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Mini-Review

A Comprehensive Review of Biological Roles and Interactions of Cullin-5 Protein

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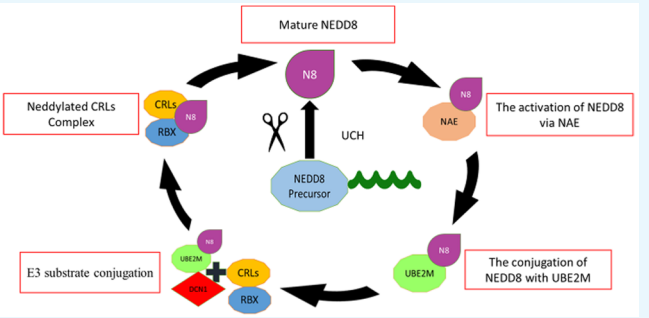
ABSTRACT: Ubiquitination is a modification of proteins that has a powerful impact on protein function along with other cellular functions. This reaction is regulated through major enzymes, including E3 ligase as a chief enzyme. The Cullin-5 ubiquitin ligase (Cul5) possesses a variety of substrates that maintain the process of ubiquitination as well as proteasomal degradation. It regulates cell development, proliferation, and other physiological tasks in the human body. Moreover, it has been discovered that the expression of Cul5 plays a significant role in specific cancer cells while affecting the progression of tumor cells. This review is based on current knowledge about Cul5 and its expression, signaling pathways, regulation, virus-related responses, and inhibitors for therapeutic strategies.



1. INTRODUCTION

The process of protein ubiquitylation comes in the category of post-translational modification, which is a reversible process.[1] Based on some recent studies, it has been concluded that this process uses both proteolytic and nonproteolytic tasks to maintain the various functions of the body. The defects in these processes can also cause many diseases.[2] The Cullin-RING E3 ubiquitin ligases (CRLs) are proteins that belong to the most prominent family of E3 ligases, which handle the ubiquitylation of proteins within the cells and control several cellular functions.[3] In mammals, CRLs comprise various subfamilies with specific Cullin proteins (Cul), including Cul1, Cul2, Cul3, Cul4a, Cul4b, Cul5, Cul7, and Cul9. These proteins act as scaffold proteins and bind with a small RING finger protein (RBX1 or RBX2).[4] Each member of the CRL family has different specific functions aside from ubiquitination actions within the cells (Figure 1). CRLs require Cul neddylation, making it possible to adapt the CRLs for simple access to the substrate. As several critical molecules that regulate a range of cell activities are the substrates of CRLs, CRLs, and the activation of neddylation consequently play an essential role in many biological mechanisms.[5] Generally, the CRL families subordinate with Cul via some definite transposable substrate receptor molecules.[6] Each receptor molecule can target multiple protein molecules for the ubiquitination reaction. The CRL subfamilies are controlled via an active covalent alteration through a ubiquitin-like protein called neural precursor cell expressed developmentally down-regulated 8 (Nedd8), which produces many conformational variations in the structures of CRLs and leads to their activation by enabling them to bind with Skp1F box molecules.[1] In the absence of Nedd8 modification, the Cul-associated and neddylation-dissociated 1 (CAND1) molecule, which is a CRL inhibitor, binds with Cul1-Rbx1, causing the formation of an inactive complex that lacks the Skp1F box structure.[7] Numerous studies have been done on the effects of CRLs in drug discovery, oncology, and virological affects. However, the data about Cul5 and its biological interaction are still lacking in science. Cul5 is a protein that was initially regarded as a vasopressin-activated calcium mobilizing (VACM-1) molecul due to having an arginine vasopressin (AVP) receptor gene in its structure. AVP mainly controls osmoregulation and regulates blood pressure within the body.[6] The current manuscript focuses on a piece of updated information related to the Cul5 substrate's involvement in various diseases, including cancers and apoptosis, in addition to its involvement in regulating several biological processes. The research focused on the roles of CRLs and the identification of their suitable substrates in addition to

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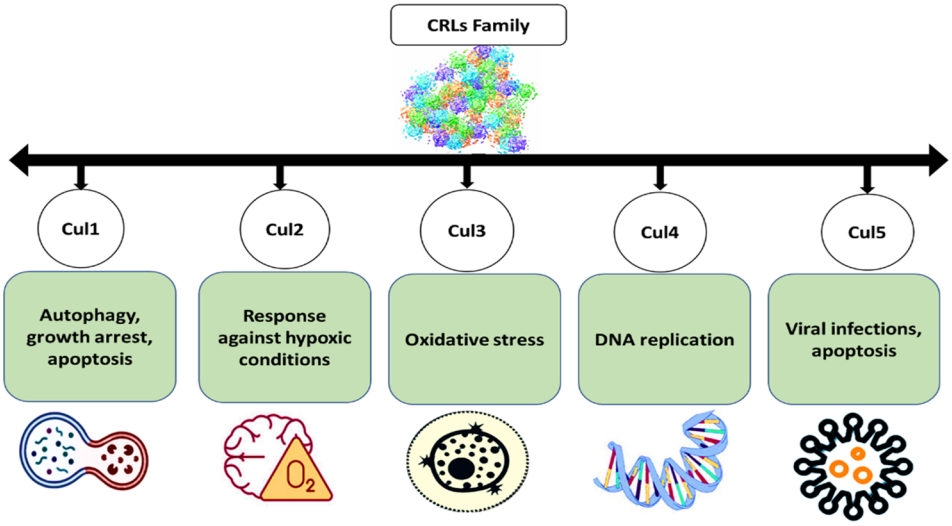

Figure 1. Diagrammatic illustration of a few members of the CRL family and their specific functions within the cell, including the regulation of apoptosis, autophagy, DNA replication, the response against hypoxia, and viral infections. Each member has specific functions and is located in different parts of cells to maintain homeostasis by protein−protein interactions.

