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Review Article

The functions and properties of cullin-5, a potential therapeutic target for cancers

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Abstract: Cullin-5 (CUL5), a scaffold protein in active cullin-RING ubiquitin ligase (CRL) complexes, is a member of the cullin family of proteins. The CUL5-type ubiquitin ligase can target multiple proteins involved in ubiquitination and proteasome degradation. CUL5 plays positive roles in regulating cell growth, proliferation and physiological and other processes in the human body. It has been found that the expression of CUL5 is significantly downregulated in various cancer cells, which affects the course of the cancers. Here, we reviewed the current data on the expression and role of CUL5 in both normal and cancer cells, its possible mechanisms, and its potential as a therapeutic target for cancers.

Keywords: Cullin-5, ubiquitin, cancer, microRNAs, regulation, therapeutics

Background

The cullin proteins are evolutionarily conserved [1]. Their N-terminal domain (NTD) is stem-like and binds to different substrates by recognizing different binding proteins and SBP. The C-terminal domain (CTD) is globular and binds to a RING finger protein (Rbx1 or Rbx2) to bind to an E2 conjugating enzyme and ubiquitin [2, 3]. Cullin proteins have been recognized to serve as a scaffold and central components of the cullin-RING ubiquitin ligase (CRL) complex [3, 4].

Cullin-5, a member of the cullin protein family, was first described as vasopressin-activated calcium mobilization receptor (VACM-1) in the renal medulla of rabbits [5]. Further analysis showed that VACM-1 was homologous to CUL5 [6]. The NTD of CUL5 links to the SOCS box protein by the adaptor complex Elongin BC, and the CTD binds to Rbx2, collectively forming functional CRL5 (SOCS-Elongin BC-CUL5-Rbx2), which participates in the recruitment of specific targets [2, 7]. There are four major classes of SOCS box proteins: the SOCS family, the WSB family, the SSB family, and the ASB family. All of them have SOCS boxes containing CUL5 boxes and BC boxes as important domains for binding to CUL5 and Elongin BC [8-10]. NEDD8 is a ubiquitin-like protein that binds to and activates CRL5. The process by which NEDD8 activates CRL5 is called neddylation, and the process of inactivating CRL5 is deneddylation [11]. CRL5 requires acetylation of the E2 NEDD8-conjugating enzyme UBE2F at its C terminus for complete activation [12-14], and Rbx2 is involved in binding to the E2 conjugating enzyme and required for the covalent attachment of NEDD8 to the CUL5 lysine residue component [15]. The removal of NEDD8 by dehydrogenation of the COP9 signalosome (CSN) complex leads to the loss of CRL5 functional activity [16, 17] (**Figure 1**).

It has been reported that the CUL5-Elongin A-Elongin BC complex can efficiently polymerize the RNA Pol II subunit Rpb1, thus affecting the cell cycle [18, 19]. In addition, CUL5 is involved in the regulation of apoptosis by regulating the phosphorylation of mitogen-activated protein kinase (MAPK) and inducing p53 mRNA and

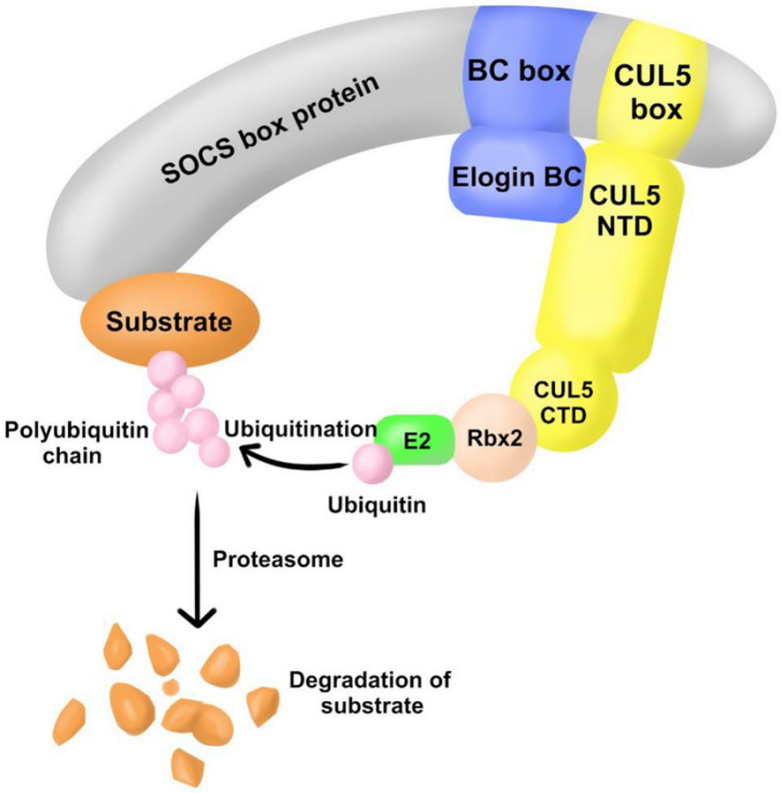

Figure 1. The structure of CRL5.CUL5 serves as a scaffold and is the central component of CRL5. The NTD of CUL5 is linked to the SOCS box protein via the adaptor complex Elongin BC. The CTD binds to Rbx2, forming the CRL5 complex (SOCS-Elongin BC-CUL5-Rbx2). The CRL5 complex belongs to the E3 ubiquitin ligase family and can recruit E2 ubiquitin conjugating enzymes. RBX2 participates in the binding of E2 enzymes. The E2 enzyme is bound to activated ubiquitin, and under the action of CRL5, ubiquitin is transferred to the substrate to form the ubiquitin chain. Subsequently, the substrate tagged by the ubiquitin chain is recognized and degraded by the proteasome.

