate cancer	[189]
	c-MYC	Degradation	Decreased malignant properties of prostate cancer	[181]
	SIRT2	Degradation	Inhibited growth of non-small cell lung cancer cells	[217]
	PR	Degradation	Suppression of oncogenic pathway in breast cancer cells	[203]
	CDC20	Degradation	Lowered viability and enhanced apoptosis in prostate cancer cells	[198]
	SETD2	Degradation	Regulation of alternative splicing	[173]
	SENp7	Degradation	Cellular senescence due to epigenetic silencing	[191]
	ERG	Degradation	Repressed neoplastic expansion and metastasis of prostate cancer cells	[28]
	ER α	Degradation	Reduced growth rate of endometrial cancer cells; Transcriptional inhibition	[205,240]
	DEK	Degradation	Less malignant traits of prostate cancer cells	[180]
	TRIM24	Degradation	Less malignant traits of prostate cancer cells	[180]
	AR	Degradation	Reduction of neoplastic proliferation among prostate cancer cells	[192,241]
	DDIT3	Degradation	Down-regulation of ER stress-induced apoptosis in prostate cancer cells	[242]
	PTEN	Degradation	Progression of kidney cancer	[218]
	DUSP7	Degradation	Progression of kidney cancer	[218]

Adaptor	Substrate	Modification	Biological impact*	Reference
KLHL2	BRMS1	Degradation	Inhibited metastatic potential of breast cancer	[243]
	PDX1	Degradation	Crucial for normal pancreatic development and differentiation; Essential regulation of glucose homeostasis	[178,244,245]
	WNK1	Degradation	Not available	[246]
	WNK3	Degradation	Not available	[246]
	WNK4	Degradation	Anti-hypertensive effect	[234,246,247]
KLHL3	NPCD	Altered protein activity	Aggravation of neurodegeneration	[69]
	WNK1	Degradation	Homeostatic blood pressure	[248]
	WNK4	Degradation	Homeostatic electrolytes and blood pressure	[249,250]
	NCC	Degradation	Maintaining electrolyte balance in distal nephron and normal blood pressure	[251]
	Claudin-8	Degradation	Help to ameliorate hypertension	[252]
KLHL6	cMyBP-C	Degradation	Heart development control	[253]
	HBXIP	Non-degradation	Cell cycle and survival controls	[106]
	RAPSYN	Degradation	Essentially controlling the function of neuromuscular junction	[254]
	Aurora B	Subcellular re-localization	Mitotic progression	[255]
	SEC31	Altered protein activity	Critical contribution to collagen export	[256]
KLHL12	D4 receptor	Altered protein activity	Not available	[257,258]
	Aurora B	Subcellular re-localization	Mitotic progression	[255]
	RBBP8	Degradation	Physiological control of DSB repair pathway	[259]
	PP2A	Degradation	Not available	[260]
	MAP1B	Degradation	Anti-degenerative and anti-apoptotic effects among neurons	[261]
KLHL16 (GAN)	TBCB	Degradation	Protecting from cytoskeletal abnormalities	[262]
	MAP8	Degradation	Maintaining normal retrograde axonal transport	[263]
	GluR6	Degradation	Contributing to the synaptic normality among neurons	[264]
	Aurora A	Altered protein activity	Necessary for mitotic entry	[150]
	MCM3	Altered protein activity	Maintenance of cell cycle stability	[84]
KLHL17 (Actinfilin)	NRF2	Degradation	Critical for oxidative stress response; Inhibited tumorigenesis and chemo-resistance in a variety of malignancies; Triggering the onset of diabetes and excessive adipogenesis	[73,265-274]
	IKK β	Degradation	Tumor-inhibitory effects in various malignancies by suppressing NF- κ B signaling; Lowered inflammatory cytokines produced by macrophage	[275,276]
	PGAM5	Degradation	Activation of pro-apoptotic behavior	[83]
	p62	Altered protein activity	Competitive inhibition of NRF2 to stimulate anti-oxidant responses; Enhanced autophagic activity	[85,277]