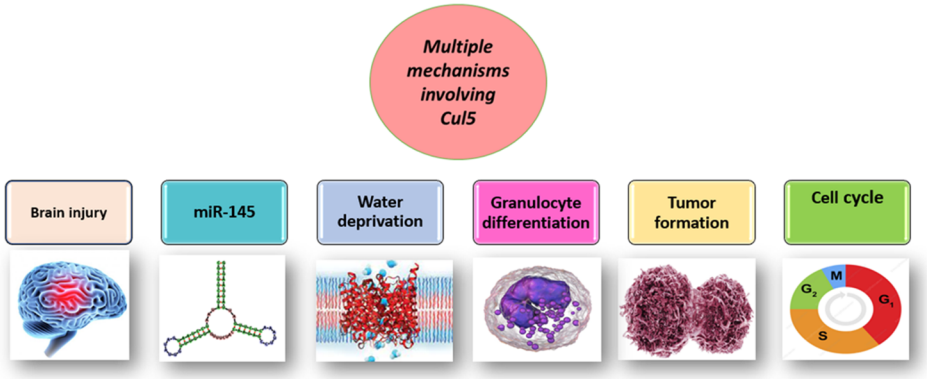

Figure 2. Diagrammatic illustration of various functions of Cul5, including its involvement in brain injury, miR-145, aqua protein regulation, granulocyte differentiation, tumor formation, and cell cycle progression.

pathways related to their reactions and proper knowledge on their regulation and expression, which will undoubtfully contribute to additional new drug targets in the future.

2. EXPRESSION OF CUL5 IN VARIOUS CELLULAR ACTIVITIES

The Cul5 protein is expressed in various body cells, including brain, kidney, and vascular endothelial cells. The expression of Cul5 is similarly subject to cell cycle control, being low or undetectable in the S phase of the cell cycle.[4] It localizes to the cytosol and the cell membrane after cytokinesis during the M phase of mitosis, showing that it may be involved in regulating cell division and the cell cycle.[8] Some previous experiments suggest that the murine Cul5 mRNA is usually expressed within the brain and in response to 48 h of water deprivation. Its ratio is elevated in regions of the body, including the kidneys, the cerebral cortex, and the hypothalamus.[4] It has also been proven that Cul5 overexpression within COS-1 cells leads to the downregulation of aquaporin-1 (AQP1); moreover, the level of Cul5 was elevated in murine skeletal muscles, heart ventricles, and mesenteric arteries in response to 24 h of water deprivation.[9] Recent research has revealed that the level Cul5 mRNA was decreased in the rat hypothalamus and increased within the cerebellum and the brainstem region because of hemorrhagic shock. In response to the induction of granulocytic differentiation by all-trans retinoic acid, both the expression of Cul5 mRNA and protein levels elevate considerably, suggesting that Cul5 may be involved in promoting granulocytic differentiation.

Moreover, some studies suggest the Cul5 is scaffold protein with an ideal dispersal of conformational situations and the neddylation process alters its conformation and stimulates it.[4] The expression of Cul5 is under-regulated in almost 82% of breast carcinoma compared to that in normal cells, whereas the overexpression of Cul5 in the T47D cells of breast cancer decreases the phosphorylation of mitogen-activated protein kinase (MAPK) and suppress cell growth. Besides this, it also leads to the downregulation of early growth response-1 (ERG-1) expression.[2] Various factors and pathways maintain the expression of Cul5. For example, resveratrol increases Cul5 expression and inhibits the growth of T47D cells, suggesting that Cul5 arbitrates its antiproliferative effect. Additionally, Cul5 suppresses maspin, a cancer suppressor agent that is essential for early embryonic growth; however, its functions have not been cleared yet.[9] These investigational reports suggest that Cul5 has a significant role in both the growth of endothelial cells and angiogenesis through the regulation of

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MAPK phosphorylation, the localization of the EGR-1, and the regulation of maspin expression.[4]

3. THE CUL5-POSSESSING UBIQUITIN FAMILY

The inhibitor of cytokine signaling proteins (SOCS), including various families like SOCS1, SOCS2, SOCS3, SOCS4, SOCS5, SOCS6, and SOCS7, interacts with Cul5 via the Cul5 box. Cul5 also interrelates with Rbx2, permitting the proteins present in the SOCS box to form a complex between Cul5 and Rbx2 (Figure 2). SOCS1 comprises a moderately conserved Cul5 box, and no interaction between SOCS1 and Cul5 has been detected.[10] The proto-oncogene (Src) belongs to the tyrosine kinase enzyme family and maintains various signaling cycles to regulate cell migration, proliferation, differentiation, and survival. Cul5 suppresses the Src gene, and when Cul5 is knockdown, the phosphorylation of tyrosine is enhanced. This suggests that it can induce some morphological modifications and affect the cell-growth mechanism.[2] Cul5 and Src both arouse the deprivation of the Src substrate molecule called p130-Cas, a Crk-associated substrate (Cas). Furthermore, the phosphorylation of Cas excites the interaction between Cas and SOCS6 and promotes the degradation of Cas.[11] Cas contributes the knockdown of Cul5 by maintaining various processes, including suppressing Src−Cas-stimulated disruption via SOCS6.[5]

4. SOME COMMON SUBSTRATES OF CUL5

The substrate receptors in the CRL5 complex are members of a superfamily of SOCS proteins that have a SOCS box containing around 40 amino acids at the C-terminus, which is found in approximately 40 other proteins.[9] There are four primary members of the SOCS box protein family found in mammalian cells, each of which is distinguished by the domain linked with the SOCS box: (1) SOCS boxes with the SH2 (Src homology 2) domain that have 8 members; (2) ankyrin domains that have 18 members; (3) the SPRY (SplA/ryanodine receptor) domain that has 4 members; and (4) the WD40 domains that have two members, WSB1 and WSB2, for SOCS boxes containing the WD-repeat (WSB) with four members.[12] In addition, there are several more SOCS box proteins, including RAB40A/B/C, MUF1, and elongin A, which are all critical in the aging process.[5] Consequently, Cul5 has several substrate receptors and theoretically generates at minimum of 37 Cul5-based substrates for the targeted ubiquitination and destruction of a wide range of substrates,[4] some of which are explored in detail in the following section and represented in Table.1.

