

Targeting NEDD8-Activated Cullin-RING Ligases for the Treatment of Cancer

Teresa A. Soucy, Peter G. Smith, and Mark Rolfe

Abstract E3 ubiquitin ligases regulate many dynamic cellular processes important for cancer cell survival. Together with ubiquitin-activating enzyme (E1) and ubiquitin-conjugating enzymes (E2s), E3s catalyze the ubiquitination of numerous protein substrates that are subsequently targeted to the 26S proteasome for degradation. The clinical success of the proteasome inhibitor bortezomib has encouraged the evaluation of other components of the ubiquitin proteasome system for pharmaceutical intervention. Targeting specific E3s is particularly attractive because there is the potential to selectively block the degradation of certain cellular proteins and possibly avoid unwanted effects on other proteins. The cullin-RING ubiquitin E3 ligases (CRLs) represent the largest subfamily of E3s. The requirement that CRLs be activated by NEDD8 modification on the cullin protein offers an "achilles heel" for modulating this entire subfamily. NEDD8-activating enzyme (NAE) catalyzes the first step in the NEDD8 pathway and as such controls the activity of CRLs. In this article, we describe the role of the NEDD8 pathway in activating CRLs and discuss the preclinical findings with a first-in-class NAE inhibitor that is currently in phase I clinical trials for both solid tumor and hematological malignancies. In addition, we speculate where NAE inhibitors may find clinical utility.

Background

Balanced protein synthesis and degradation maintain normal healthy cellular function. Failure to maintain protein homeostasis within the cell can lead to unrestrained cellular proliferation and/or failure to undergo programmed cell death, which can lead to the development of cancer (1, 2).

In eukaryotic cells, the ubiquitin-proteasome system (UPS) is responsible for maintaining cellular homeostasis by regulating the degradation of unwanted proteins (3). These proteins may be misfolded or damaged proteins or "short-lived" proteins that have served their function in regulating cellular processes such as cell signaling, cell cycle progression, and apoptosis (4). Proteins are marked for degradation by poly-ubiquitination, the process by which the proteins are "tagged" with poly-ubiquitin chains, providing the signal for recognition and degradation via the 26S proteasome. Ubiquitination is carried out via a multistep enzymatic cascade (Fig. 1). In the first step, ubiquitin is activated by ubiquitin-activating enzyme (UAE or E1), in an ATP-dependent reaction (5). The "activat-

ed" ubiquitin is then transferred via a transthioylation reaction to an ubiquitin-conjugating enzyme (E2). The ubiquitin charged E2 then collaborates with an ubiquitin ligase (E3) to promote the conjugation of a poly-ubiquitin chain onto the specific target substrate recruited by the E3. E3s can be subdivided into three broad classes on the basis of whether they have a HECT (homologous to E6-AP carboxy terminus), RING (Really Interesting New Gene) finger, or U-box domain-containing protein at their core (6). HECT E3s form a thioester intermediate with ubiquitin, which is conjugated to the substrate directly from the ubiquitin-charged E3 (7). In contrast, RING-finger containing E3s serve as a scaffold for transfer of the ubiquitin to the target substrate directly from the ubiquitin-charged E2. There are two major subclasses within the RING-finger E3s: those in which the RING-finger and substrate binding domains are contained on the same polypeptide (e.g., MDM2) (ref. 8) and cullin-containing RING-finger E3s (CRLs), which will be discussed in more detail below (9). The U-box E3s represent a smaller family of proteins that have been shown to have ubiquitin ligase activity. The U-box domain is structurally similar to the RING-finger domain however it lacks the metal chelating residues characteristic of RING domains (10).

Owing to the complexity of the UPS and its critical role in regulating biological processes involved in tumor growth and survival, several hypotheses for therapeutic intervention in this system have been proposed (11). The first success in therapeutically targeting the UPS was clinically shown with the proteasome inhibitor bortezomib (Millennium Pharmaceuticals, Inc., Cambridge, MA). Bortezomib is a peptide boronic-acid inhibitor of the proteasome approved for the treatment of multiple myeloma and relapsed mantle cell lymphoma (12, 13). Because proteasome inhibition can interfere with the

Authors' Affiliations: Discovery, Millennium Pharmaceuticals, Inc., Cambridge, Massachusetts

Received 2/10/09; accepted 2/20/09; published OnlineFirst 6/9/09.

Note: T.A. Soucy and P.G. Smith contributed equally to this work.

