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The Emerging Role of the COP9 Signalosome in Cancer

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Abstract

In the last several years, multiple lines of evidence have suggested that the COP9 signalosome (CSN) plays a significant role in the regulation of multiple cancers and could be an attractive target for therapeutic intervention. First, the CSN plays a key role in the regulation of Cullin-containing ubiquitin E3 ligases that are central mediators of a variety of cellular functions essential during cancer progression. Second, several studies suggest that the individual subunits of the CSN, particularly CSN5, might regulate oncogenic and tumor suppressive functions independently of, or coordinately with, the CSN holocomplex. Thus, deregulation of CSN subunit function can have a dramatic effect on diverse cellular functions, including the maintenance of DNA fidelity, cell cycle control, DNA repair, angiogenesis, and microenvironmental homeostasis that are critical for tumor development. Additionally, clinical studies have suggested that the expression or localization of some CSN subunits correlate to disease progression or clinical outcome in a variety of tumor types. Although the study of CSN function in relation to tumor progression is in its infancy, this review will address current studies in relation to cancer initiation, progression, and potential for therapeutic intervention.

Cullin-Based E3s and Cancer

Before one can appreciate the scope in which the COP9 signalosome (CSN) can regulate tumorigenic processes, it is essential to briefly discuss the protein complexes that they regulate. Cullins are scaffold proteins that serve as assembly centers for the recognition components of a large variety of ubiquitin E3 ligases and their cognate ubiquitin E2 enzymes (Fig. 1; reviewed in refs. 1,2). Cullins can be post-translationally modified on a conserved lysine via an isopeptide bond to the small protein Nedd8 in a manner similar to ubiquitin conjugation. This “neddylation” is thought to be required for the assembly of the ubiquitin E3-substrate complex and the ubiquitin-conjugated E2.

Cullins are required for the degradation of key proto-oncogenes and tumor suppressors; therefore, a major goal for cancer biology will be to determine how this regulation becomes altered during tumorigenesis (3). There are seven Cullins expressed in humans that potentially couple to a large number of different E3 recognition components, although only a few of these complexes have been studied in any detail. Indeed, just the recognition components in the F-box, BTB, and SOCS/BC families number ~600 in humans, suggesting that a significant portion of the proteome could be regulated by the CSN. Importantly, several of these Cullin-based E3s (Cul-E3) have major significance in multiple aspects of cancer initiation and progression that include DNA replication fidelity, cell cycle control, apoptosis, immune response, adhesion, motility, and angiogenesis (3). Other factors, such as gene amplification of Cul4A in 16% of primary breast cancers and potentially in several other tumors and evidence

that 47% of primary breast cancers overexpress Cul4A, are indications of Cullin significance in oncogenesis (4,5).

Genetic evidence in lower organisms also suggests that Cullins are major mediators of processes required for oncogenesis. For instance, loss-of-function mutations of *Cullin-1* in *Caenorhabditis elegans* result in a shortened G₁ phase and hyperplasia in all tissues (1–3). Cul1 is also required for correct cell cycle exit in the worm. Cul2 positively regulates the cell cycle in *C. elegans* and is expressed primarily in proliferating cells. Disruption of Cul2 expression induces G₁ arrest of germ cells and deregulated mitotic chromosome condensation (1–3). Cul3 depletion in *C. elegans* results in abnormal microfilament and microtubule organization (1–3). Importantly, Cul4 maintains genomic stability by temporally restricting DNA replication licensing in *C. elegans* (1–3). Cul4 knockdown results in massive DNA re-replication and S-phase accumulation of the Cdt1 replication licensing factor, which is also a target of Cul1 E3s in mammals.

Multiple Cul-E3s are known to regulate the 26S-dependent destruction of both tumor suppressors and proto-oncogenes (Table 1). The regulation of Cul-E3 recognition of these proteins is predominantly dependent on post-translational modification(s) of the target protein (i.e., phosphorylation and prolyl hydroxylation) or the regulated expression of the Cul-E3 recognition component. As shown in Table 1, many key oncogenic/tumor suppressor pathways are currently known to be regulated by Cul-E3s, such as Rb/E2F, cyclin/cyclin-dependent kinase, Myc, Notch, β -catenin, nuclear factor- κ B, transforming growth factor- β , p53, Hedgehog, growth factor receptors, and pVHL. Notably, these Cul-E3 targets are central mediators of cell proliferation, apoptosis, adhesion, DNA repair, and oxygen homeostasis critical for tumor growth. Thus, there is substantial genetic evidence from model organisms and human cell lines that establish the Cullins as key regulators of cellular processes central to tumor development.