Table 1. List of Cul5 Substrates and Their Receptors Involved in Various Functions of the Cell

substrate family	abbreviation	receptors involved	references
Kaposi's sarcoma-associated herpesvirus LANA	KSHV LANA	pVHL and BUB1	10
		p53	9
heat shock protein 90	HSP90	ERBB2	16
		HIF1α	10
		AKT and CDK4	1
ankyrin repeat and SOCS box containing 2	ASB2	AK3	9
		filamin and SMAD9	17
		A/B	10
ankyrin repeat and SOCS box containing 3	ASB3	TNF-R2	18
ankyrin repeat and SOCS box containing 4	ASB4	IRS4	19
		ID2	10
ankyrin repeat and SOCS box containing 6	ASB6	APS	2
ankyrin repeat and SOCS box containing 7	ASB7	DDA3	1
ankyrin repeat and SOCS-box containing 8	ASB8	IKKβ	19
		NSP1α	10
suppressor of cytokine signaling 1	SOCS1	GMRβC and VAV	10
		JAK2, TRAF6, and TEL-JAK2	9
		FAK, IRAK, and IRF7	20
		CDH1	20
suppressor of cytokine signaling 2	SOCS2	GHR and SOCS3	10
		PYK2	9
		STK38	15
		FLT3	20
suppressor of cytokine signaling 3	SOCS3	IDO	10
		JAK1, TRAF6, integrin-β1, and CD33	3
		p65	1
suppressor of cytokine signaling 4	SOCS4	EGFR	9
suppressor of cytokine signaling 5	SOCS5	EGFR	9
suppressor of cytokine signaling 6	SOCS6	p130Cas, p56lck	1
		SIN1	9
		C-KIT	10
SOCS box containing WD-40 protein	WSB1	ATM, D2, HIPK2, and LRRK2	3
		pVHL	10

4.1. Heat Shock Protein 90 (HSP90) as a Substrate of Cul5. The heat shock protein 90 (HSP90) facilitates the activation and stabilization of almost 350 client proteins.[13] Numerous oncogenic kinases are HSP90 clients that participate in a wide variety of normal cellular functions and are hyperactivated, amplified, or overexpressed in cancers. An ATP-driven chaperone cycle controlled by various cochaperones is required for the HSP90-mediated activation and stability of customer proteins. This is accomplished via an ATP-driven chaperone cycle.[1] The deprivation of HSP90 mediated by Cul5 is not based on the function of either the elongin B or C as indicated by directing elongin C, which can fix Cul5 but not the SOCS box inside substrate receptors that affect ErbB2 denaturation.[4] According to a study, the Cul5 and the HSP90 chaperone complex were recently linked. As a client protein of HSP90, HSP90 helps fold other proteins into their final functional state. According to the available research, several malignancies have been shown to express HSP90 abnormally. They also showed that Cul5 interacts with both the HSP90 chaperone complex and ErbB2, an HSP90 client.[2] After Cul5 was recruited to ErbB2's plasma membrane location, polyubiquitination and proteasomal degradation were induced. In addition, they found that ErbB2 Cul5 degradation occurs without regard to elongin B or C function.[14]

4.2. TRIAD1 as a Substrate of Cul5. The two RING finger proteins and double RING finger-linked 1 (TRIAD1) possess a domain named RING-between-RING (RBR), which noticeably prohibits the formation of myeloid colony cells. Despite the RBR ligase's biological importance, its activity is still poorly known. Some previous research shows that the mice deficient in TRIAD1 died because of the extreme

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Table 2. Roles of Cul5 Substrates in Various Cellular Signaling Mechanisms

substrate of Cul5	functions of the substrate	receptor	references
TRAF6	TRAF6 regulates inflammatory responses in myeloid immune cells, which triggers adaptive immunological responses and maintains the homeostasis of microenvironments. This is essential since it helps keep the body's immune system functioning correctly.	SOCS	3
	liposaccharide signaling regulation		10
GHR	growth hormone signaling regulation	SOCS	19
TRII	hyperactivity and migration of tumor cells via SOCS silencing	SOCS	4
iNOS	production of nitric oxide	SOCS	2
CDH1	blocks mitosis in melanoma cells	SOCS	1
pVHL	promotes tumor metastasis	WSB1	9
p53	facilitates viral propagation	BZLF1	1
IRS4	decreases insulin signaling	ASB4	12
SNAIL	tumor metastasis enhancement and negative regulation of the epithelial−mesenchymal transition (EMT)	SPSB3	1