Requests for reprints: Teresa A. Soucy, Millennium Pharmaceuticals, Inc. Discovery, 40 Landsdowne Street, Cambridge, MA 02139. Phone: 617-551-3719; Fax: 617-551-8906; E-mail: teresa.soucy@mpi.com.

© 2009 American Association for Cancer Research.

doi:10.1158/1078-0432.CCR-09-0343

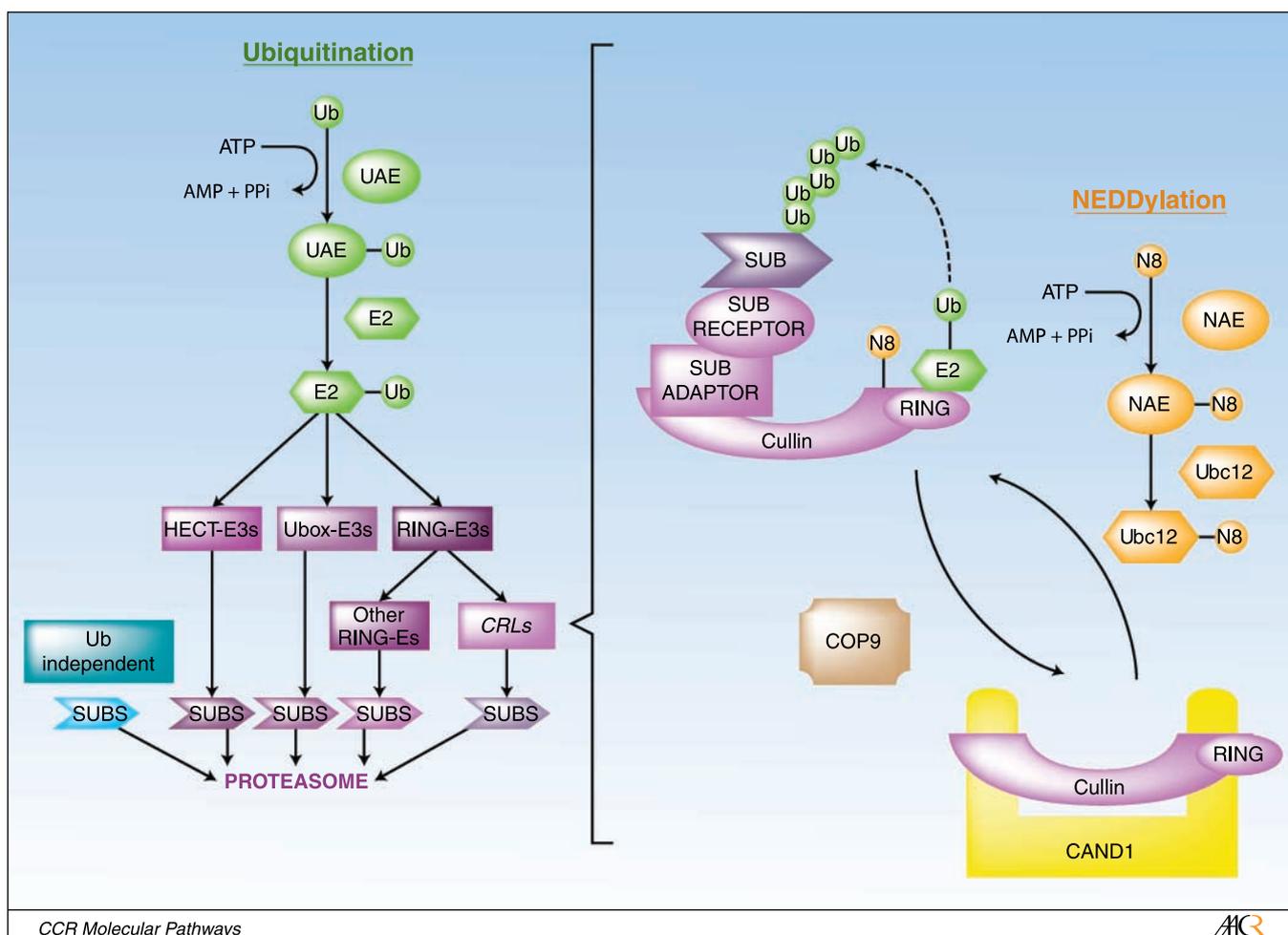


Fig. 1. NEDD8 modification is required to activate cullin-RING ubiquitin ligases (CRLs). Protein degradation by the UPS is a highly regulated process. Proteasomes degrade proteins that are tagged with poly-ubiquitin chains. The formation of the poly-ubiquitin chain on a protein is catalyzed by a pyramidal cascade of enzymes; a single E1-activating enzyme activates Ub (UAE), transfers it via a transthiolation reaction to one of dozens of E2 enzymes that in turn collaborate with specific E3 ligases to catalyze the formation of a poly-ubiquitin chain on a substrate protein recruited by that E3. E3 ligases can be subdivided into three broad subclasses; HECT, U-box, and RING-finger domain E3s with CRLs representing the largest subfamily of RING-finger E3s. A key feature of CRLs is that the cullin subunit must be modified on a conserved lysine by the ubiquitin-like protein NEDD8 to activate holoenzyme activity. NEDD8 activation and conjugation to cullin proteins is catalyzed via an enzymatic cascade that is homologous to ubiquitination involving NEDD8's E1 (NAE) and E2 (Ubc12). Removal of NEDD8 from cullin is catalyzed by the COP9 signalosome. Deneddylation facilitates dissociation of CRL components. The cullin-RING core is sequestered in an inactive state by binding to CAND1 until it is recruited to form a new CRL.

turnover of thousands of poly-ubiquitinated protein substrates, multiple mechanisms of action have been attributed to the activity of bortezomib. These include the inhibition of nuclear factor κ B (NF- κ B) signaling, disruption of cell cycle regulator proteins, stabilization of proapoptotic proteins, aggresome formation leading to cell death, activation of the unfolded protein response, and induction of endoplasmic reticulum stress (14). The pleiotropic effects of proteasome inhibition make decoupling these diverse events and attributing them to activity challenging; however, it is likely the broad interference with multiple cellular pathways accounts for the activity of proteasome inhibitors. The clinical success of bortezomib has led to the pursuit of other proteasome inhibitors and indeed Millennium has identified a second generation proteasome inhibitor, MLN9708, that differs from bortezomib in both physicochemical and pharmacokinetic properties giving the potential for a broader use in oncology treatments. Other groups are investigating the use of irreversible proteasome inhibitors (15). Thus,