protein expression [20]. The expression level of CUL5 mRNA is different under different conditions. For example, in 24-hour water-deprived rats, the levels of CUL5 mRNA in the heart, skeletal muscle and mesenteric arteries increased significantly, while those in the kidneys decreased significantly [21, 22]. CUL5 also has a function in cell differentiation and proliferation. For example, decreased CUL5 expression is associated with small-cell lung cancer metastasis and breast cancer occurrence [23]. Meanwhile, CUL5 protein can induce the differentiation of leukemia cells [24].

Current studies on CUL5 focus on its expression in cancer cells and its roles in carcinogenesis and the development of cancer. CUL5 inhibits the proliferation and metastasis of cancer cells and promotes apoptosis in a variety of cancer cells [24-29]. However, in a small number of cancer cells, such as colon cancer and hepatitis B virus-induced liver cancer cells, CUL5 shows the opposite effect [30, 31]. The specific reasons remain to be further studied. In addition, due to the high specificity of CUL5 for substrates, CUL5 is also a new drug target for the treatment of cancer [2]. It has been reported that both hematoxylin and resveratrol can target CUL5 to control its expression and inhibit the proliferation and invasion of cancer cells [32, 33]. At present, a variety of drugs targeting CUL5 are still under investigation. It can be predicted that CUL5, as a target of drug action, will play an invaluable role in future cancer treatment.

Functions of cullin-5 in normal organisms

CUL5 plays a significant role in migration, DNA damage and proliferation via the ubiquitination and degradation of different target proteins in various normal cells [34-37]. Furthermore, CUL5 in different tissues engages in different physiological regulations, mainly regulating angiogenesis [38], downregulating aquaporins [21, 22] and inhibiting autophagy [39]. Nevertheless, the functions and mechanisms of CUL5 have not been revealed thoroughly. Further examination and validation of CUL5 may lend further insight into how organisms adjust their physiological balance. The main functions and mechanism of CUL5 in normal organisms are presented in **Table 1** and **Figure 2** below.

Regulating the activity of cellular life

Inhibiting migration: During growth, cells need to move between other cells through the extracellular matrix (ECM), which attaches to intra-

Table 1. Functions and molecular mechanism of CUL5 in normal cells

	Effectors/ Targets	Mechanism	Function	Reference
CUL5	pYCas	CUL5↑/pYCas↑/FAs↑	inhibiting migration	[34]
	Dab-1	CUL5↑/Dab-1↑/Src↑, Fyn↓ and Reln↓	decelerating neuron migration, cortical layering	[46]
	Rpb1	CUL5↑/Rpb1↓	responding to DNA damage	[35]
	AC	CUL5↑/AC↓/cAMP↓	inhibiting proliferation	[36, 54, 55]
	MAPK	CUL5↑/MAPK↓	inhibiting proliferation	[20]
	DDA3	CUL5↑/DDA3↓/stabilize MT	inhibiting proliferation	[9]
	β-TrCP1	CUL5↑/β-TrCP1↓	inhibiting cell growth	[59]
	AQP-1/2	CUL5↑/AQP-1/2↓	balancing hydrosalinity	[21, 22]
	DEPTOR	CUL5↑/DEPTOR↓	inhibiting autophagy	[39]

↑: upregulation; ↓: downregulation; CUL5: cullin-5; pYCas: phosphorylated Cas; FAs: focal adhesions; Dab-1: Disabled-1; Reln: Reelin; Rpb1: RNA polymerase II's largest subunit; MAPK: mitogen-activated protein kinase; MT: microtubule; DDA3: differential display and activation by P53, also known as PSRC1; β-TrCP: β transducin repeat-containing protein; AQP: aquaporin; DEPTOR: DEP domain-containing mTOR-interacting protein.