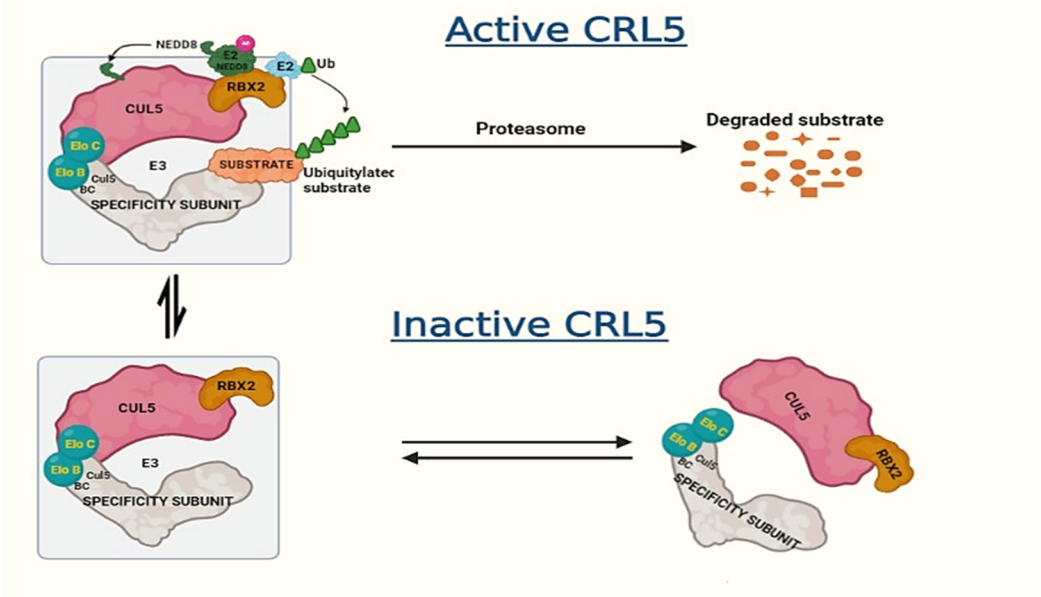

Figure 3. A schematic diagram of Cul5 ubiquitin ligase function and the interaction with proteins, illustrating the Cul5 complex that includes the ubiquitin, Rbx2, elongin B/C complexes, and SOCS box proteins. Ubiquitin (Ub) is initially activated in an ATP-dependent event that creates a thioester intermediate, which includes the carboxy-terminal glycine region of ubiquitin and the active site cysteine sequence on the ubiquitin-activating enzyme E1. Afterward, ubiquitin is transported to the cysteine active site of ubiquitin-conjugating enzyme E2. When using RING-finger E3s, ubiquitin is directly transported from E2 to the target molecule. Afterward, the ubiquitin chain substrate is recognized and degraded via the proteasome. Cul5 may assemble with the RING subunit RBX2 in the CRL5 family. RBX2 is necessary for the direct binding of NEDD8 to either lysine residue of Cul5. The CRL5 is activated once Cul5 is post-translationally changed by NEDD8 (neddylation). Meanwhile, the deneddylation through the COP9 signalosome (CSN) removes NEDD8 from CUL5 and thus inactivates it.

immune response of multiple organs.[2] Moreover, it has also been proved that when TRIAD1 binds with neddylated Cul5 and Rbx2 it enhances ubiquitin ligase activity. To gain insights into TRIAD1 functionalities, scientists performed an anti-GFP immunoprecipitated mass spectrometry analysis using cell lysates with stable GFP or GFP-tagged TRIAD1 expression. In a comparison investigation, these immunoprecipitated compounds were identified as TRIAD1 interaction partners, namely Cul5 and UBCH7. Immunoblotting has shown the development of the endogenous TRIAD1−Cul5 complex.[3]

4.3. DEPTOR as a Substrate of Cul5. A number of proteins associated with mTOR and involved in the creation of two complexes known as mTORC1 and mTORC2 have been discovered in previous years. It was discovered that DEPTOR, an exciting and modulating mTOR interaction partner, was one of these proteins, possessing an mTOR-interacting domain.[15] Several biochemical processes, including cell proliferation, apoptosis, autophagy, and the ER stress response, are influenced by DEPTOR. As a result, it seems to serve an essential function in regulating cellular homeostasis. Surprisingly, DEPTOR causes autophagy by blocking mTOR activity, which is known to be a negative regulator of autophagy.[10] Generally, it accumulates because of starvation and leads to the initiation of the autophagy response. Based on some investigations, it has been suggested that Cul5 targets t DEPTOR to facilitate proteasomal destruction under nutrient-rich circumstances, and the knockdown of Cul5 leads to the initiation of an autophagy response.[16] Besides that, under normal development circumstances DEPTOR is ubiquitinated through Cul5 and degraded, resulting in the induction of mTOR and the suppression of autophagy. As a result, when the autophagy pathway is activated, AMBRA1 (autophagy and beclin 1 regulator 1) helps stabilize DEPTOR by reducing Cul5 activity, reinforcing the suppression of mTOR activity as

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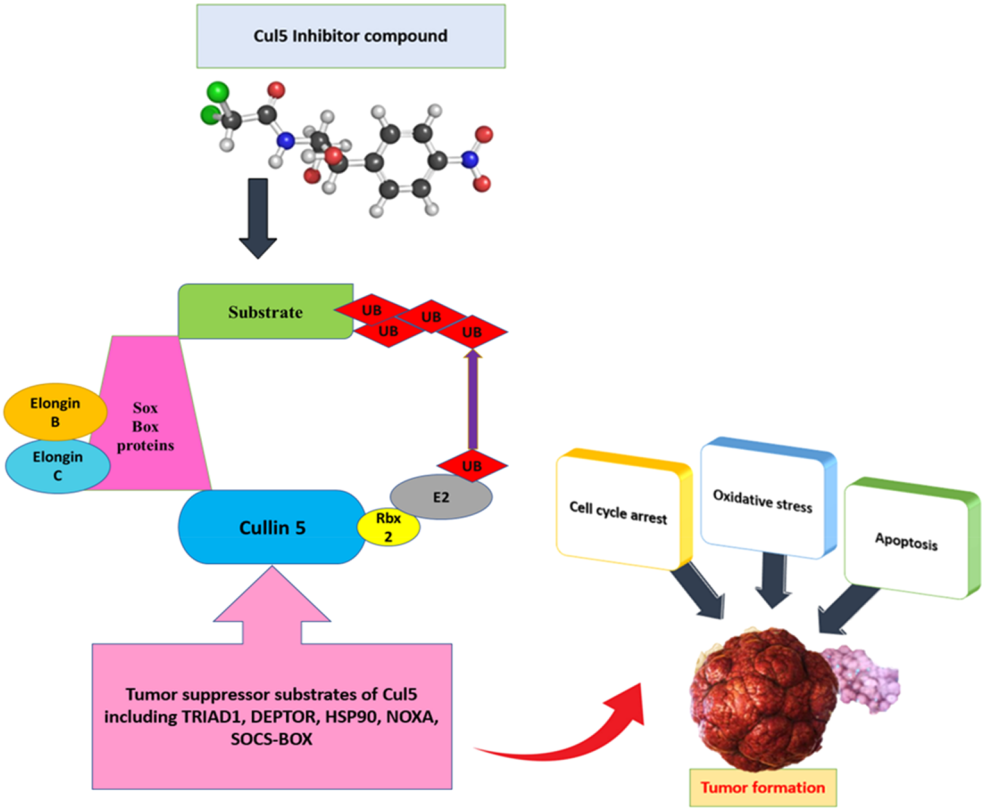