based on the clinical success of bortezomib, the pursuit of additional drug targets in the UPS for the treatment of cancer is ongoing.

Ubiquitin-Like Protein Pathways

The enzymes that catalyze ubiquitin conjugation were the first to be discovered, however homologous pathways for ubiquitin-like proteins (Ubls) have been discovered in recent years (16). Ubls are proteins that are related by sequence and structure to ubiquitin and examples of some of the better studied Ubls include NEDD8 (17), SUMO (18), ATG8/12 (19), and ISG15 (20). These Ubls are conjugated to a diverse array of substrates via biochemical mechanisms that are homologous to ubiquitination, each Ubl having its specific E1, E2, and in some cases an E3. The functional consequences of the modification are distinct depending on the Ubl and substrate involved.

Cullin-RING Ligases and the NEDD8 Pathway

The CRLs represent the largest subclass of RING domain E3s (21). At their core, they contain one of the isoforms of the cullin family of proteins (CUL1, 2, 3, 4A, 4B, 5) tightly bound to the RING finger-containing protein RBX1 or 2. These elongated cullin-RING scaffolds bind charged-E2s at their C-terminus via RBX1's RING domain and can assemble with numerous adaptors and/or substrate receptor modules through their N-terminus (ref. 22) (Table 1). By recruiting the substrate and ubiquitin-conjugation machinery, CRLs serve as a scaffold to facilitate ubiquitination of the substrate.

A key feature of CRL E3s is that the cullin protein in the multi-subunit complex must be covalently modified by a single copy of the Ubl, NEDD8, ("neddylated") to activate holoenzyme ubiquitin ligase activity (Fig. 1) (refs. 17, 23, 24). Neddylation, similar to ubiquitination, is initiated by a specific E1^{NEDD8}, NAE (25). NAE first uses ATP to form a NEDD8 adenylate and then transfers NEDD8 from the adenyl group to a specific cysteine within NAE forming an "activated" NAE-NEDD8 thioester. The activated NEDD8 is then transferred to the active site cysteine of UBC12, the E2 specific for the NEDD8 pathway. Finally, NEDD8 is conjugated on a conserved lysine near the C-terminal end of the cullin protein. Recent studies suggest that SCCRO (DCN1), a protein that has been shown to interact with UBC12 and cullins, acts as a scaffold-type E3 ligase for cullin neddylation (26, 27).