COP9 Signalosome

The CSN was discovered in *Arabidopsis* over a decade ago and has been shown to comprise eight core subunits in mammals (6–9). These subunits bear remarkable homology to the 19S lid of the 26S proteasome and the translation initiation complex eIF3 and are currently postulated to play a largely undetermined role in protein degradation (reviewed in refs. 10–12). The CSN has been of considerable interest to cancer biologists and oncologists in recent years for several reasons. Perhaps most importantly, the CSN has been found to play a central and necessary role in the degradation of multiple proteins that are known regulators of disease progression in diverse cancers (Table 2). Although most of the proteins shown in Table 2 interact with the CSN5 subunit, it is unclear whether all these proteins are degradation targets or if they play other roles in modulating undetermined CSN subunit functions [i.e., PGP9.5, migration inhibitory factor (MIF), and TRC8]. These data are further supported in knockout/mutational studies in a variety of organisms that suggest that the CSN is involved in pleiotropic functions (including cell cycle progression, radiation sensitivity, genome stability, and cell survival) that largely overlap known Cullin-regulated phenotypes (10–13). Further, several components of the CSN have also been found to be directly associated with proto-oncogenes and tumor suppressors and can regulate their function (Table 2).

The most thoroughly studied function of the CSN is as a metalloproteinase capable of cleaving the protein Nedd8 covalently bound to the Cullin family of proteins (Fig. 1; refs. 10–12). This function has been proposed to be mediated by the catalytic subunit CSN5 but requires the assembly of the entire CSN holocomplex (10–12,14–28). The deneddylation of Cullins is required for Cullin-mediated degradation of E3 substrates. Currently, it is thought that neddylation stimulates the assembly of competent E3-substrate complexes with their cognate

E2 enzymes and that deneddylation facilitates the turnover of these complexes while maintaining E3 stability, thus recycling the E3s for further rounds of ubiquitylating activity (Figs. 1 and 2; refs. 10–12,29,30).

Function of CSN Subcomplexes

One of earliest observations of the CSN was that various CSN-subunit-containing complexes migrate differentially during native size fractionation chromatography or electrophoresis (31). Commonly, there is a 550- to 450-kDa band corresponding to the CSN holocomplex, but invariably there are multiple smaller complexes containing some but not all CSN subunits. There are also several supercomplexes thought to contain assembled CSN-Cul-E3 complexes and perhaps other 26S-associated proteins. The smaller complexes are of particular interest, as recent evidence has associated these complexes with deregulated function and oncogenic transformation (32,33).

CSN5 is a common component of many of the small CSN complexes and can also exist in a monomeric form (reviewed by ref. 34). CSN5 proteolytic activity is required for the CSN-directed deneddylating of the Cullins. Its overexpression, which seems to be common in a variety of tumors (Table 3), has been noted to increase the small CSN5 complexes without affecting CSN5 association with the CSN holocomplex. Other CSN subunits also form smaller complexes as well, but the function of these subcomplexes remains virtually unstudied. Importantly, CSN5 is found in both the cytosol and the nucleus of mammalian cells, whereas the CSN holocomplex has been reported to be predominantly perinuclear/nuclear and perhaps associated with the nucleolus. One of the key studies suggesting that CSN5-containing small complexes might regulate oncogenic processes was the observation that CSN5 sequestered the cyclin-dependent kinase inhibitor p27 in the cytosol, preventing nuclear accumulation and cell cycle arrest (Fig. 3; ref. 35). CSN5 overexpression can also lead to the deregulation of a variety of oncogenic processes leading to the hypothesis that CSN5 can constrain the degradation of target proteins in cells when its expression level, or perhaps undetermined forms of regulation, permit it to form non-CSN-associated holocomplexes. How CSN5 can interact with such a large number of proteins is currently undetermined. There seems to be no common domains within these proteins, suggesting that perhaps a common post-translational modification may occur or that a common structural feature is present that is not easily deduced by comparing secondary structure. One other possibility could be a redox-induced protein modification or stimulation of the unfolded protein response, as many of the proteins that interact with CSN5 are redox-responsive proteins.