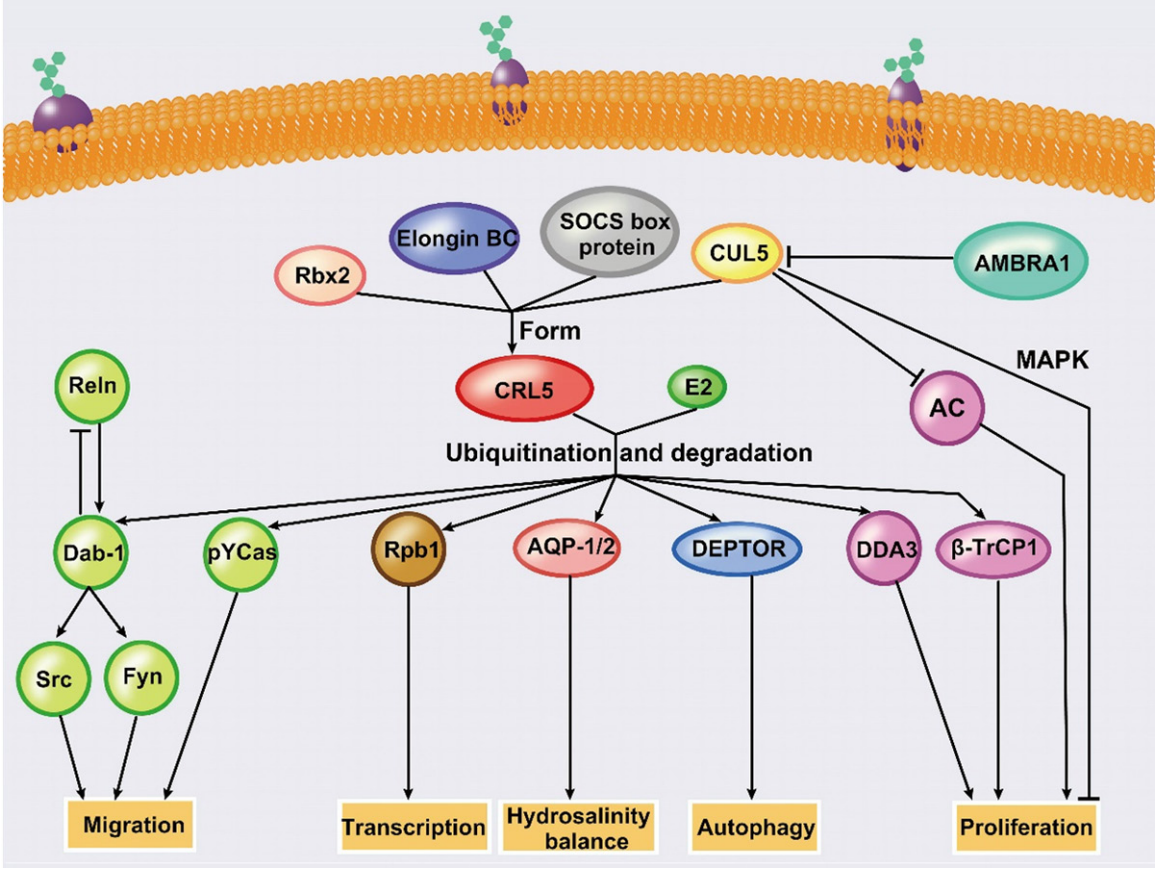

Figure 2. The main functions and mechanism of CUL5 in normal cells. CUL5 plays an important role in normal cell activities. CUL5, along with a SOCS box protein, Elongin BC and Rbx2, form the CRL5 complex and recruit the E2 enzyme. Then, the CRL5-E2 complex ubiquitinates and degrades componentsassociated with cellular activities, such as Dab-1, pYCas, Rpb1, AQP-1/2, DEPTOR, DDA3, and β-TrCP1. The degradation of key components alters or affects downstream signaling pathways and further regulates cell migration, transcription, autophagy and proliferation. Meanwhile, CUL5 can directly decrease the activity of AC and regulate the phosphorylation process of MAPK to affect cell proliferation. In addition, CUL5 can be inhibited by AMBRA1.

cellular focal adhesions (FAs) and actin to initiate cell migration [40]. As part of FAs, Cas (p130Cas) is an FA protein that can be phosphorylated by Src. The overexpression of phosphorylated Cas (pYCas) stimulates FA decomposition, which is conducive for recycling FA at

the leading edge of the cell to enhance cell migration [41]. CUL5 can combine with SOCS6 and Elongin BC to form the E3 Elongin ligase, which targets pYCasfor degradation through the SH2 domain in SOCS6 [41], stabilizes FAs and inhibits cell migration [34]. Without CUL5, epithelial cell proliferation and migration and fibroblast transformation become more dynamic [10]. In addition, CUL5 combines with different SOCS proteins, such as SOCS2, SOCS4 and SOCS5, to target different phosphorylated proteins to regulate adhesion kinetics. Therefore, the migration activities of cells operate normally [34].

In the central nervous system, migration and differentiation in distant sites promote the formation of multiple layers of the mammalian neocortex from the inside out [42]. For example, undifferentiated projection neurons migrate from the ventricular region to the bottom of the cortical layer and then migrate upward to the top and stop [43]. The correct stratification depends on three substances, the extracellular protein Reelin (Reln), the intracellular signaling protein Disabled-1 (Dab-1), and the E3 ubiquitin ligase CUL5 [44-46]. Reln stimulates tyrosine phosphorylation of Dab-1 to activate Dab-1, which activates kinases Src and Fyn and then initiates downstream signal transduction pathways [47] and provides a negative feedback signal to terminate Reln signal transduction [46, 48]. Dab-1 is recognized by CUL5 after signal transduction and then undergoes ubiquitination and degradation to prevent excessive migration [46]. CUL5 has two roles in neuronal migration: slowing the rate of neuronal migration through the cortex and allowing cells to shift down as the cortex grows. CUL5-deficient neurons have a shorter resting time and move faster than their wild-type counterparts; instead of moving down, they stay on top of the cortical plate for longer periods of time, which determines the final position of the neurons [43].