Although it has been known for many years that NEDD8 modification of cullins is essential for ubiquitination of CRL substrates, the mechanism by which this modification enhances the transfer of Ub from the recruited E2-Ub to the substrate is just now being elucidated. Immunoprecipitation and nuclear magnetic resonance studies have suggested that neddylation is required for recruitment of ubiquitin-charged E2s (28, 29). More recently, structural studies have yielded additional insight by showing that NEDD8 conjugation induces a large conformational change in the C-terminal domain of CUL5, which results in the positioning of the E2 and substrate in closer proximity (30).

NEDD8 is removed from cullins by the isopeptidase activity of the metalloprotease CSN5/JAB1 subunit of the COP9 signalosome (31). Dynamic neddylation and deneddylation of CRLs represent an important mechanism by which the activation cycle of CRLs is regulated. Neddylation promotes the association of the cullin-RING core and the substrate recognition module that make up an active CRL, whereas deneddylation promotes dissociation of these components and association of the cullin-RING core with CAND1 (cullin asso-

ciated NEDD8 dissociated-1), a large protein that sterically inhibits assembly of CRL complexes (32, 33). This neddylation/deneddylation cycle facilitates the recycling of the cullin-RING core (34). In doing so, the core is made available for association with other substrate adaptor/receptor combinations facilitating the ubiquitination of many different substrates as required by the cell. The COP9 signalosome also associates with a de-ubiquitinase enzyme UBP12 that protects the CRL from self-ubiquitination (35).

Clinical-Translational Advances

Inhibiting NEDD8-activating enzyme as an anticancer strategy.

In principle, targeting enzymes in the UPS upstream of the proteasome could effect specific inhibition of the degradation of one or a few key proteins. Targeting a specific E3 would have the potential to selectively stabilize only cellular proteins that are regulated by this E3 and may in turn lead to reduced toxicity and an improved therapeutic index compared with inhibition of the proteasome. For example, *cis*-imidazoline derivatives (nicknamed "Nutlins") have been developed by Roche that specifically disrupt protein-protein interactions between HDM2 and the tumor suppressor p53 (36). HDM2, the RING-finger E3 that targets p53 for ubiquitination and subsequent degradation, is associated with poor prognosis of several human cancers when expressed at high levels (37). Nutlins have been shown to reactivate the p53 pathway in cancer cell lines leading to apoptosis (38). Moreover, they have been shown to suppress the growth of human tumor xenografts in mice without significant toxicity to normal tissues.

CRLs represent a subclass of E3 ligases that control the ubiquitination and turnover of many key substrates with important roles in cell cycle control, NF- κ B signaling, DNA replication and repair, oxidative stress response, and hypoxia signaling (Table 1). These cellular processes are relevant to tumor growth and survival providing a rationale for targeting CRLs via inhibition of NAE as an anticancer strategy.

MLN4924 (Millennium Pharmaceuticals, Inc.) was recently discovered as a first-in-class, potent, and selective inhibitor of NAE (39). In human tumor derived cell lines, MLN4924 was shown to inhibit selectively the modification of cullin proteins by NEDD8 resulting in increased levels of CRL substrates but not substrates ubiquitinated by other noncullin RING E3 ligases. Consequently, MLN4924 was shown to suppress a subset of bulk intracellular protein turnover compared with proteasome inhibition in cultured cells. MLN4924 was shown to inhibit the growth of a variety of human tumor cell lines including those derived from solid (colon, lung) and hematological

Table 1. Examples of CRL substrates with important roles in cancer

Cullin	Substrate adaptor/receptor module	Example substrate receptors	Example substrates	Reference
CUL1	SKP1/F-box	SKP2, β -TrCP, FBW7	p27, p-I κ B α , β -catenin, cyclin E, c-MYC, c-JUN, mTOR	(23, 24, 45–50)
CUL2,5	Elongin-BC/ SOCS-box	VHL	HIF1 α	(51)
CUL3	BTB domain	KEAP1	NRF2	(52)
CUL4A	DDB1/ DCAF	DDB2/CDT2	CDT1, p27	(43, 53)

(myeloma, lymphoma) malignancies suggesting that MLN4924 may have broad activity. In most of the cell lines evaluated the most prominent phenotype contributing to cell death was disruption of S-phase regulation, a somewhat surprising finding considering the numerous cell cycle regulators affected by NAE inhibition.