Adaptor	Substrate	Modification	Biological impact*	Reference
KLHL20	PALB2	Altered protein activity	Prohibited homologous recombination in GI cells	[278]
	ULK1	Degradation	Governing autophagic termination	[138]
	VPS34	Degradation	Governing autophagic termination	[138]
	PML	Degradation	Potentiates development of prostate cancer; Increased metastatic capability in colon cancer	[139,143]
	DAPK	Degradation	Anti-apoptotic effects in multiple myeloma cells; Increased metastatic capability in colon cancer	[66,143]
	Beclin-1	Degradation	Governing autophagic termination	[138]
	Coronin 7	Altered protein activity	Modulating post-Golgi trafficking	[135]
	PDZ-RhoGEF	Degradation	Driving neurite outgrowth	[134]
	Aurora B	Subcellular re-localization	Completion of mitotic events	[145,279]
	PLK1	Subcellular re-localization	A faithful mitotic progression guaranteed	[280]
KLHL25	ACLY	Degradation	Repression of the neoplastic characteristics of lung cancer cells	[132]
KLHL42	4E-BP1	Degradation	Enhanced translational activity	[281]
	p60	Degradation	Permitting faithful mitotic procedures	[149]
	c-MYC	Degradation	Inhibited tumorigenesis of glioma	[75]
	sAnk1.5	Degradation	Core impact towards normal musculogenesis	[282]
	Notch1	Degradation	Physiological control of cardio-vascularogenesis	[107]
KCTD10	HDAC1	Degradation	Down-regulated Hedgehog-dependent proliferation of neural progenitors and medulloblastoma cells	[116]
KCTD13	RhoA	Degradation	Necessary for brain development; Physiological control of actin cytoskeleton structure and cellular movement; Preventing from vascular dysfunction and hypertension	[283–286]
KCTD17	Trichoplein	Degradation	Necessary for cilogenesis in multiple systems	[287]
KCTD21	HDAC1	Degradation	Down-regulation of Hedgehog-induced transcriptional activity and suppressing growth of medulloblastoma cells	[127]
KBTBD2	p85α	Degradation	Activation of PI3K pathway and enhanced insulin sensitivity	[153]
KBTBD6	TIAM1	Degradation	Restricted migration of breast carcinoma cells	[131]
KBTBD7	TIAM1	Degradation	Restricted migration of breast carcinoma cells	[131]
KBTBD8	Neurofibromin	Degradation	Elevated proliferation in glioblastoma	[288]
	TCOF1	Altered protein activity	Facilitating neural crest specification	[289]
	NOLC1	Altered protein activity	Driving neural crest specification	[289]
	PDCD4	Degradation	Increased translation of anti-apoptotic proteins	[155]
	RhoA	Degradation	Oriented cell migration	[290]
BTBD26 (IBTKα)				
BTBD34 (TNFAIP1)				
RhoBTB2	MSI2	Degradation	Suppression of breast carcinogenesis	[125]

Adaptor	Substrate	Modification	Biological impact*	Reference
RhoBTB3	MUFI	Degradation	Not available	[291]
	Cyclin E	Degradation	Activating cell cycle progression	[292]
ZBTB16	Atg14L	Degradation	Blocking the formation of autophagosomes	[293]
ZBTB28	CBF1	Degradation	Supporting angiogenic sprouting	[237]

Note:

*: The biological impact refers to the cellular alterations secondary to the ubiquitinated modification on substrates, rather than its original functionality.