Responding to deoxyribonucleic acid damage

CUL5 is able to combine with Elongin to form a complex that regulates RNA polymerases and participates in responding to DNA damage [18]. As a class of molecules that transmit genetic information in cells, RNA polymerases play an important role in cell growth and proliferation through transcriptional regulation [49]. In eukaryotes, RNA polymerase II (Pol II) is regulated during mRNA synthesis by some transcription factors, such as Elongin and CSB [18, 50]. Elongin is a heterotrimer composed of subunits A, B and C [50]. Among them, Elongin A is the transcriptionally active subunit, and the Elongin BC complex, formed by Elongin B and C, regulates the activity of Elongin A [50]. In mammals, Elongin A links the CUL5 and RING finger proteins via the Elongin BC complex to form a multisubunit complex [51]. By profiling DNA damage simulated by ultraviolet radiation, some researchers have found that the colocalization of CUL5 and Elongin A in the nucleus is enhanced and that ubiquitination and degradation of Pol II's largest subunit (Rpb1) occur [18, 35]. In addition, in cells containing Cockayne syndrome B (CSB) protein, CSB protein encourages the recruitment of the ubiquitin ligase consisting of Elongin A and CUL5 to DNA damage sitesand induces stalling of Pol II [52]. This may explain why Pol II is phosphorylated and ubiquitinated and then degraded by proteasomes when DNA damage occurs [18].

Inhibiting proliferation

CUL5 exhibits antiproliferative effects in vitro and in a variety of cell lines [36]. Overexpression of CUL5 decreases the activity of adenylate cyclase (AC) and the production of cAMP [36, 53]. Through the mechanism of MAPK phosphorylation, CUL5 participates in the degradation process of various proteins related to cell proliferation, thus inhibiting cell proliferation [20]. The ability of CUL5 to inhibit cell proliferation is affected by its carboxy-terminal nuclear localization signal (NLS) "pat7", which begins with proline, and this consensus signal sequence is conserved among VACM-1/CUL5 across species. NLS mutation affects the localization of CUL5 in the nucleus and reduces its ability to inhibit cell proliferation [54]. When using *Drosophila* eggs to study cell differentiation and morphogenesis, it was found that downregulation of CUL5 led to excessive production of germ cells [55]. During mitosis, dynamic changes in microtubules (MTs) are involved in the process of evenly distributing chromosomes to two daughter cells [56]. DDA3, a kind of MT-related protein, together with other proteins, regulates mitotic spindle dynamics [57]. If DDA3 is knocked out, the tension of sister kinetochores at metaphase will be attenuated, and the rate of late chromosome segregation is slowed down, which shows that DDA3, as a

destabilizing protein of MTs, enhances mitotic spindle dynamics by promoting MT dynamic assembly [57]. The ASB7-CUL5-Elongin BC complex has been identified to play a role in ubiquitinating and degrading DDA3, thereby weakening the mitotic drive and promoting anti-proliferative effects [9].

F-box proteins, a family of proteins containing F-box motifs, have substrate recognition specificity during ubiquitin-mediated proteolysis and participate in various physiological processes, such as cell phase transition, signal transduction and growth [58]. One recent study reported that β-TrCP1 (F-box protein 20) and SAG-CUL5 can form a complex that shortens β-TrCP1's half-life, negatively regulates the expression of β-TrCP1 and inhibits cell growth and survival by ubiquitinating β-TrCP1 [59].

Participating in physiological regulation

Regulating angiogenesis: CUL5 is widely distributed in humans, found in such locations as the placenta, skeletal muscle, brain, kidney, heart and other tissues, and may be involved in regulating endothelial permeability [60, 61]. Thalidomide, a drug that inhibits cell proliferation by inhibiting angiogenesis, can reduce the amount of CUL5 in the nucleus during the growth of human endothelial cells [38]. The anti-proliferative effect of thalidomide was inhibited in human endothelial cells transfected with anti-CUL5 siRNA and in rat endothelial cells with CUL5 mutation [38]. These results suggest that CUL5 may be involved in the mechanism by which thalidomide interferes with angiogenesis [38].

Downregulating aquaporin

Aquaporin-1 (AQP-1) is highly expressed in the vascular endothelium to regulate water permeability [62]. In vivo, CUL5 is expressed in kidney collecting tubular cells and vascular endothelial cells [61]. In COS-1 cells in vitro, the expression of CUL5 cDNA decreases the levels of endogenous AQP-1 mRNA and AQP-1 protein, suggesting that CUL5 can regulate the expression of AQP-1 at both the transcriptional and post-translational levels through glycosylation of VACM-1 via MAPK phosphorylation [21, 53]. The level of CUL5 mRNA in the vascular tissue of 24-hour water-deprived rats was significantly elevated. Although there was no significant decrease in the AQP-1 level, the concentration of AQP-1 was negatively correlated with the ratio of CUL5 to NEDD8-modified CUL5 [21]. These results suggest that the hypertonic stress of water deficiency in vivo increases the level of CUL5 protein, which is induced by NEDD8 after translation and participates in the regulation of water balance.

AQP-2 is located in the plasma membrane at the apical end of the renal collecting duct and regulates water permeability [63]. The expression of CUL5 in vivo is controlled by hydration [64]. The changes in CUL5 protein levels are region-specific and were negatively correlated with AQP-2 protein levels in kidneys isolated from dehydrated rats [22, 53, 64]. As a key component of the E3 ubiquitin ligase, CUL5 can enhance or attenuate the ubiquitination process to regulate the degradation of AQP-2, thereby regulating the concentration of AQP-2 [22, 65, 66]. In addition, CUL5 indirectly regulates the concentration and function of AQP-2 by modulating its posttranslational modifications, subcellular localization and interactions with other proteins in the cell. For example, the translocation of microfilaments related to AQP2 to the apical plasma membrane is related to Rab GTPases, regulatory molecules controlled by CUL5, and as client protein for CUL5 E3 ligases, HSP70 participates in the processes of translocation and degradation during AQP2 internalization [22].