The potential for antitumor activity was shown in human colon and lung tumor xenograft models in immunocompromised mice. Single doses of MLN4924 resulted in dose- and time-dependent inhibition of NEDD8 conjugation to cullin proteins in addition to increased levels of CRL substrates demonstrating NAE inhibition in tumor tissue. Repeated dosing of MLN4924 affected tumor growth inhibition in these models and indeed regressions were observed in a lung tumor model at doses and schedules that were well tolerated. These preclinical findings have supported transition of MLN4924 into clinical development, and multiple phase I trials for evaluation of MLN4924 in hematological and solid tumor malignancies are currently ongoing.

Avenues for Strategic Use of an NAE Inhibitor

It is expected that most, if not all CRL substrates will be modulated by NAE inhibition; however, the genetic background of different cell types could plausibly determine the cellular consequences of such inhibition. The most frequent cellular consequence of NAE inhibition observed in cultured cells was the disruption of S-phase regulation, namely DNA re-replication, leading to DNA damage and cell death (39). One could speculate that combining MLN4924 with other S-phase active agents may lead to a significant increase in activity. It will be of interest to define the role of the DNA-damage response in determining sensitivity of cells to MLN4924 inhibition because many tumor cells have aberrant DNA-damage response pathways.

In recent years, an important role of investigational oncology has been the development of chemotherapies directed at specific molecular abnormalities within cancer cells. Certain cancers are dependent for their survival on specific aberrant signaling pathways, with one such example being in Diffuse Large B-Cell Lymphoma (DLBCL). Approximately half of all DLBCLs display a constitutively active NF- κ B gene transcription signature (40, 41), a consequence of deregulated I κ B kinase (IKK) activity. IKK phosphorylates the NF- κ B inhibitor, I κ B α , making it a substrate of the NEDD8-activated CRL1^{BTRCP} (24, 46). By inhibiting NAE, turnover of I κ B α is prevented and NF- κ B signaling is inhibited, thus making this pathway a potentially attractive point of intervention with MLN4924 in DLBCLs with constitu-

tively active NF- κ B signaling. Furthermore, induction of NF- κ B signaling is thought to be an important mechanism of resistance to many commonly used chemotherapeutic agents (42). Thus, in addition to potential single agent activity of MLN4924 in NF- κ B-dependent cancers, it is intriguing to hypothesize that when used in combination, MLN4924 may prove useful to overcome NF- κ B-related mechanisms of resistance thereby increasing the clinical utility of other chemotherapeutics.

Chronic activation of the WNT/ β -catenin signaling pathway is a common feature of colon and some types of breast cancer. A recent report has linked Wnt-signaling to down-regulation of the tumor suppressor p27 via CRL4^{DDB1} ubiquitination and degradation (43). These data suggest that cancers that originate from Wnt-stimulated progenitor cells may be particularly susceptible to MLN4924 through the ability to inhibit CRL4 activity. More recently, it has been shown that CRL1^{BTRCP}-mediated degradation of the BimEL tumor suppressor may be a mechanism of resistance to chemotherapy-induced apoptosis (44), suggesting another avenue for use of an NAE inhibitor.

Ultimately, where NAE inhibitors will be efficacious will be revealed from the MLN4924 clinical trial outcome. In the meantime, studies evaluating NAE inhibitors in cancer cells with different genetic backgrounds could identify molecular abnormalities that make certain cancer types particularly sensitive to NAE inhibition. In addition, approaches such as synthetic-lethal RNAi screens with MLN4924 may identify pathways that synergize with NAE inhibition thereby allowing further rationalization of combination therapies and as such may facilitate clinical trial design.

Conclusion

The cullin-RING subfamily of ubiquitin ligases plays important roles in many cellular processes necessary for cancer cell survival. The role of the NEDD8 pathway in modulating the activity of CRLs has afforded an innovative way of targeting this class of enzymes through inhibition of the first step in the pathway catalyzed by NAE. The first-in-class inhibitor of NAE, MLN4924, has been shown to induce cancer cell death and inhibit the growth of tumors in xenograft models. MLN4924 is currently in multiple phase I clinical trials in both solid tumor and hematological malignancies.

Disclosure of Potential Conflicts of Interest

All authors are employees of Millennium Pharmaceuticals.