Figure 4. Schematic representation of targeting Cul5 for anticancer drug discovery. Cul5 has many specific substrates like DEPTOR, TRIAD1, NOXA, SOCS, and HSP90. Therefore, novel inhibitors can be discovered by targeting specific pathways involving these substrates and their linkage to tumorigenesis.

a result.[10] Specifically, it was revealed that elongin B creates contacts with Cul5 but not with Cul2, which results in the ubiquitination and breakdown of DEPTOR and, as a result, has an unfavorable effect on autophagy while still inversely regulating autophagy. According to these findings, studies have shown that RBX2 may function in conjunction with either Cul1 or Cul5 to enhance the ubiquitination and degradation of DEPTOR, which in turn stimulates tumor cell proliferation, survival, and migration both *in vitro* and *in vivo* using prostate and lung tumor types.[15]

4.4. NOXA as a Substrate of Cul5. NOXA is a BH3-only protein member and plays an essential role in the cell response to the anticancer drug.[3] The activation of mitochondrial and intrinsic pathways by NOXA is critical for the survival of cancer cells. Xu and his team have shown that the neddylation and ubiquitination complexes of UBE2F−Cul5−RBX2 and UBE2M−Parkin/DJ-1 enzymes regulate apoptosis from NOXA turnover.[6] First, UBE2F, one of two neddylators of the ubiquitin-conjugated enzyme (E2), collaborates with RBX2 serving as the E3 neddylator, which initiates Cul5 neddylation around the triplet of a lysine residue (Lys724).[1] Cul5 activity depends on the presence of this enzyme. In the second step, neddylated Cul5 interacts with RBX2, which is now acting as an E3 ubiquitin ligase, and an unidentified substrate receptor to connect an E3 ubiquitin ligase, which cooperates in the ubiquitination of E2/UbcH10 and UBE2S to encourage NOXA polyubiquitination through the K11 linkages for proteasomal degradation, thereby protecting cells from apoptosis.[15] UBE2F degradation interferes with Cul5 neddylation, resulting in CRL deactivation and the consequent NOXA accumulation, which is necessary for apoptosis induction in the cell. Cul5 operates as an oncogenic gene in this context because it targets NOXA for destruction.[9]

5. CULLIN-5 TARGETING FOR THERAPEUTIC PURPOSES

As the CRLs determine the substrate's specificity, they are regarded as a novel class of effective drug targets for therapeutic uses.[3] Using the degradation of various substrate molecules, CRLs control many processes, including cell cycle progression and its dysregulation, leading to various types of malignancies (Table.2). Most scientists are nowadays focused on developing anticancer targets via the use of CRLs. Some of them have now reached preclinical trials.[9] Cul5 is involved in various pathways, including apoptosis, cell cycle events, cellular proliferation, DNA damage response, and the progression of tumor cell development. Therefore, the effective modulation of Cul5 activity for therapeutic uses will be benefitted by understanding the structural organization of this protein and its mechanisms of binding with substrates,[16] such as that shown in Figure 3. Indeed, the crystal-structured organization of Cul5 has been solved now. Likewise, the small-molecule inhibitors of CRLs primarily act by disrupting the compatibility of the substrate and its subunits.[1] As the CRLs have many specific substrates, the proper identification of suitable substrates whose degradation affects the biological performance of CRLs is vital for targeting strategies. For this reason, it is essential to determine whether the E3 performs its function of leading to the polyubiquitination reaction and the degradation of substrates in normal conditions of the cell. Understanding the biological functions of CRL, identifying particular substrates and recruitment mechanisms, and having

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knowledge CRL assembly and control will lead to the discovery of novel therapeutic targets (Figure 4).[3]

5.1. Antiproliferative Effects of Cul5. Various cell lines and in vitro studies have shown that Cul5 has antiproliferative effects. When Cul5 is overexpressed, it has been been shown to decrease the efficiency of adenylate cyclase (AC) and the synthesis of cyclic AMP (cAMP). Cul5 contributes to the breakdown process of many proteins associated with cell proliferation, and as a result Cul5 inhibits cell proliferation. This is accomplished through the pathway of MAPK phosphorylation.[12] According to recent findings, the complex composed of SOCS (ASB7), Cul5, and elongin BC is involved in the ubiquitination and degradation of DDA3, decreasing the mitotic drive and boosting the antiproliferative effects. Cul5 is thought to also be involved in the control of endothelial permeability.[15] While human endothelial cells are growing, thalidomide, a medication that limits cell proliferation by limiting angiogenesis, has been shown to decrease the quantity of Cul5 present in the nuclei of the cells. Using anti-Cul siRNA, we could reduce the antiproliferative effects of thalidomide in both human endothelial cells and rat endothelial cells that had been genetically modified to lack Cul5.[21]