Paradoxically, both Ras and FOXO4 activation results in the down-regulation of CSN5 (36, 37). Whether these are feedback mechanisms that result in cellular attempts to regulate cell cycle or other CSN5-mediated processes is not known. Hypoxia also results in the CSN5 transcript inhibition, suggesting that CSN5 is an important modulator of environmental homeostasis (38).

Chromosome Instability, DNA Damage, and Repair

Multiple subunits in the CSN are implicated in the DNA damage sensitivity and repair (39, 40). Chromosome instability has been linked with the rapid degradation of the DNA damage-binding protein (DDB2) through the proteasome pathway. Following irradiation, DDB2 binds damaged DNA and thus facilitates nucleotide excision repair of DNA lesions (41). DDB2 degradation is regulated by Cul-E3s and is thus implicated as a target of the CSN. The CSN complex negatively regulates the ubiquitin ligase activity in DDB2 and CSA complexes, potentially compromising their stability. Importantly, this regulation by CSN was dependent on CSN5, with knockdown of CSN5 reducing its repair activity by ~50% (41). Thus,

deregulated Cullin activation by aberrant CSN function could compromise DDB2 protein levels, thus contributing to DNA damage-induced mutagenesis.

Another factor potentially affected by CSN misregulation is Cdt1 (21,42), a replication initiator protein, regulated by the CSN following irradiation damage. Cdt1 interacts with the origin recognition complex, facilitating loading of the replication helicases called minichromosome maintenance complex to chromatin. Assembly of the pre-replicative complex occurs during G₁ phase of the cell cycle. Once initiation of DNA synthesis occurs, reinitiation is prevented until the next G₁ phase. Therefore, regulation of Cdt1 is important for maintaining genomic integrity. Altered CSN function would potentially result in overexpressed Cdt1 levels and genomic instability. Further, elevated Cdt1 levels have been shown to correlate with increased DNA damage and re-replication leading to activation of ATM/ATR, Chk2 kinases, and p53. Activation of these cell cycle checkpoints would induce p21 and arrest re-replication. However, continued Cdt1 elevation would continue to promote cell cycle progression leading to further genomic instability.

Interestingly, CSN interaction with p53 has been shown to target this tumor suppressor for degradation, greatly affecting the ability of the cells to respond to DNA damage and proceed through cell cycle or signal for apoptosis. Therefore, Cullin inactivation, due to altered CSN function, would most likely compromise p53 function leading to altered cell cycle and apoptotic functions.

Cell Cycle

Several studies have implicated the CSN in the regulation of proteins critical for the regulation of cellular proliferation. In one example, p27^{KIP1} was shown by Tomoda et al. (35,43) to interact with CSN5, promoting the degradation of p27. Overexpression of CSN5 as well as other CSN components also facilitated p27 nuclear export and degradation. Cullin recognition and regulation is another potential mechanism of altering cellular proliferation by the CSN. Inhibition of Cullin regulation, through either improper activation or cycling of the Nedd8 modification, can have a dramatic effect in response to cancer cell growth and progression. A few interesting associations that could become altered via altered CSN function are the cyclin-dependent kinase inhibitors p27 (35,43), p57 (18), and p21 (14) and their transcriptional regulators (i.e., SMAD4 and SMAD7; refs. 44–47) as well as several cyclins (cyclin D1, cyclin E, and cyclin B1; refs. 17,20,24,28). Theoretically, inhibition of Cullin activity would promote accumulation of the cyclin-dependent kinase inhibitors, restoring control to cell cycle and inhibiting cell proliferation. At least for p27, this does not occur, as CSN5 overexpression mislocalizes p27 to the cytosol and facilitates its degradation (Fig. 3). This highlights a key point in studying the CSN in oncogenesis in that deregulation of the CSN particularly by subunit overexpression can have varied results depending on specific E3 ligase and substrate mechanisms on protein degradatory regulation.