Abbreviations: SPOP: speckle-type POZ protein, DAXX: death domain-associated protein 6, SRC-3: steroid receptor co-activator protein 3, Gli3: Gli family zinc finger 3, BMI1: B Lymphoma Mo-MLV Insertion Region 1 Homolog, PIPKI β : phosphatidylinositol-5-phosphate 4-kinase type II beta, HDAC6: histone deacetylase 6 protein, INF2: inverted formin 2, c-MYC: myc proto-oncogene protein, SIRT2: sirtuin 2, PR: progesterone receptor, CDC20: Cell division cycle protein 20, SETD2: SET domain containing 2, SENP7: setrin specific peptidase 7, ERG: ETS transcription factor, ER α : estrogen receptor α , DEK: DEK proto-oncogene, TRIM24: tripartite motif containing 24, AR: androgen receptor, DDIT3: DNA-damage inducible transcript 3, ER: endoplasmic reticulum, PTEN: phosphatase and tensin homolog, DUSP7: dual specificity phosphatase 7, BRMS1: breast cancer metastasis suppressor 1, PDX1: pancreatic and duodenal homeobox 1, KLHL21: kelch like family member 21, KCTD2: potassium channel tetramerization domain containing 2, RhoBTB2: Rho related BTB domain containing 2, MSI2: musashi 2, IBTK α : inhibitor of Bruton tyrosine kinase isoform α , PDCD4: programmed cell death 4, KLHL12: kelch like family member 12, SEC31: secretory 31, KEAP1: kelch-like ECH-associated protein 1, MCM3: minichromosome maintenance complex component 3, IKK β : I-kappaB kinase beta, NF- κ B: nuclear factor kappa B, BCL2: B-cell leukemia/lymphoma 2, PGAM5: phosphoglycerate mutase family member 5, KLHL15: kelch like family member 15, RBBP8: RB binding protein 8, DSB: DNA double strand breaks, NRF2: nuclear factor erythroid 2 like 2 (also known as NFE2L2), KLHL20: kelch like family member 20, ULK1: unc-51 like autophagy activating kinase 1, VPS34: vacuolar protein sorting 34, KBTBD8: kelch repeat and BTB domain containing 8, TCOF1: treacle ribosome biogenesis factor 1, NOLC1: nucleolar and coiled-body phosphoprotein 1, KCTD5: potassium channel tetramerization domain containing 5, ZNF711: zinc finger protein 711, MCM7: minichromosome maintenance complex component 7, FAM193B: family with sequence similarity 193 member B, KCTD11: potassium channel tetramerization domain containing 11, HDAC1: histone deacetylase 1 protein, KBTBD6: kelch repeat and BTB domain containing 6, KBTBD7: kelch repeat and BTB domain containing 7, TIAM1: T-cell lymphoma invasion and metastasis 1, PML: promyelocytic leukemia, DAPK: death-associated protein kinase, KCTD17: potassium channel tetramerization domain containing 17, KLHL3: kelch like family member 3, WNK1: WNK lysine deficient protein kinase 1, WNK4: WNK lysine deficient protein kinase 4, NCC: solute carrier family 12 member 3 (also called as SLC12A3), RhoA: ras homolog family member A, KLHL22: kelch like family member 22, PLK1: polo like kinase 1, KLHL2: kelch like family member 2, WNK3: WNK lysine deficient protein kinase 3, RhoBTB3: Rho related BTB domain containing 3, MUF1: leucine rich repeat containing 41 (also known as LRRC41), KCTD6: potassium channel tetramerization domain containing 6, sAnk1.5: small ankyrin-1 isoform 5, NPCD: neuronal pentraxin chromo domain, KLHL8: kelch like family member 8, RAPSYN: receptor associated protein of the synapse, GAN: gigaxonin (also known as KLHL16), MAP1B: microtubule associated protein 1B, TBCB: tubulin folding cofactor B, MAP8: microtubule associated protein 1S (also known as MAP1S), eEF1A1: eukaryotic translation elongation factor 1 alpha 1, D4 receptor: dopamine type 4 receptor, GluR6: glutamate ionotropic receptor kainate type subunit 2 (also defined as GRIK2), KLHL25: kelch like family member 25, ACYL: ATP-citrate lyase, TNFAIP1: TNF alpha induced protein 1 (also known as BTBD34), PALB2: partner and localizer of BRCA2, ZBTB16: zinc finger and BTB domain containing 16, Atg14L: autophagy related 14 (also called as Atg14), KCTD13: potassium channel tetramerization domain containing 13, KCTD10: potassium channel tetramerization domain containing 10, BCL6: B cell leukemia/lymphoma 6, Tdt: terminal deoxynucleotidyl-transferase, 4EBP1: eukaryotic translation initiation factor 4E binding protein 1 (also known as ARHGAP11), KLHL17: kelch like family member 17, KCTD21: potassium channel tetramerization domain containing 21, HDAC1: histone deacetylase 1, KLHL42: kelch like family member 42, KLHL18: kelch like family member 18, EglN2: egl-9 family hypoxia inducible factor 2, BET: Bromodomain and extraterminal domain, BRD2: bromodomain containing 2, BRD3: bromodomain containing 3, BRD4: bromodomain containing 4, KBTBD2: kelch repeat and BTB domain containing 2, PI3K: phosphoinositol-3-kinase, KLHL6: kelch like family member 6, HBXIP: late endosomal/lysosomal adaptor, MAPK and MTOR activator 5 (also called LAMTOR5), cMyBP-C: cardiac myosin binding protein C.