5.2. DNA Damage Response and Cul5. Studies have revealed that Cul5 limits the activity of the transcription factor Src. Src is a powerful tumor-inducing protein involved in cell cycle control and DNA damage repair.[12] Moreover, the Cul5 can form a complex with elongin, which modulates RNA polymerases and contributes to DNA damage.[7] Some transcription factors, including elongin and cockayne syndrome B (CSB) protein, influence the activity of RNA polymerase II (Pol II) during the synthesis of mRNA (mRNA) in eukaryotes. Elongin is comprised of three subunits: A, B, and C. Elongin is a heterotrimeric protein. Elongin A is the most transcriptionally active of these subunits, while the elongin BC complex, composed of elongin B and C, modulates the activity of elongin A. Elongin A is a transcriptionally active subunit.[9] In mammals, elongin A connects the Cul5 and RING finger proteins through the elongin BC complex, resulting in a multisubunit complex with other proteins. Some researchers revealed that DNA damage because of ultraviolet radiation increases the colocalization of Cul5 and elongin A in the nucleus and leads to the ubiquitination and degeneration of Pol II's largest subunit (Rpb1) by causing DNA damage.[2] Furthermore, in cells the CSB protein promotes the assembly of the ubiquitin−ligase complex comprising elongin A and Cul5 to DNA repair sites, resulting in the stalling of Pol II. When DNA damage occurs, Pol II is phosphorylated, ubiquitinated, and then consumed by proteasomes, which may explain why proteasomes degrade Pol II. The ubiquitin−proteasome system controls BIK, a pro-apoptotic protein that only binds to BH3. However, this control's mechanism and physiological effects are still a mystery.[2] Cul5-ASB11 was recently discovered to be the E3 ligase responsible for the ubiquitination and degradation of BIK. Genotoxic drugs reduce the activity of the IRE1−XBP 1s−ASB11 axis and stabilize BIK, which help trigger the cell's apoptotic response in response to DNA damage.[22]

5.3. Cul5 and Cell Cycle Events. MicroRNA-7 (miR-7) targets the gene of Cul5. Moreover, human hepatocellular carcinoma cells may undergo an easier G1/S transition when miR-7 is downregulated, which enhances the expression of Cul5 and aids in the G1/S transition.[1] When cell differentiation and morphogenesis were studied in *Drosophila* eggs, it was shown that a downregulation of the Cul5 gene resulted in the excessive generation of germ cells. Dynamic alterations in microtubules (MTs) take part in uniformly dispersing the chromosomes between two daughter cells during the cell cycle event.[12] Mitotic spindle dynamics are controlled by DDA3, a type of MT-related protein that works with other proteins. It has been shown that when DDA3 is knocked out, the strain between sister kinetochores at metaphase is reduced, and the percentage of delayed chromosome segregation is decreased. This suggests that DDA3 is a mitotic spindle-destabilizing protein that increases mitotic spindle dynamics by enhancing the dynamic spindle assembly. Moreover, the suppression of Cul5 also blocked the development of cell colonies and led to cell cycle arrest.[8]

5.4. Cellular Migration and Cul5. The extracellular matrix (ECM) facilitates cell movement between neighboring cells during growth. The ECM connects with intracellular focal adhesions (FAs) and actin to trigger cell migration. Cas (p130Cas) is an FA protein that is a component of FAs and can be phosphorylated via Src.[23] When the phosphorylated Cas (pYCas) gene is overexpressed, it accelerates FA breakdown, which is favorable to recycling FA at the trailing edge of the cell and hence enhances cell migration.[20] In combination with SOCS6 and elongin BC, Cul5 can create the E3 elongin ligase (also known as the E3 elongin ligase). This enzyme targets pYCas for degradation via the SH2 domain of SOCS6, stabilizes FAs, and inhibits cellular migration. The proliferation and migration of epithelial cells and the transformation of fibroblasts become more dynamic in the absence of Cul5.[1] In addition, Cul5 forms complexes with other SOCS proteins, including SOCS2, SOCS4, and SOCS5, to target specific phosphorylated proteins and modulate adhesion kinetics in various types of cell. As a result, the cell migration activities continue to function correctly. Moreover, cellular migration and differentiation in distant regions in the nervous system support the establishment of several levels of Cul5 in the mammalian neocortex, which is essential for learning and memory.[6] Undifferentiated projection neurons, for example, migrate from the ventricular area to the bottom of the cortical layer then move upward to the top of the layer and end the migratory activity. Cul5 can form a complex with elongin, which modulates RNA polymerases and contributes to DNA damage.[24]

5.5. Cul5 and Apoptosis. Many malignancies have increased levels of the anti-apoptotic proteins MCL1 and Bcl-xL. Some cancer models are inherently resistant to inhibitors targeting MCL1, although these drugs are now in clinical trials.[21] Multiple flow cytometry-based genome-wide CRISPR screens probing two medicines that actively (MCL1i) and indirectly (CDK9i) target MCL1 were conducted to identify the underlying mechanisms of resistance to MCL1 inhibition. Cells were resensitized to MCL1 inhibition by Cul5. Researchers found that the Cul5 complex controls the levels of the pro-apoptotic BH3-only proteins Bim and the Noxa genes.[12] In contrast to the MCL1 inhibitor, the accumulation of Noxa as result of the depletion of Cul5 components restored sensitivity to the CDK9 inhibitor. Hence, the discovery of Cul5's new involvement in the death of cancer cells and the resistance to many anticancer drugs offers the possibility of better therapy combinations.[1] The ubiquitination and degradation of NOXA by neddylated Cul5 is critical for preventing its overaccumulation and maintaining an adequate

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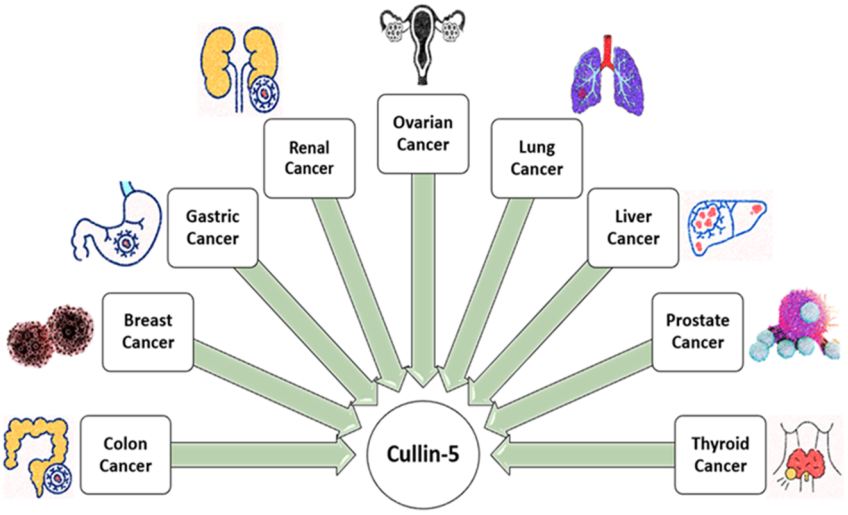

Figure 5. Representation of human cancers associated with the Cul5 protein, including breast cancer, gastric cancer, colon cancer, thyroid cancer, liver cancer, prostate cancer, lung cancer, ovarian cancer, and renal cancer.