Microenvironment Homeostasis and Angiogenesis

Several factors that regulate microenvironmental homeostasis associate with the CSN and are specifically bound by CSN5, becoming either degraded or stabilized as a result. Hypoxia-inducible factor-1 α (HIF-1 α), in particular, has been shown by several groups to interact directly with CSN5 (32,49). Subsequent CSN5 binding to HIF-1 α interferes with hydroxylation via the prolyl hydroxylases leading to stabilization in the cell (Fig. 3). Overexpression of this CSN component leads to enhanced stabilization, with knockdown resulting in normal regulation of HIF-1 α and subsequent degradation (32). Interestingly, endogenous CSN5 is also constitutively bound to the recognition component of the ubiquitin E3 ligase, the pVHL tumor suppressor that regulates HIF-1 α degradation. Because pVHL is postulated to control other

undetermined regulators of oxygen homeostasis independently of HIF, CSN5 deregulation could also affect these processes.

Other potential factors regulated by altered CSN function include Notch1 and Notch4 (50) that maintain cell-cell interaction while also promoting cell growth and progression and serving as critical mediators of angiogenic processes. Notch regulation is primarily controlled by TrCP association and Cull1; therefore, misregulated Notch degradation could be altered by deregulated CSN function.

Finally, CSN5 also interacts with MIF (51,52). Several studies have indicated increased MIF expression in precancerous, cancerous, and metastatic tumors with increased expression correlating with tumor aggressiveness and contributing to tumor neovascularization. Hypoxic and hypoglycemic stresses have also been shown to strongly induce MIF expression and are key indicators of initiation of angiogenesis and correlate with vascular endothelial growth factor expression. Interestingly, Kleemann et al. (51) described how the association between MIF and CSN5 allows MIF to enter the cell, where it then negatively regulates CSN5 function. Thus, in this case, MIF is a potential regulator of CSN function and not the reverse, suggesting that microenvironmental stresses could possess feedback mechanisms that potentially regulate CSN function and, by extension, facilitate adaptive growth.

Clinical Studies Relevant to CSN

Several studies that are summarized in Table 3 have correlatively associated either CSN5 overexpression or cytosolic CSN5 expression to disease progression or clinical outcome. CSN5 is located on human chromosome 8q, which is frequently amplified in a large variety of cancers. CSN5 amplification has been identified in one study in hepatocellular carcinomas, with knockdown of CSN5 resulting in impaired ability of these cells to proliferate (53). To date, no published reports on CSN subunit amplification or deregulation of expression other than those described for CSN5 have been reported.

Conclusion and Perspectives

Current genetic studies from model organisms and molecular biology of the CSN clearly support the role of this complex in many functions important for tumor initiation and progression. The exact nature of how the CSN affects such a multitude of oncogenic processes, however, is only just beginning to be understood. For example, a paradox is evident in multiple published works in which the CSN is critical for degradation, yet overexpression of certain subunits yields loss-of-function phenotypes. CSN5, in particular, seems to function as a dominant negative of CSN function either by interacting with ubiquitin E3 substrates or the ubiquitin E3 ligases independent of the CSN holocomplex. These promiscuous interactions are thought to sterically alter E3 recognition, facilitate target mislocation, or alter CSN holocomplex recognition preventing Cullin turnover. These observations are pertinent to clinical oncology, as many cancers exhibit CSN5 overexpression. Further, the misregulation of specific CSN subunits could have diverse effects on different Cul-E3s and their substrates leading to the stability of some proteins and the degradation of others. How this complex regulatory network is selected for and adapted during oncogenesis will be a challenge for future studies.

The fact that the targets of the CSN, the Cullin complexes and their E3 substrates, are frequently deregulated during tumorigenesis strongly suggests that the CSN itself might become deregulated during tumor progression. Initial studies in human cancers have largely supported this hypothesis, although much work remains to be done. Whether the CSN is a good therapeutic target remains controversial. Proponents argue that the pleiotropic functions that such inhibitors could affect in cancers are sufficient to pursue drug studies; opponents,

however, suggest that inhibition could result in further genomic instability resulting in more aggressive cancers and might have large toxic effects on normal tissues (3,54). Knockdown of CSN5 in murine xenografts does significantly affect tumor growth, however, suggesting that this therapeutic paradigm may be worth pursuing (55).