Inhibit autophagy

Autophagy is a survival mechanism that degrades damaged or unnecessary components in cells and provides energy and components to synthesize new substances, thereby maintaining cell homeostasis [67]. AMBRA1 binding to CUL4 or CUL5 and forming a whole complex is a key factor involved in autophagy [68, 69]. CUL4 and CUL5 can act as autophagic modulators to regulate the initiation and termination of autophagy [39]. mTORC1 inhibits autophagy [70], and DEPTOR is an inhibitor of mTORC1 that can inhibit the function of mTORC1 and induce autophagy [71]. Overexpression of CUL5 can cause a significant decrease in DEPTOR levels [39]. Autophagy stimulation dissociates AMBRA1 from CUL4 and causes it to bind to CUL5, which inhibits CUL5 activity and reduces DEPTOR degradation; accumulated DEPTOR then induces autophagy [39].

Roles of cullin-5 in cancers

Cullin-5 in digestive system cancers

Low expression of CUL5 is associated with malignant phenotypes in gastric cancer and non-HBV-induced HCC. Nonetheless, in colon cancer and HCC induced by HBV infection, the opposite is true: CUL5 is overactivated in cells; these contradictory results require further insight.

In recent years, although the treatment of gastric cancer has improved, the mortality rate is still the third highest, and the early diagnosis of gastric cancer still lacks effective markers [72]. Recently, it was found that miR-19a expression was enhanced in gastric cancer tissues and that miR-19a directly targeted and downregulated CUL5 expression, promoting the proliferation and invasion of gastric cancer cells [25]. ABKRD9, a newly identified E3 substrate receptor subunit belonging to the ASB protein family, can form a complex with CUL5, Elongin B, Elongin C and other subunits to ubiquitinate and degrade the inosine monophosphate dehydrogenase 2 (IMPDH2) isoform, which mediates cell proliferation [73]. ANKRD9 is related with gastric cancer susceptibility [73]. Downregulation of CUL5 in human gastric cancer cells reduces the formation of the ABKRD9-Elongin BC-CUL5 complex, which causes anchorage-independent proliferation and accelerates tumor progression [73].

In colorectal cancer (CRC) cells, endoplasmic reticulum protein 29 (ERp29) is an endoplasmic reticulum-resident protein involved in ER stress (ERS) [30, 74]. ERS inhibits cell growth and the ability of CRC to metastasize [75]. The increased expression of ERp29 during ERS in CRC counteracts the inhibitory effect of ERS on cells [30]. ERp29 functions via CUL5. The expression of CUL5 in CRC cells mirrors that of ERp29. CUL5 colocalizes with ERp29, which may be related to the occurrence of colorectal cancer [30].

Hepatocellular carcinoma (HCC) is the most common primary malignant liver tumor [76]. Among hundreds of risk factors inducing HCC, hepatitis B virus (HBV) infection is the most common [77]. Hepatitis B virus X protein (HBX) is an HBV protein that can transactivate oncogenes and induce HCC tumors [78]. MiR-145 is associated with HCC, and CUL5 is the target of miR-145 [31]. In HBX-transfected cells, the level of miR-145 is decreased, and the level of CUL5 is increased. At the same time, the overexpression of HBX significantly increases the proportion of cells in the G2/M phase and decreases the proportion of cells in the G0/G1 phase, which inhibits cell apoptosis [31]. It is worth noting that the overexpression of CUL5 does not inhibit proliferation, which may be related to the role of HBX, but these ideas require further study [31]. In HCC tissues without HBV infection, miR-7 and CUL5 are both downregulated [27]. Because miR-7 positively regulates the expression of CUL5, low miR-7 levels participate in the formation of the HCC malignant phenotype by reducing endogenous CUL5 levels [27].

Cullin-5 in female reproductive system cancers

In female reproductive system cancer cells, CUL5 is generally downregulated by different kinds of microRNAs, showing malignant proliferation and strong invasiveness.

Endometrial adenocarcinoma, also known as corpus carcinoma, is a malignant tumor of endometrial epithelial cells and the second most common gynecological cancer [79]. MiR-182 is overexpressed in endometrial adenocarcinoma and serous endometrial adenocarcinoma and directly targets and inhibits CUL5 [28]. Moreover, the levels of CUL5 in the highly invasive serous type are decreased, suggesting that low CUL5 expression promotes tumor cell proliferation [28].

Cervical cancer ranks fourth in incidence and mortality, and it is the main cause of death in cancer patients in 42 countries [72]. High miR-19a/b and low CUL5 expression, which are also found in gastric cancer, are found in human cervical cancer cells, leading to high proliferation and invasiveness [25].

Ovarian cancer is one of the most common gynecological malignancies in the world, accounting for 2.5% of all female malignancies [72]. MiR-27 is overexpressed in both ovarian cancer tissues and ovarian cancer cell lines [26]. CUL5 is the target of miR-27, and overexpression of CUL5 can induce G2/M blockade [26]. Overexpression of miR-27a in ovarian cancer tissues and cell lines inhibits CUL5, pre-

venting CUL5 from inducing cell cycle checkpoint blockade, which abolishes the inhibition of cancer cell proliferation and leads to substantial proliferation of cancer cells [26].