References

- Reinstein E, Ciechanover A. Narrative review: protein degradation and human disease: the ubiquitin connection. *Ann Intern Med* 2006; 145:676–84, PubMed.
- Ciechanover A. Intracellular protein degradation: from a vague idea through the lysosome and the ubiquitin proteasome system and onto human diseases and drug targeting. *Cell Death Differ* 2005;12:1178–90, PubMed doi:10.1038/sj.cdd.4401692.
- Hershko A, Ciechanover A. The ubiquitin system. *Annu Rev Biochem* 1998;67:425–79, PubMed doi:10.1146/annurev.biochem.67.1.425.
- Hershko A. The ubiquitin system for protein degradation and some of its roles in the control of the cell division cycle. *Cell Death Differ* 2005; 12:1191–7, PubMed doi:10.1038/sj.cdd.4401702.
- Haas AL, Rose IA. The mechanism of ubiquitin activating enzyme: A kinetic and equilibrium analysis. *J Biol Chem* 1982;257:10329–37, PubMed.
- Ardley HC, Robinson PA. E3 ubiquitin ligases. *Essays Biochem* 2005;41:15–30, PubMed doi: 10.1042/EB0410015.
- Kee Y, Huijbrechtse JM. Regulation of catalytic activities of HECT ubiquitin ligases. *Biochem Biophys Res Commun* 2007;354:329–33, PubMed doi:10.1016/j.bbrc.2007.01.025.
- Sun Y. Targeting E3 ubiquitin ligases for cancer therapy. *Cancer Biol Ther* 2003;2:623–9, PubMed.
- Willems AR, Schwab M, Tyers M. A hitchhikers guide to the cullin ubiquitin ligases: SCF and its kin. *Biochim Biophys Acta* 2004;1695:133–70.
- Hatakeyama S, Nakayama KI. U-box proteins as a new family of ubiquitin ligases. *Biochem Biophys Res Commun* 2003;302:635–45, PubMed doi:10.1016/S0006-291X(03)00245-6.
- Nalepa G, Rolfe M, Harper JW. Drug discovery in the ubiquitin proteasome system. *Nat Rev Drug Discov* 2006;5:596–613, PubMed doi: 10.1038/nrd2056.