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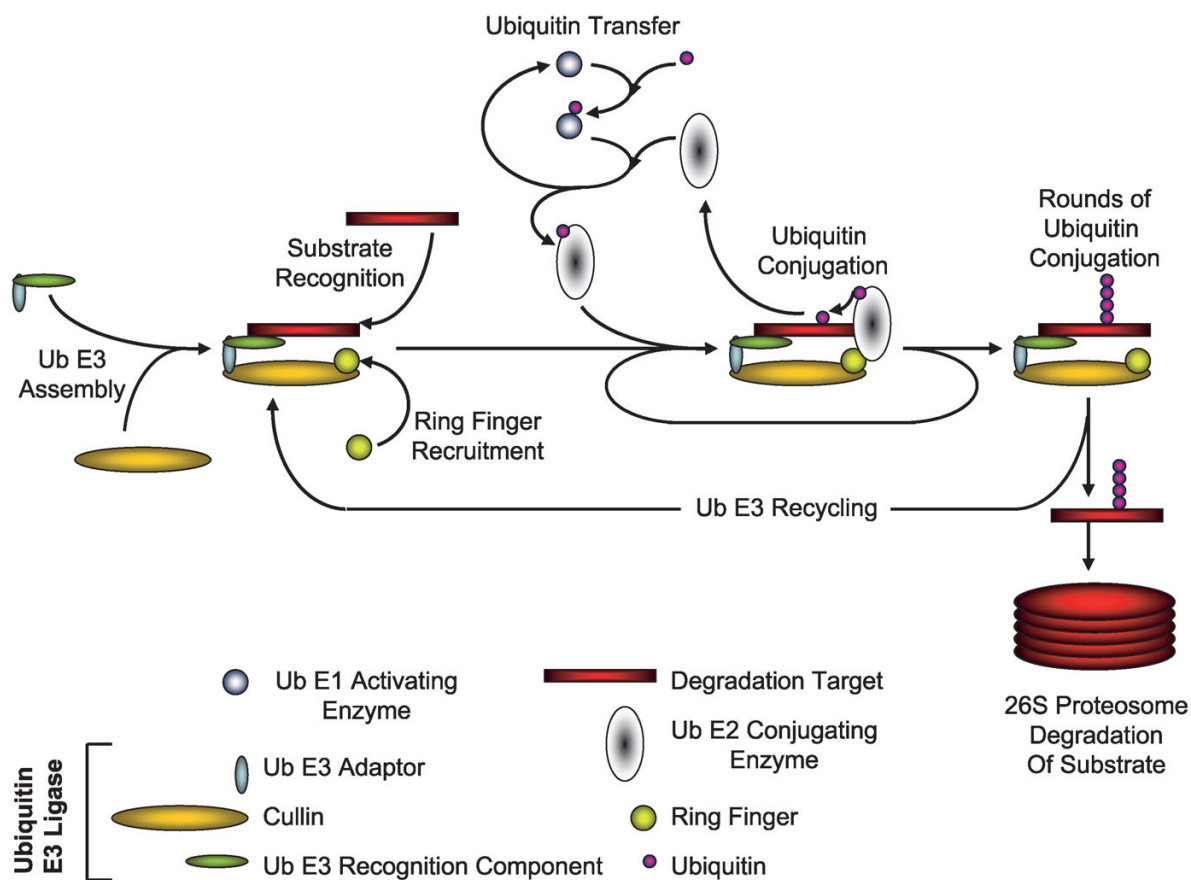


FIGURE 1.
The Cullin-containing ubiquitylation cycle.