Cullin-5 in leukemia

B cell chronic lymphocytic leukemia (B-CLL) is the most common leukemia in adults and has a highly variable clinical course [80]. In B-CLL, genomic aberrations are important independent predictors of disease progression and patient survival [80]. Deletion of chromosome region 11q22-q23 is a common type of chromosome aberration in B-CLL [81]. In the general population, CUL5 is involved in regulating the stability of p53 and controlling the apoptosis of normal lymphocytes by controlling the formation of E3 ubiquitin ligases and the degradation of P53 and apoptosis-related proteins in lymphocytes [24]. In patients with 11q22-q23 deletion, CUL5 gene expression is reduced, which impairs the ability of B-CLL lymphocytes to undergo apoptotic death, especially in high-risk B-CLL [24]. Therefore, CUL5 participates in the occurrence of B-CLL by inducing apoptosis resistance.

The human myeloid cell line HL-60 is a promyelocytic lymphocyte line derived from patients with acute promyelocytic leukemia (APL) and has been widely used as a model for studying granulocyte differentiation. The expression of CUL5 mRNA and protein is significantly increased during the differentiation of HL-60 cells induced by all-trans retinoic acid (ATRA) [82], which indicates that CUL5 may play an important role in granulocyte differentiation, but the specific mechanism needs further research.

Cullin-5 in other cancers

Although different types of cancer have different origins, it is generally suggested that the downregulation of intracellular CUL5 is associated with malignancy.

Breast cancer is a malignant tumor occurring in the epithelial tissues of the breast gland, and most cases occur in women and endanger their physical and mental health, accounting for 1/4 of all cancers diagnosed in women [72]. In breast cancer cells, CUL5 expression is generally low, and the downregulation is unrelated to genetic aberrations in CUL5, CUL5 neddylation and phosphorylation abnormalities in breast cancer cells, indicating a probable relation with posttranslational modifications [83, 84]. In addition, research shows that CUL5 participates in regulating estrogen-dependent growth by decreasing the levels of estrogen receptor (ER-alpha) in the nucleus [53]. When CUL5 is overexpressed, it inhibits the growth of breast cancer cells by reducing MAPK phosphorylation, reducing early growth response elements (EGR-1) and increasing Fas-L mRNA [29, 85].

Angiosarcoma, a malignant hemangioma originating in blood or lymphatic vascular endothelial cells, often occurs in the skin of the head and face and is very aggressive, as it can be transferred through the blood; the prognosis of patients is very poor, and its cause is still unknown [86]. In in vitro human dermal microvascular endothelial cell (HDMEC) and angiosarcoma cell lines, the circ_0024169/CUL5 ratio is found to be lower than that in normal cells [87]. The same results occurred in both suppurative granulomas and endovascular sarcomas in vivo [87]. The ratio of circ_0024169/CUL5 has potential as a diagnostic biomarker for angiosarcoma [87].

Clear cell renal cell carcinoma (ccRCC) is the most common histologic subtype of renal carcinoma, accounting for 70-75% of cases, and is currently considered to be a lesion produced by cells lining the proximal tubules of the nephron [88]. CUL5 is downregulated in cancerous cells, which also have increased DNA damage, mitotic errors and excessive replication of centrioles [89]. Decreased or absent expression of the CUL5 gene is significantly associated with a decreased survival rate in patients [89].

Metastasis is the leading cause of death in small-cell lung cancer (SCLC) patients [90]. CUL5 and SOCS3 are the two most critical components that mediate cancer cell metastasis [23]. In cancer cells from patients with SCLC metastasis, the expression of CUL5 and SOCS3 was decreased significantly, which prevented the formation of the E3 ligase complex and the degradation of integrin 1 and activated downstream focal adhesion kinase/Src (Fak/Src) signaling pathways, which ultimately led to SCLC metastasis [23]. Furthermore, SCLC cases lacking CUL5 are sensitive to the Src inhibitor dasatinib [23].

UBE2F promotes lung cancer cell survival and is overexpressed in non-small-cell lung carcinoma [15]. The UBE2F/Sag/CUL5 complex activates CRL5 through the modification of CUL5 by NEDD8, which causes CRL5 to degrade the pro-apoptotic protein Noxa and inhibit apoptosis [15].

Clinical significance

Low levels of CUL5 expression have been validated in mostly malignant tumors, except for CRC [30], HCC (associated with HBV infection) [31], APL [82] and NSCLC [15]; hence, it exhibits a relationship with poor prognoses. CUL5 has real potential as a novel potential therapeutic target for gastric cancer [25, 73], HCC (not related to HBV infection) [27], endometrial adenocarcinoma [28], cervical cancer [25], ovarian cancer [26], B-CLL [24], breast cancer [83], angiosarcoma [87], ccRCC [88] and SCLC [23]. The expression and functions of CUL5 in cancer cells are summarized in **Table 2** and **Figure 3** below.