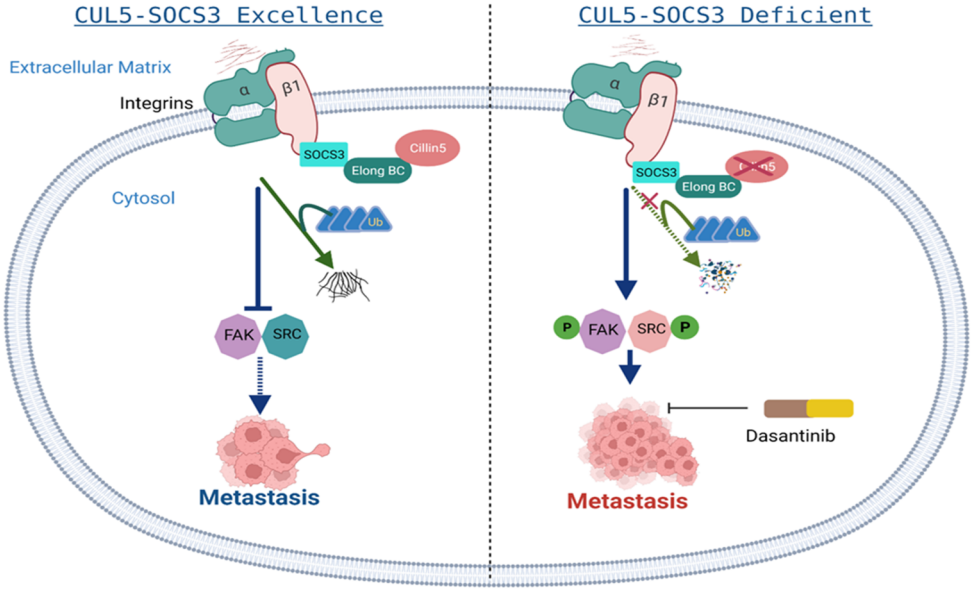

Figure 6. Importance of Cul5 and SOCS3-mediated integrin 1 turnover in regulating SCLC metastasis. The lack of Cul5 and SOCS3 prevented integrin 1 from degrading, which stabilized integrin 1 and triggered downstream FA/Src signaling that ultimately led to SCLC metastasis. Dasatinib, a FDA-approved Src inhibitor, is effective against SCLCs that lack Cul5. This shows that Cul5 and SOCS3 have therapeutic implications.

action time.[2] Researchers have shown that the peroxiredoxin PRDX1, a potential antioxidant highly expressed in CRC tissues, may prevent apoptosis and TRAF6 ubiquitin−ligase activity. In recent research, scientists discovered that PRDX1 suppresses CRC cell apoptosis via downregulating the NOXA gene.[8] The PRDX1 increases NOXA ubiquitination and degradation through Cul5 neddylation. Furthermore, the oligomerization of PRDX1 is required for Cul5 neddylation because silencing PRDX1 or blocking PRDX1 oligomerization significantly reduces Cul5 neddylation and subsequent NOXA degradation.[12] Thus, in CRC PRDX1 is essential for maintaining intracellular homeostasis by strengthening te UBE2F−Cul5-mediated degradation of NOXA, as shown by CRC cells' resistance to etoposide therapy. These results suggest that targeting PRDX1 may be a viable approach to overcoming CRC DNA damage resistance.[6]

5.6. Cul5 and Cancer. Despite advancements in stomach cancer treatment in recent years, the mortality rate remains the third-highest among cancers, and adequate markers for the early detection of gastric cancer are still lacking. Cul5 is now being studied for its overexpression in cancer cells and its involvement in carcinogenesis and cancer formation (Figure 5).[21] Cul5 inhibits cancer cell proliferation and metastasis while promoting apoptosis in a range of normal cells by ubiquitinating and degrading multiple target proteins. A recent study identified the increased expression of miR-19a in gastric cancer tissues and showed that miR-19a directly targeted and dysregulated Cul5 expression, increasing the proliferation and migration of carcinoma cells.[1] The ankyrin repeat domain 9 protein (ANKRD9) is associated with a higher risk of developing stomach cancer. Reducing the amount of the ABKRD9−elongin BC−Cul5 complex formed in human gastric cancer cells limits proliferation and tumor growth.