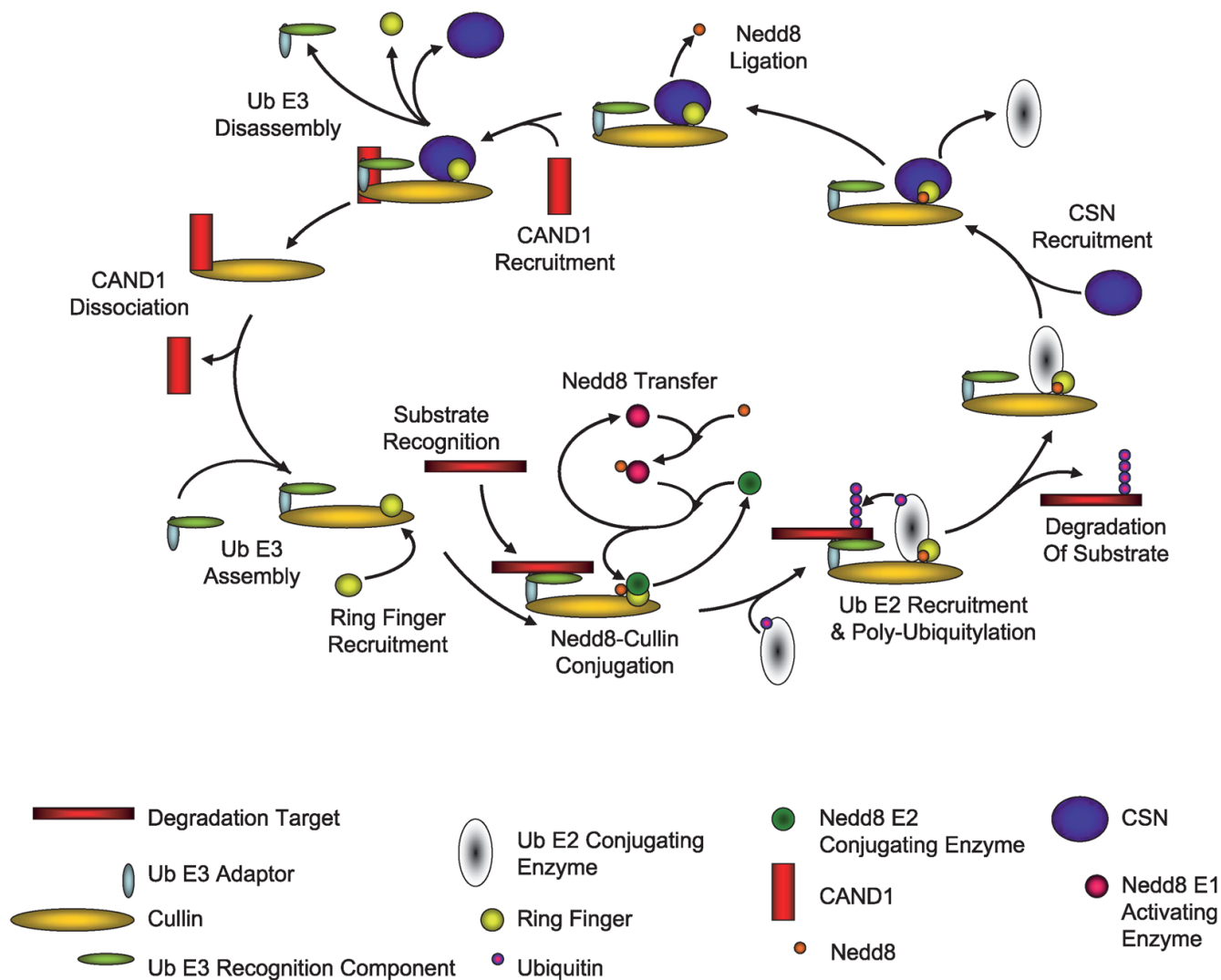


FIGURE 2.
Regulation of Cul-E3s by the CSN.