Advances in anticancer therapies associated with cullin-5

Heat shock protein 90 (HSP90) is a highly conserved chaperone protein in eukaryotic cells that interacts with a variety of client kinases or transcription factors [91]. In tumor cells, HSP90 is overexpressed due to the abundant presence of various client proteins, and HSP90 stabilizes these carcinogenic client proteins and maintains tumor progression; thus, HSP90 has also become a target of anticancer therapy [92]. However, the widespread low expression of CUL5 in tumor cells attenuates the efficacy of HSP90 inhibitor monotherapy [93].

HSP90 inhibitors promote ubiquitination and degradation of cancer-causing client proteins, among which CRAF, a client protein of HSP90, is involved in tumorigenic MAPK signaling. One of the independent degradation pathways involving CRAF requires the participation of CUL5, Elongin B and Elongin C [94].

17-N-allylamino-17-demethoxygeldanamycin (17-AAG), a derivative of geldanamycin, is an effective inhibitor of HSP90 [95]. Low expression of CUL5 in tumor cells may adversely affect the efficacy of 17-AAG. By delivering gold nanoparticles (AuNPs) containing CUL5 DNA, the sensitivity of human breast cancer cells to 17-AAG can be enhanced, indicating that CUL5 is involved in the mechanism of action of 17-AAG, which may be the reason why 17-AAG presents low efficacy as a monotherapy [95]. However, 17-AAG-induced degradation of UHRF1, a regulator involved in DNA methylation and the maintenance of cell proliferation, is independent of CUL5 [96].

As a fatal malignant tumor with almost the same incidence and mortality rates, pancreatic cancer has a five-year relative survival rate of only 9%. How to effectively control its malignant progression remains to be studied [79]. Sanguinarine, a kind of plant alkaloid, has certain anticancer effects [97]. In human orthotopic adenocarcinoma cells and pancreatic cancer cells, sanguinarine can increase the levels of CUL5 mRNA and protein and inhibit cell proliferation [32].

Resveratrol, a natural component of the human diet, was shown to regulate apoptosis and cell proliferation by controlling the expression of CUL5 in a breast cancer cell line. Thus, CUL5 can be used as a target of resveratrol in the treatment of breast cancer [33].

Conclusion

CUL5-type ubiquitin ligases play a vital role in physiological regulation by specifically recognizing different substrates and mediating the targeted degradation of proteins to regulate different signaling pathways, thereby widely participating in numerous cell activities. Due to the differential expression of CUL5 in normal cells and cancer cells, CUL5, as a protein that inhibits malignant proliferation and invasion of tumors, is a potential therapeutic target of great value. Nevertheless, exploration of the exact mechanism of CUL5 in some signaling pathways is required, and the unknown pathways involved in CUL5 remain to be discovered. The diversity of microRNAs regulating the CUL5 gene and CUL5's targeted substrates indicates that CUL5 is extensively involved in regulating cellular activities. Further profiling studies on the physiological functions and mechanisms of CUL5 will help hewn out new approaches to treat cancer.

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Table 2. Functions and molecular mechanism of CUL5 in cancer cells

	Type	Molecule	Mechanism	Function	Reference
CUL5	Gastric cancer	miR-19a, ANKRD9	miR-19a↑/ABKRD9-Elongin BC-CUL5↓	promoting proliferation, invasion and anchorage-independent growth	[25, 73]
	Hepatocellular carcinoma (associated with HBV infection)	miR-145	miR-145↓/CUL5↑	inhibiting apoptosis	[31]
	Hepatocellular carcinoma (not associated with HBV infection)	miR-7	miR-7↓/CUL5↓	Formation of a malignant phenotype in HCC	[27]
	Endometrial adenocarcinoma	miR-182	miR-182↑/CUL5↓	promoting proliferation	[28]
	Cervical cancer	miR-19a/b	miR-19a/b↑/CUL5↓	promoting proliferation and invasion	[25]
	Ovarian cancer	miR-27a	miR-27a↑/CUL5↓	inhibiting G2/M blockade	[26]
	B cell chronic lymphocytic leukemia	-	Chromosome region 11q22-q23↓/CUL5↓	inducing apoptosis resistance	[24]
	Acute promyelocytic leukemia	-	CUL5↑	regulating granulocyte differentiation	[82]
	Breast cancer	-	CUL5↓	promoting proliferation	[29, 83-85]
	Angiosarcoma	circ_0024169	circ_0024169 / CUL5 ratio↓	acting as a diagnostic biomarker	[87]
	Clear cell renal cell carcinoma	-	CUL5↓	increasing DNA damage, promoting mitotic disorder	[88]
	Small-cell lung cancer	SOCS3 integrinβ1	SOCS3↓/CUL5↓/integrinβ1↑	promoting metastasis	[23]
	Non-small-cell lung cancer	UBE2F	UBE2F↑/activate CRL5/Noxa↓	inhibiting apoptosis	[15]

-: no data; ↑: upregulation; ↓: downregulation; miR: microRNA; ANKRD9: ankyrin repeat domain-containing protein 9; circ: circRNA; SOCS: suppressor of cytokine signaling; UBE2F: ubiquitin conjugating enzyme E2 F.

