

Radiosensitization of Cancer Cells by Inactivation of Cullin-RING E3 Ubiquitin Ligases

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Abstract

Although radiotherapy represents one of the most effective treatment modalities for patients with cancer, inherent and/or acquired resistance of cancer cells to radiotherapy is often an impediment to effective treatment. Diverse strategies have been developed to improve the efficacy of radiotherapy. The ubiquitin-proteasome system (UPS) operates in numerous vital biologic processes by controlling the protein turnover in cells. Ubiquitination is central to the UPS pathway, and it relies on the E3 ubiquitin ligases to catalyze the covalent attachment of ubiquitin to its protein substrates. Cullin-based RING ligases (CRLs) are the largest family of E3 ligases that are responsible for the ubiquitination and destruction of numerous cancer-relevant proteins. Its deregulation has been linked to many human cancers, making it an attractive target for therapeutic intervention. This review discusses how targeting the ubiquitin-proteasome system, particularly CRLs, is an exciting new strategy for radiosensitization in cancer and, specifically, focuses on MLN4924, a recently discovered small-molecule inhibitor of the NEDD8-activating enzyme, which is being characterized as a novel radiosensitizing agent against cancer cells by inactivating CRL E3 ubiquitin ligases.

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Introduction

Cancer is a large group of highly complex diseases with dramatically different biologic behaviors. Even within cancers of the same organ, the extent of therapeutic response varies considerably, making it unlikely that any single agent would cure all cancers or even cancers of a single organ. Radiation therapy represents one of the most clinically effective forms of treatment [1]. It is frequently applied as a single treatment modality with curative intent or, more often, combined with surgery and/or chemotherapy to maximize the therapeutic effect [2]. Treatment outcome of patients with cancer receiving radiotherapy has improved in recent decades, mainly because of optimized therapeutic plans and technological advancements in the precise delivery of radiation to the targeted tumor tissues [3]. However, in many patients, disease recurs locally after radiotherapy. Although some treatment failures can be explained by the traditionally accepted clinical factors, such as tumor stage and grade, many failures remain unexplained [1]. It is now increasingly recognized that multiple biologic factors of tumors may contribute to radioresistance and, thereby, have a potential role in determining treatment outcome of patients. Examples include the intrinsic radioresistance of tumor cells, the existence of radioresistant cancer stem cells, repopulation of surviving cells after radiotherapy, repair of radiation-induced damage, the vasculature, as well as the extent of hypoxia and inflammation within tumors [1]. These factors associated with radioresistance have been extensively

studied in both the preclinical and clinical settings, leading to the development of diverse strategies, including targeted agents to overcome or modulate them with the goal of improving radiotherapy efficacy.

The ubiquitin-proteasome system (UPS) is responsible for the timely degradation of many regulatory proteins within the cell [4] and also mediates various nondegradative functions [5]. Abnormal regulation of UPS has been implicated in a growing number of human diseases, notably in cancer [6]. Ubiquitination plays a central role in the UPS pathway and relies on the E3 ligases to catalyze the covalent attachment of ubiquitin to its protein substrates, which usually confers a recognition signal for proteasome targeting [4,7]. Cullin-based RING ligases (CRLs) are the largest family of E3 ubiquitin ligases that control the ubiquitination and proteasomal degradation of numerous cancer-relevant proteins [8], thus representing potential therapeutic targets in cancer [9,10]. Here, we provide an overview of CRL E3 ligases and discuss how general targeting of the UPS as well as selective targeting of CRL E3 ligases are being used for radiosensitization of cancer cells.

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Ubiquitin and CRL E3 Ligases

Posttranslational modification of proteins by ubiquitin or ubiquitin-like proteins (e.g., NEDD8, SUMO-1, SUMO-2, SUMO-3, FUBI, HUB1, ISG15, FAT10, URM1, UFM1, ATG12, and ATG8) represents one of the most prevalent mechanisms for regulating most aspects of cell physiology [4,7,11,12]. As a *bona fide* modifier, ubiquitin is a highly conserved protein of 76 amino acids that can be covalently attached to other proteins through a stepwise cascade of three enzymes, i.e., E1 (ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzyme), and finally E3 (ubiquitin ligase), thereby influencing protein fate and function [4]. Ubiquitination typically acts as a degradation signal for the 26S proteasome (poly-ubiquitylation) [13] and also serves non-proteolytic roles (Lys63-linked poly- or mono-ubiquitylation) in regulating the nuclear factor kappaB (NF- κ B) signaling pathway [14,15], DNA replication and repair [16,17], as well as intracellular trafficking [13,18].