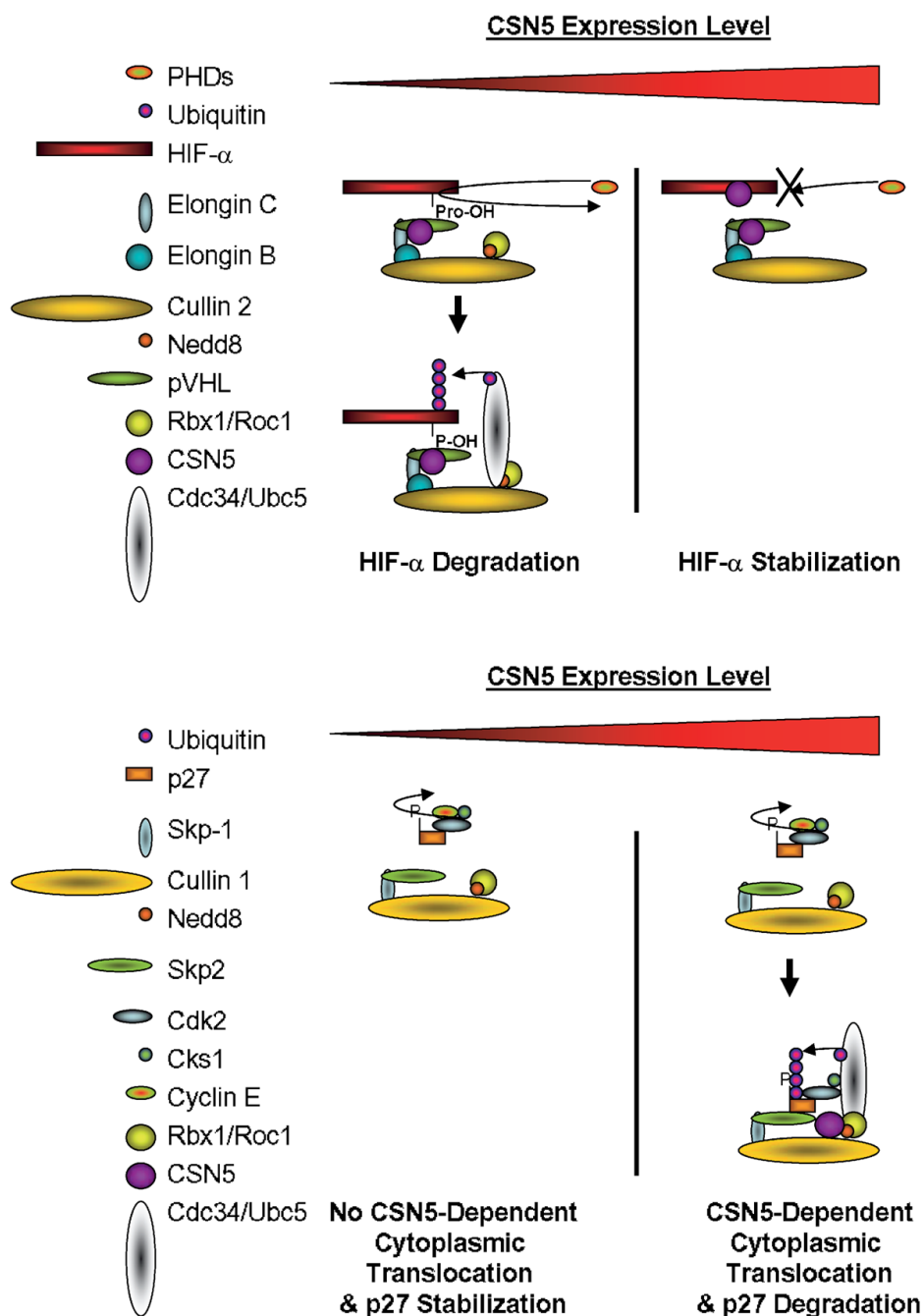


FIGURE 3.
Examples of how normal Cul-E3s can become deregulated by high CSN5 protein expression.

Table 1**Cullin-Regulated Proteins Associated with Cancer**

Cullin type	E3 recognition component (reference)	Relevant target for ubiquitylation during tumor development
Cullin 1	Skp2 (14–19,21–28)	p27 ^{KIP1} , p57 ^{KIP2} , p21 ^{WAF1} , Rb-related p130, Cdt1, c-Myc, Orc1, B-myb, E2F1, cyclin D1, cyclin E, E7
Cullin 1	Fbw7/hCdc4 (20,50,56,57,59,60)	Cyclin E, Notch1, Notch4
Cullin 1	TrCP (46,47,61–70)	IκBα, Cdc25A, SMAD4, β-catenin, Emi1, ATF4/CREB2, nuclear factor-κB/p105, nuclear factor-κB/p100, Discs large tumor suppressor, Wee1, SMAD3, SMAD4
Cullin 1	NIPA (48)	Cyclin B1
Cullin 2	pVHL (71–73)	HIF-1α, HIF-2α, Med8, Rpb1
Cullin 2	SOCS (74,75,100)	Tel-Jak2, Vav, IRS-1, IRS-2
Cullin 3	Keap1 (76)	Nrf2
Cullin 3	Cdl3Mel-26 (77)	Mei-1/katanin
Cullin 3	Unknown (79,80)	Cubitus interruptus, RhoBTB2
Cullin 3	Btb3p/Bpoz2 (78)	Unknown
Cullin 4A	DDB1 (81,82)	DDB2, CSA
Cullin 4A	Unknown (83)	Cdt1
Cullin 5	ASB2 (84)	Unknown
Cullin 5	E4orf6-E1B55k (85)	p53