Table 4

Summary of evidence for tumor suppressive Cullin 3-based E3 ligases.

Adaptor	Pathological evidence (<u>Cancer relevant human specimens</u>)	Biochemical evidence (<u>Cancer relevant substrates</u>)
Keap1	Mutation [86,87]	NRF2, MCM3, IKK β PGAM5, p62, PALB2 [73,83–85,276,278]
KLHL6	Mutation [106]	HBXIP [106]
KLHL9	Decreased expression [129]	Aurora B [255]
KLHL16	Mutation [130]	MAP1B [261]
KCTD6	Decreased expression [127]	NA
KCTD10	Decreased expression [112]	Notch1 [107]
KCTD11	Decreased expression [115]	HDAC1 [116]
KCTD21	Decreased expression [115]	HDAC1 [127]
RhoBTB2	Decreased expression and mutation [125,126]	MSI2 [125]
RhoBTB3	Decreased expression [126]	MUF1, Cyclin E [291,292]
LZTR1	Mutation [128]	NA
KLHL2	NA	WNK1/3/4 [246]
KLHL3	NA	WNK1/4, Claudin-8 [248,249,252]
KLHL13	NA	Aurora B [255]
KLHL15	NA	RBBP8 [259]
KLHL25	Normal [132]	ACLY, 4E-BP1 [132,281]
KCTD13	NA	RhoA [283]
KBTBD6	NA	TIAM1 [131]
ZBTB28 (BAZF)	NA	CBF1 [237]

Note: The neoplastic evidences of Cullin 3 adaptors include physiological, pathological and biochemical contents that correspond to mouse model (knockout or transgenic), clinical sample analysis and substrate respectively. The neoplastic role of each adaptor is determined by the overall consideration of three categories of evidences, with physiological evidences the most significantly weighing. Generally, if the physiological evidences dominate, level 1 of evidence quality will then refer to the specific adaptor, so as to pathological and biochemical evidences via a descending order.

Table 5

Summary of evidence for oncogenic Cullin 3-based E3 ligases.

Adaptor	Pathological evidence (<u>Cancer relevant human specimens</u>)	Biochemical evidence (<u>Cancer relevant substrates</u>)
KLHL20	Increased expression [139]	ULK1, PML, DAPK, PDZ-RhoGEF, Beclin-1, VPS34 [134,138,143]
KLHL21	Increased expression [147]	Aurora B [145,279]
KLHL22	Mutation [294]	PLK1 [280]
KLHL42	Increased expression [148]	p60 [149]
KLHL18	NA	Aurora A [150]
KBTBD2	NA	p85 α [153]
IBTK α	NA	PDCD4 [155]

Table 6

Summary of evidence for context-dependent neoplastic role of Cullin 3-based E3 ligases.