In humans, there are two E1 enzymes, at least 38 E2 enzymes [19], and hundreds of E3 enzymes [8]. The E3 ligases are responsible for substrate specificity [8] and are subdivided into two major classes characterized by the presence of either a HECT or a RING domain within them [4,8,20]. RING domain-containing E3 ligases have more than 600 members, comprising about 95% of human E3 ligases [8]. Among the RING-based E3 ligases, the CRLs are the largest family of multiunit ubiquitin ligases that control the turnover of approximately 20% of all ubiquitinated proteins through proteasome-mediated degradation [21]. Within the CRL complex, cullin serves as a molecular scaffold and interacts at its C terminus with the RING finger protein, creating the catalytic core of the ligase, whereas its N terminus interacts directly or indirectly (through an adapter protein) with the substrate-recognition subunit (SRS). It is this SRS that confers specificity toward its substrate proteins [22]. In human and mouse, there are eight cullins (cullins 1–3, 4A, 4B, 5, 7, and 9) [22] and two RING family members, RING box protein-1 (RBX1) and RBX2, also known as sensitive to apoptosis gene (SAG) [8,23,24]. Both RBX1 and RBX2 are capable of binding to six members of the human cullin family (cullins 1–3, 4A, 4B, and 5) under overexpressed conditions [25] and demonstrate *in vitro* E3 ubiquitin ligase activity when complexed with cullin 1 [26]. A potential difference between RBX1 and RBX2 lies in that RBX1 is constitutively expressed, whereas RBX2/SAG seems to be stress-induced [27]. Furthermore, RBX1 preferentially interacts with cullin 2, whereas RBX2 is selectively associated with cullin 5, under physiological conditions [28]. The best characterized CRL complex is the SKP1-cullin 1–F-box protein (SCF) E3 ligase [29–31]. SCF is also known as CRL1 [32], where cullin 1 tethers both the RING finger protein RBX1/RBX2 and the adaptor protein SKP1, which, in turn, binds to the F-box protein [e.g., FBXW7, beta-transducin repeat containing protein (β -TrCP) and SKP2] [33]. The human genome contains approximately 69 F-box proteins that can potentially form a complex with cullin 1 [34]. Conceivably, the availability of two RBX family members, along with eight cullins, hundreds of substrate receptors, and many adaptor proteins allows for the assembly of a multitude of CRLs in eukaryotic cells, imparting these enzymes with key regulatory functions in protein homeostasis.

Notably, most CRLs, if not all, are activated by neddylation through attachment of ubiquitin-like protein (NEDD8) to cullin, therefore preventing the inhibitory binding by the cullin-associated neddylation-dissociated protein (CAND1) [35–38]. The conformation-based mechanisms that explain these activating roles of neddylation have been discussed previously [36,38,39]. Neddylation, through a process analogous to ubiquitination, involves an enzymatic cascade

through the sequential activity of E1, E2, and E3, resulting in the covalent attachment of NEDD8 to its substrates. The NEDD8 cascade is currently known to contain a single E1, NEDD8-activating enzyme (NAE), two E2s, UBE2M (also known as UBC12), UBE2F [40–43], and a few candidate E3s, such as RBX1 [44,45], SCCRO (DCN1) [46], and IAP [47]. Owing to the critical role of neddylation in the activation of CRLs, it provides an alternative approach to modulate CRL activity by controlling the NEDD8 cascade, as discussed below.

Targeting the UPS and Radiosensitization

Aberrant UPS function has been strongly associated with cancer, and its pharmacologic inhibition has proved efficacious in the treatment of cancers [48]. Bortezomib (Velcade, PS-341) is the first commercially available proteasome inhibitor approved for clinical use in treating selected human cancers [49,50], whereas next-generation compounds, such as carfuzomib, MLN9708, and CEP18770, are in clinical development [51]. By blocking the active sites in the 20S proteasome, bortezomib disrupts the entire UPS. Although bortezomib has demonstrated clinical efficacy as a single agent and in combination with chemotherapy in multiple myeloma and mantle cell lymphoma, its overall success has been limited because of a lack of response in other malignancies and drug-associated toxicity [52–54].