12. Richardson PG, Barlogie B, Berenson J, et al. A Phase 2 Study of Bortezomib in relapsed refractory myeloma. *N Engl J Med* 2003;348:2609–17, PubMed doi:10.1056/NEJMoa030288.
13. Kane RC, Dagher R, Farrell A, et al. Bortezomib for the treatment of mantle cell lymphoma. *Clin Cancer Res* 2007;13:5291–4, PubMed doi:10.1158/1078-0432.CCR-07-0871.
14. Orlowski RZ, Kuhn DJ. Proteasome inhibitors in cancer therapy: lessons from the first decade. *Clin Cancer Res* 2008;14:1649, doi:10.1158/1078-0432.CCR-07-2218.
15. Demo SD, Kirk CJ, Aujay MA, et al. Anti-tumor activity of PR-171, a novel irreversible inhibitor of the proteasome. *Cancer Res* 2007;67:6383–91, PubMed doi:10.1158/0008-5472.CAN-06-4086.
16. Kerscher O, Felberbaum R, Hochstrasser M. Modification of proteins by ubiquitin and ubiquitin-like proteins. *Annu Rev Cell Dev Biol* 2006;22:159–80, PubMed doi:10.1146/annurev.cellbio.22.010605.093503.
17. Pan ZQ, Kentsis A, Dias DC, Yamoah K, Wu K. NEDD8 on cullin: building an expressway to protein destruction. *Oncogene* 2004;23:1985–97, PubMed doi:10.1038/sj.onc.1207414.
18. Geiss-Friedlander R, Melchior F. Concepts in sumoylation a decade on. *Nat Rev Mol Cell Biol* 2007;8:947–56, PubMed doi:10.1038/nrm2293.
19. Xie Z, Klionsky DJ. Autophagosome formation: core machinery and adaptations. *Nat Cell Biol* 2007;9:1102–9, PubMed doi:10.1038/ncb1007-1102.
20. Andersen JB, Hassel BA. The interferon regulated ubiquitin-like protein ISG15 in tumorigenesis, friend or foe? *Cytokine Growth Factor Rev* 2006;17:411–21, PubMed doi:10.1016/j.cytogr.2006.10.001.
21. Petroski MD, Deshaies RJ. Function and regulation of cullin-RING ubiquitin ligases. *Nat Rev Mol Cell Biol* 2005;6:9–20, PubMed doi:10.1038/nrm1547.
22. Zheng N, Schulman BA, Song L, et al. Structure of the Cul1-Rbx1-Skp1-F-boxSkp2 SCF ubiquitin ligase complex. *Nature* 2002;416:703–9, PubMed doi:10.1038/416703a.
23. Podust VN, Brownell JE, Gladysheva TB, et al. A Nedd8 conjugation pathway is essential for proteolytic targeting of p27Kip1 by ubiquitination. *Proc Natl Acad Sci U S A* 2000;97:4579–84, PubMed doi:10.1073/pnas.090465597.
24. Read MA, Brownell JE, Gladysheva TB, et al. Nedd8 modification of cul-1 activates SCF(beta (TrCP))-dependent ubiquitination of Ikkappa-Balpha. *Mol Cell Biol* 2000;20:2326–33, PubMed doi:10.1128/MCB.20.7.2326-2333.2000.
25. Gong L, Yeh ET. Identification of the activating and conjugating enzymes of the NEDD8 pathway. *J Biol Chem* 1999;274:12036–42, PubMed doi:10.1074/jbc.274.17.12036.
26. Kurz T, Chou Y-C, Willems AR, et al. Dcn1 functions as a scaffold-type E3 ligase for cullin neddylation. *Mol Cell* 2008;29:23–35, PubMed doi:10.1016/j.molcel.2007.12.012.
27. Kim AY, Bommeljé CC, Lee BE, et al. SCCRO (DCUN1D1) is an essential component of the E3 complex for neddylation. *J Biol Chem* 2008;283:33211–20.
28. Kawakami T, Chiba T, Suzuki T, et al. NEDD8 recruits E2-ubiquitin to SCF E3 ligase. *EMBO J* 2001;20:4003–12, PubMed doi:10.1093/emboj/20.15.4003.
29. Sakata E, Yamaguchi Y, Miyauchi Y, et al. Direct interactions between NEDD8 and ubiquitin E2 conjugating enzymes upregulate cullin-based E3 ligase activity. *Nat Struct Mol Biol* 2007;14:167–8, PubMed doi:10.1038/nsmb1191.
30. Duda DM, Borg LA, Scott DC, et al. Structural insights into NEDD8 activation of cullin-RING ligases: conformational control of conjugation. *Cell* 2008;134:995–1006, PubMed doi:10.1016/j.cell.2008.07.022.
31. Wei N, Serino G, Deng X-W. The COP9 signalosome: more than a protease. *Trends Biochem Sci* 2008;33:592–600.
32. Zheng J, Yang X, Harrell JM, et al. CAND1 binds to unneddylated Cul1 and regulates the formation of SCF ubiquitin E3 ligase complex. *Mol Cell* 2002;10:1519–26, PubMed doi:10.1016/S1097-2765(02)00784-0.
33. Bornstein G, Ganoth D, Hershko A. Regulation of neddylation and deneddylation of cullin1 in SCFSkp2 ubiquitin ligase by F-box protein and substrate. *Proc Natl Acad Sci U S A* 2006;103:11515–20, PubMed doi:10.1073/pnas.0603921103.
34. Lo SC, Hannink M. CAND1-mediated substrate adaptor recycling is required for efficient repression of Nrf2 by Keap1. *Mol Cell Biol* 2006;26:1235–44, PubMed doi:10.1128/MCB.26.4.1235-1244.2006.
35. Wee S, Geyer RK, Toda T, et al. CSN facilitates cullin-RING ubiquitin ligase function by counteracting autocatalytic adaptor instability. *Nat Cell Biol* 2005;7:387–91, PubMed doi:10.1038/ncb1241.