Adaptor	Physiological evidence (Cancer relevant mouse models)	Pathological evidence (Cancer relevant human specimens)	Biochemical evidence (Cancer relevant substrates)
SPOP	Conditional <i>Spop</i> ^{-/-} in prostate: spontaneous development of prostatic intraepithelial neoplasia	Mutation, decreased expression or overexpression [186,220,295]	BRD2/3/4, HDAC6, INF2, DAXX [189,218,239,295]
KCTD2	NA	Either increased or decreased expression [75,159]	c-Myc [75]
BTBD34 (TNFAIP1)	NA	Mutation or overexpression [160,161]	RhoA [290]
ZBTB16 (PLZF)	NA	Either increased or decreased expression [162,163]	Atg14L [293]
KBTBD7	NA	NA	TIAM1, Neurofibromin [131,288]
KBTBD8	NA	NA	TCOF1, NOLC1 [289]

Table 7

Functional summary of SPOP in mammalian systems.

System	Category	Functional implications Animal evidence	Non-animal evidence
General impact	Physiology	NA	<ol style="list-style-type: none">1 Regulation of alternative splicing [173]2 DNA damage response [174]3 Stable X chromosome inactivation [165]4 Hedgehog pathway silencing [170]5 Phosphoinositide transduction [171]
	Pathology	NA	<ol style="list-style-type: none">1 Triggering cellular senescence [191]2 Induction of apoptosis [172]
Nervous system	Physiology	Auxiliary role in dorsoventral patterning of embryonic spinal cord (Systemic KO mouse (<i>Spop^{-/-}</i>)) [175]	NA
	Tumorigenesis	NA	Tumor suppressor in glioma [215]
Cardiovascular system	Physiology	NA	Homeostasis of endothelial cell function [176]
Respiratory system	Tumorigenesis	NA	Tumor suppressor in non-small cell lung cancer [217]
Digestive system	Tumorigenesis	Tumor suppressor in colorectal cancer (Xenografts in nude mouse) [213]	<ol style="list-style-type: none">1 Tumor suppressor in colorectal cancer [239]2 Tumor suppressor in liver cancer [211]3 Tumor suppressor in gastric cancer [210]
Urological system	Tumorigenesis	<ol style="list-style-type: none">1 Tumor suppressor in prostate cancer (Systemic KO mouse (<i>Spop^{-/-}</i>), conditional KO in mouse prostate (<i>Spop^{-/-}</i>) and xenografts in nude mouse) [181,196]2 Tumor promoter in ccRCC (Xenografts in nude mouse) [223]	<ol style="list-style-type: none">1 Tumor suppressor in prostate cancer [29]2 Tumor promoter in ccRCC [223]
Gynecological system	Physiology	Normalizing decidualization of endometrial stromal cells in mice [179]	Normalizing decidualization of endometrial stromal cells [179]
	Tumorigenesis	Tumor suppressor in breast cancer (Xenografts in nude mouse) [166]	<ol style="list-style-type: none">1 Tumor suppressor in ovarian cancer cells [201]2 Tumor suppressor in breast cancer cells [203]3 Tumor suppressor in endometrial cancer cells [205]
Musculoskeletal system	Physiology	Promotion of skeletal development (Systemic KO mouse (<i>Spop^{-/-}</i>) and conditional KO in mouse limb (<i>Spop^{-/-}</i>)) [177]	NA

System	Category	Functional implications	Non-animal evidence
Endocrine system	Physiology	Animal evidence Improved blood homeostasis and function of pancreatic β cells <i>Spop^{+/-}/pdx^{+/-}</i> [178]	Improved blood homeostasis and function of pancreatic β cells [178]

Abbreviations: NA: not available, KO: knockout, ccRCC: clear-cell renal cell carcinoma.

Table 8

Compounds that directly target Cullin 3-based E3 ligases.

Adaptor	Chemical	Category	Function
SPOP	Compound 6b	Inhibitor	Inhibition of malignant properties of ccRCC [223]
Keap1	CDDO-Me	Inhibitor	Protective impacts against chronic kidney disease [296]; Anti-tumor activity among various cancers [227]
	DMP	Inhibitor	Neuroprotective effects against neuroinflammation [297]; Anti-tumor activities among various cancers [228]
	MMP	Inhibitor	Neuroprotective effects against neuroinflammation [297]; Tumor preventive role [298]

Note: The chemicals mentioned in the table directly target the adaptors, while those influence the protein-protein interactions between adaptors and substrate/scaffold are not included.