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Primary malignant liver tumors such as hepatocellular carcinoma (HCC) account for most cases. Hepatitis B virus (HBV) infections are the most prevalent risk factor of developing HCC, among hundreds of other variables.[25] A protein produced by the HBV can transactivate oncogenes and promote HCC. miR-145 is related to the development of HCC, while Cul5 has the miR-145 target. According to an experiment, the HBX-transfected cells exhibited a decrease in the amount of miR-145 and an increase in the level of Cul5.[21] The overexpression of HBX, on the other hand, dramatically enhances the number of cells in the G2/M stage and dramatically reduces the percentage of cells within the G0/G1 phase, both of which are important for cell survival and apoptosis prevention.[9] It is important to note that an overexpression of Cul5 does not result in inhibited cell proliferation, which may be related to the activity of HBX; however, further investigation into these theories is required. Moreover, the miR-7 and Cul5 are downregulated in HCC tissues that are not infected with HBV.[12] Given that miR-7 positively influences the production of Cul5, reduced miR-7 levels contribute to the development of the HCC malignant phenotype by lowering the levels of endogenous Cul5. Recently, a pan-cancer assessment of Cul5 showed a significant relationship between Cul5 interpretation and inflammatory cell infiltration from a clinical tumor sample perspective and the clinical prognosis or tumor mutational load, which can increase the grasp of the Cul5 molecular mechanism during tumorigenesis.[1] The metastasis of small-cell lung cancer (SCLC) is the most common cause of mortality, and more excellent knowledge of the molecular pathways driving SCLC metastasis might enhance its therapeutic therapy. According to the findings, a lack of Cul5 or SOCS3 hindered the E3 ligase complex's functional assembly and prevented integrin 1 from degrading, which stabilized integrin 1 and triggered downstream FA/Src signaling that ultimately led to SCLC metastasis.[9] Research based on 128 individuals with SCLC found that high integrin 1 levels were related to a poor prognosis and a low degree of Cul5 and SOCS3 expression. Dasatinib, an FDA-approved Src inhibitor, was particularly effective against SCLCs that lacked Cul5 (Figure 6). This study highlights the importance of Cul5 and SOCS3-mediated integrin 1 turnover in regulating SCLC metastasis, which may have therapeutic implications[4] (Figure 3).

5.7. Aquaporin Downregulation by Cul5. Cul5 regulates angiogenesis, downregulates aquaporins, and inhibits autophagy in diverse tissues. Aquaporin-1 (AQP-1) is a protein that is abundantly expressed in the vascular endothelium and is responsible for regulating water permeability.[2] In vivo, Cul5 is found in kidney collecting tubular cells and vascular endothelial cells, among other places. The expression of Cul5 cDNA in COS-1 cells in vitro results in a decrease in the thresholds of the endogenous AQP-1 mRNA and protein, indicating that Cul5 can enforce the expression of AQP-1 at both the transcriptional and post-translational stages through the glycosylation of VACM-1 and the phosphorylation of MAPK, as previously reported.[4] The amount of Cul5 mRNA inside the vascular tissue of rats denied water for 24 h dramatically increased. However, even though there was no statistically significant drop in AQP-1 levels, the concentration of AQP-1 was found to be adversely linked with the proportion of Cul5 with NEDD8-modified Cul5.[4] Taking these findings together, they suggest that the hypertonic stress of water deprivation in vivo raises the level of the Cul5 protein, which itself is produced by NEDD8 after translation and is involved in regulating the water balance. Meanwhile, the AQP-2 is a protein found in the cell membrane at the terminal portion of the collecting ducts and is responsible for regulating water permeability.[9] Hydration has been shown to influence the activity of Cul5 in vivo. Changes in the levels of the Cul5 protein were found to be localized and negatively associated with changes in the AQP-2 protein levels in kidneys isolated from dehydrated rats.[1]

5.8. Cul5 and Autophagy Responses. Autophagy is a cell survival process that destroys damaged or unneeded components in cells while also providing energy and components to synthesize new substances, thus preserving cell homeostasis.[4] AMBRA1 binding to Cul4 and Cul5 and creating a complex is a critical step in the autophagic process. For the time being, research on the functions of CRLs in regulating the autophagy machinery has concentrated mainly on the ULK1 complex and the beclin-1−class III PI3K complexes, both of which are involved in the early stages of autophagy.[1] In contrast to their functions in autophagy, CRLs have only a limited role in other aspects of autophagy. Considering that the whole mechanism of autophagy is meticulously regulated, it will be fascinating and informative to learn more about the functions of CRLs in controlling other autophagy machinery, including ATG9 and its recycling system as well as two ubiquitin-like peptide conjugation systems.[4] Cul4 and Cul5 can work as autophagic modulators, regulating both the start of and the termination of autophagy, respectively. Given the critical function for autophagy in maintaining cellular homeostasis, it should come as no surprise that the entire process is adequately supervised and regulated. Multiple kinds of post-translational changes, including phosphorylation, ubiquitination, and acetylation, have been identified in the control of autophagy. Overexpression of Cul5 has been shown to result in a substantial reduction in DEPTOR levels.[15] As a result of the autophagy stimulation, AMBRA1 dissociates from Cul4 and attaches to Cul5. This suppresses Cul5 activity and decreases the rate of DEPTOR breakdown, resulting in an accumulation of DEPTOR that subsequently promotes autophagy.[16]

6. CONCLUSION

CRLs require Cul neddylation, making it possible to adapt their access to the substrate. CRLs and the activation of neddylation consequently play an essential role in many biological mechanisms. Cul5, due to its higher specificity for substrate molecules, is regarded as a promising drug target molecule. The multifunctional Cul5 protein family participates in the formulation of E3 ligase complexes and various other cellular biological processes. The Cul5 protein family has several substrates that maintain the ubiquitination process and the destruction of proteasomes. For ubiquitin-dependent degradation, Cul5 delivers the ubiquitin protein to its target substratum protein. A functional link between Cul5 and clinical disorders, particularly HIV, is emerging, affecting muscle function and stem-cell homeostasis, autonomy, and differentiation. In several areas of the cellular response to HSP90, research has revealed the relevance of Cul5. HSP90 is a chemical chaperone for the functioning and stability of its substrate proteins. The suppression of Cul5 was also discovered to suppress the development of cell colonies and promote cell cycle arrest. Some researchers recently analyzed the genetic change and molecular gene expression features in

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33 cancers and investigated the Cul5 gene. Initially, the pan-cancer assessment of Cul5 showed a significant relationship between Cul5 interpretation and inflammatory cell infiltration from a clinical tumor-sample perspective and the clinical prognosis or tumor mutational load, which can increase the grasp of the Cul5 molecular mechanism during tumorigenesis. Moreover, understanding and researching the roles of CRLs, identifying of their suitable substrates beside pathways related to their reactions, and having proper knowledge on their regulation and expression will undoubtably contribute to additional new drug targets in the future.

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Notes

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