Table 2**CSN5-Interacting Proteins Associated with Cancer**

CSN5-binding proteins	Stability of CSN5-binding protein		Known Cullin target?	Known to be polyubiquitin?
	CSN5 RNA1 or AS	CSN5 overexpression		
p53 (86)	Unknown	Unknown	Yes	Yes
HIF-1 α (32,49)	Degraded	Stabilized	Yes	Yes
<i>c-jun</i> (58)	Unknown	Unknown	Yes	Yes
JunD (58)	Unknown	Unknown	Yes	Yes
SMAD4 (45)	Unknown	Degraded	Yes	Yes
SMAD7 (44)	Stabilized	Degraded	Unknown	Yes
ID1 (88)	Unknown	Unknown	Unknown	Yes
ID3 (88)	Unknown	Unknown	Unknown	Yes
Estrogen receptor α (106)	Unknown	Degraded	Unknown	Yes
Progesterone receptor (90)	Unknown	Unknown	Unknown	Yes
SRC-1 (90)	Unknown	Unknown	Unknown	Yes
Bcl-3 (109)	Unknown	Unknown	Unknown	Yes
p27 (35)	Unknown	Degraded	Yes	Yes
α_v Integrin (92)	Unknown	Unknown	Unknown	Unknown
β_2 Integrin (93)	Unknown	Unknown	Unknown	Unknown
rLHR (94)	Unknown	Degraded	Unknown	Yes
Thioredoxin (95)	Unknown	Unknown	Unknown	Unknown
DNA topoisomerase II α (96)	Degraded	Stabilized	Unknown	Yes
IRE1 (97)	Unknown	Unknown	Unknown	Yes
pVHL (32)	Unknown	Unknown	Unknown	Yes
TRC8 (98)	Unknown	Unknown	Unknown	Unknown
PGP9.5 (99)	Unknown	Unknown	Unknown	Unknown
MIF (51)	Unknown	Unknown	Unknown	Unknown

Table 3**CSN5 Overexpression Correlating Tumor Progression or Clinical Outcome**

Prognostic indicator	Cancer (reference)	Increased expression associated with poor clinical outcome
CSN5	Pancreatic ductal adenocarcinoma (101)	Not evaluated
CSN5	Hepatocellular carcinoma (53)	Gene amplification (76%)
CSN5	Hepatocellular carcinoma (102)	Not evaluated
CSN5	Laryngeal squamous cell carcinoma (87)	Indicator of disease-free and overall survival
CSN5	Oral squamous cell carcinoma (103)	Indicator of lymph node metastasis and poor prognosis
CSN5	Lung adenocarcinoma (104)	Indicator of disease state but not clinical outcome
CSN5	Breast ductal carcinoma <i>in situ</i> (105)	Expression is higher in lesions with necrosis
CSN5	Node-negative breast cancer (89)	Associated with tumor size but not disease-free survival
CSN5	Invasive breast carcinoma (107)	Indicator of disease progression and relapse
CSN5	Melanoma (108)	Not evaluated
CSN5	Rhabdomyosarcoma (91)	Not evaluated
CSN5	Pituitary carcinomas (110)	Not evaluated
CSN5	Neuroblastoma (111)	Localization associated with tumor differentiation
CSN5	B-cell non-Hodgkin's lymphomas (112)	Not evaluated
CSN5	Malignant lymphoma (thyroid; ref. 113)	Predictor of tumor grade and proliferative index