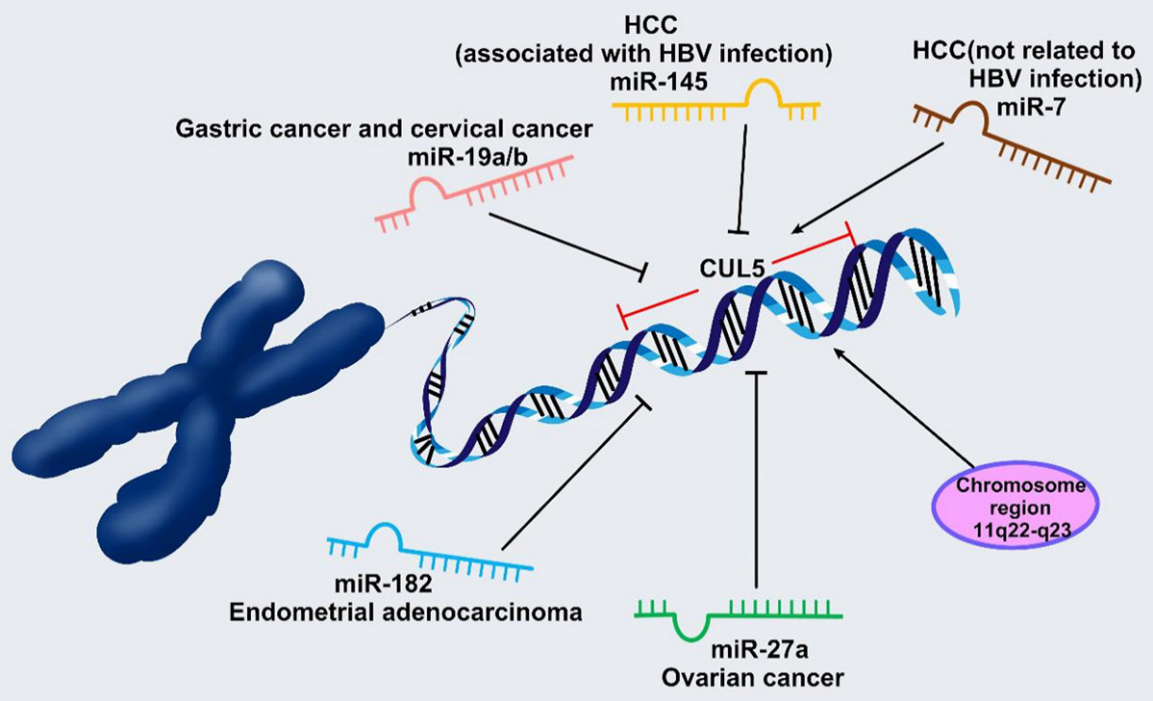

Figure 3. The expression of the CUL5 gene regulated by miRNAs. MiRNAs are involved in various types of cancers and malignancies. MiR-19a/b inhibits the expression of the CUL5 gene and is associated with gastric cancer and cervical cancer. Simultaneously, miR-145, miR-182, and miR-27a also inhibit the CUL5 gene and are related to HCC (associated with HBV infection), endometrial adenocarcinoma, and ovarian cancer, respectively. Conversely, the expression of the CUL5 gene can be promoted by miR-7, which is related to HCC development (not associated with HBV infection). Moreover, the chromosome region 11q22-q23 also promotes the expression of the CUL5 gene.

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Disclosure of conflict of interest

None.

Abbreviations

CUL5, cullin-5; CUL4, cullin-4; CRLs, cullin-RING ubiquitin ligase; NTD, N-terminal domain; CTD, C-terminal domain; SBP, substrate-binding protein; RBX1/2, RING finger protein; SOCS, suppressor of cytokine signaling; CRL5, cullin-5-RING ubiquitin ligase; WSB, WD repeat and SOCS box; SSB, SPRY repeat and SOCS box; ASB, ankyrin repeat and SOCS box; NEDD8, neural precursor cell expressed developmentally downregulated 8; CSN, COP9 signalosome; MAPK, mitogen-activated protein kinase; FAs, focal adhesions; Cas, Crk-associated substrate; pYCas, phosphorylated Cas; Reln, Reelin; Dab-1, Disabled-1; Pol II, RNA polymerase II; Rpb1, RNA polymerase II's largest subunit; CSB, Cockayne syndrome B; AC, adenylate cyclase; NLS, nuclear localization signal; MT, microtubule; DDA3, differential display and activation by P53, also known as PSRC1; SAG, sensitive to apoptosis gene, also known as Rbx2; β-TrCP, β transducin repeat-containing protein; AQP, aquaporin; mTORC, mammalian target of rapamycin; DEPTOR, DEP domain-containing mTOR-interacting protein; miRNA, microRNA; IMPDH, inosine monophosphate dehydrogenase; ANKRD9, ankyrin repeat domain-containing protein 9; CRC, colorectal cancer; Erp, endoplasmic reticulum protein; ERS, ER stress; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HBX, hepatitis B virus X protein; B-CLL, B cell chronic lymphocytic leukemia; APL, acute promyelocytic leukemia; ATRA, all-trans retinoic acid; EGR-1, early growth response element 1; Fas-L, Fas ligand; HDMEC, human dermal microvascular endothelial cell; circ, circRNA; ccRCC, clear cell renal cell carcinoma; SCLC, small-cell lung cancer; Fak, focal adhesion kinase; UBE2F, ubiquitin conjugating enzyme E2 F; HSP90, heat shock protein 90;

CRAF, cytosolic Raf-1; 17-AAG, 17-N-allylamino-17-demethoxygeldanamycin; AuNPs, gold nanoparticles; UHRF1, ubiquitin-like with PHD and ring finger domains 1.

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