36. Vassilev LT. Small molecule antagonists of p53-MDM2 binding: research tools and potential therapeutics. *Cell Cycle* 2004;3:419–21, PubMed.
37. Zhang, Wang H. MDM2 oncogene as a novel target for human cancer therapy. *Curr Pharm Des* 2000;6:393–416, PubMed doi:10.2174/1381612003400911.
38. Vassilev LT, Vu BT, Graves B, et al. *In vivo* activation of the p53 pathway by small molecule antagonists of MDM2. *Science* 2004;303:844–8, PubMed doi:10.1126/science.1092472.
39. Soucy TA, Smith PG, Milhollen MA, et al. An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. *Nature* 2009;458:732–6.
40. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 2000;403:503–11, PubMed doi:10.1038/35000501.
41. Feuerhake F, Kutok JL, Monti S, et al. NFkB activity, function, and target-gene signatures in primary mediastinal large B-cell lymphoma and diffuse large B-cell lymphoma subtypes. *Blood* 2005;106:1392–9, PubMed doi:10.1182/blood-2004-12-4901.
42. Nakanishi C, Toi M. Nuclear factor-κB inhibitors as sensitizers to anticancer drugs. *Nat Rev Cancer* 2005;5:297–309, PubMed doi:10.1038/nrc1588.
43. Miranda-Carboni GA, Krum SA, Yee K, et al. A functional link between Wnt signaling and SKP2-independent p27 turnover in mammary tumors. *Genes Dev* 2008;22:3121–34, PubMed doi:10.1101/gad.1692808.
44. Dehan E, Bassermann F, Guardavaccaro D, et al. βTRCP- and Rsk1/2-mediated degradation of BimEL inhibits apoptosis. *Mol Cell* 2009;33:109–16, PubMed doi:10.1016/j.molcel.2008.12.020.
45. Tsvetkov LM, Yeh KH, Lee SJ, et al. p27(Kip1) ubiquitination and degradation is regulated by the SCF(Skp2) complex through phosphorylated Thr187 in p27. *Curr Biol* 1999;9:661–4, PubMed doi:10.1016/S0960-9822(99)80290-5.
46. Winston JT, Strack P, Beer-Romero P, et al. The SCFbeta-TRCP-ubiquitin ligase complex associates specifically with phosphorylated destruction motifs in Ikkappa-Balpha and beta-catenin and stimulates Ikkappa-Balpha ubiquitination *in vitro*. *Genes Dev* 1999;13:270–83, PubMed doi:10.1101/gad.13.3.270.
47. Koepp DM, Schaefer LK, Ye X. Phosphorylation-dependent ubiquitination of cyclin E by the SCFFbw7 ubiquitin ligase. *Science* 2001;294:173–7, PubMed doi:10.1126/science.1065203.
48. Yada M, Hatakeyama S, Kamura T. Phosphorylation-dependent degradation of c-Myc is mediated by the F-box protein Fbw7. *EMBO J* 2004;23:2116–25, PubMed doi:10.1038/sj.emboj.7600217.
49. Nateri AS, Riera-Sans L, DaCosta C, et al. The ubiquitin ligase SCFFbw7 antagonizes apoptotic JNK signaling. *Science* 2004;303:1374–8, PubMed doi:10.1126/science.1092880.
50. Mao JH, Kim IJ, Wu D, et al. FBXW7 targets mTOR for degradation and cooperates with PTEN in tumor suppression. *Science* 2008;321:1499–502, doi:10.1126/science.1162981.
51. Liakopoulos D, Büsgen T, Brychzy A, et al. Conjugation of the ubiquitin-like protein NEDD8 to cullin-2 is linked to von Hippel-Lindau tumor suppressor function. *Proc Natl Acad Sci U S A* 1999;96:5510–5, PubMed doi:10.1073/pnas.96.10.5510.
52. Kobayashi A, Kang MI, Okawa H, et al. Oxidative stress sensor Keap1 functions as an adaptor for Cul3-based E3 ligase to regulate proteasomal degradation of Nrf2. *Mol Cell Biol* 2004;24:7130–9, PubMed doi:10.1128/MCB.24.16.7130-7139.2004.
53. Jin J, Arias EE, Chen J, et al. A family of diverse Cul4-Ddb1-interacting proteins includes Cdt2, which is required for S phase destruction of the replication factor Cdt1. *Mol Cell* 2006;23:709–21, PubMed doi:10.1016/j.molcel.2006.08.010.

Clinical Cancer Research

Targeting NEDD8-Activated Cullin-RING Ligases for the Treatment of Cancer

Teresa A. Soucy, Peter G. Smith and Mark Rolfe

Clin Cancer Res 2009;15:3912-3916.

Updated version Access the most recent version of this article at:
<http://clincancerres.aacrjournals.org/content/15/12/3912>

Cited articles This article cites 53 articles, 21 of which you can access for free at:
<http://clincancerres.aacrjournals.org/content/15/12/3912.full#ref-list-1>

Citing articles This article has been cited by 24 HighWire-hosted articles. Access the articles at:
<http://clincancerres.aacrjournals.org/content/15/12/3912.full#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://clincancerres.aacrjournals.org/content/15/12/3912>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.