In the setting of radiation therapy, general proteasome inhibition has been shown to impart tumor cell radiosensitization in many preclinical models and is thought to involve modulation of proteins involved in apoptosis, cell cycle, and DNA double-strand break repair [55–57] (see Table 1). In particular, bortezomib has demonstrated radiosensitizing properties in a number of tumor cell models and in association with stabilization of p38 mitogen-activated protein kinase (MAPK) [58] and inhibition of NF- κ B [59] or cancerous inhibitor of protein phosphatase 2A [60]. Other proteasome inhibitors in clinical (marizomib) and preclinical studies (MG-132) also showed radiosensitizing activity in glioma, prostate, and lung cancer cells [61–63] (Table 1). Whereas bortezomib combined with chemoradiation is an active area of clinical investigation, initial studies suggest that toxicity is a significant concern [64,65]. Taken together, these studies with general proteasome inhibitors have provided proof-of-concept that proteasome inhibition is a worthwhile strategy for sensitizing tumor cells to radiation and chemotherapy but underscore the importance of developing agents with better selectivity.

One approach to circumvent the toxicity associated with general proteasome inhibitors is to directly target the E3 ligases, because each E3 ligase is responsible for a subset of cellular protein substrates. In contrast to general proteasome inhibition that has a broad impact on total cellular proteolysis, specific E3 ligase inhibition is expected to selectively stabilize a subset of cellular proteins, thus avoiding unwanted stabilization of other cellular proteins that may have deleterious effects on normal cells. Therefore, it is expected that a greater therapeutic window could be achieved with agents that target specific components of the UPS rather than the entire UPS.

Targeting CRL Components and Radiosensitization

CRLs represent the largest known class of E3 ubiquitin ligases and are fundamental in controlling protein homeostasis, thus regulating various biologic processes including cell cycle progression, gene transcription, signal transduction, and DNA replication among others [8,32,66]. Not surprisingly, deregulation of CRL has been associated with uncontrolled proliferation, genomic instability, and cancer [66]. Among the components of CRL, some have been defined as oncogenes

Table 1. Radiosensitization of Human Cancer Cells by UPS Inhibitors.

	Developmental Stages	Radiosensitization Activity	Cancer Types	References
<i>UPS inhibitors</i>				
Bortezomib	Clinical use	Yes	Prostate, gastric, cervical, rectal, esophageal, lung, and liver cancers; lymphoma; and central nervous system malignancies	[58–60,64,116–118,129]
Marizomib	Phase 1	Yes	Glioma	[63]
MG-132	Preclinical	Yes	Prostate and lung cancers; Hodgkin's lymphoma; and melanoma	[55,61,62,130,131]
<i>CRL inhibitor</i>				
MLN4924	Phase 1	Yes	Pancreatic, lung, and breast cancers	[91,119]

(e.g., SKP2) that are frequently amplified and/or overexpressed in cancers, whereas others act as tumor suppressors (e.g., FBXW7) that are often found to be mutated in cancer [48,67,68]. The oncogenic properties of some CRLs make them potential targets for therapeutic intervention. The CRL components with attractive potential as radiosensitizing targets in cancer cells are discussed below.

RBX1/RBX2

Our previous and recent studies showed that RBX1 and RBX2, two family members of the RING component of CRL found in human and mouse, are frequently overexpressed in many types of human cancer [69–71]. In multiple human cancer cell lines, knockdown of either RBX1 or RBX2 suppresses cancer cell growth and survival [70,71]. This impaired growth and survival in response to RBX1 or RBX2 knockdown appears to involve the induction of apoptosis and senescence or only apoptosis in the case of RBX2 [70,71]. Similarly, ectopic expression of RBX2 protects cells from apoptosis induced by a variety of stresses including metal ions and redox compounds [23,72], nitric oxide [73], neurotoxin and 1-methyl-4-phenylpyridinium [74], heat shock [75], UV irradiation [76], and ischemia/hypoxia both *in vitro* [77] and *in vivo* [78,79]. Taken together, these results support the notion that cancer cells are more reliant on RBX1/RBX2 overexpression for their survival, thus more sensitive to RBX1/RBX2 targeting. Because every individual member of CRL ligase family requires either RBX1 or RBX2 for activity, targeted inhibition of RBX1/RBX2 would lead to general inactivation of the entire family of CRL ligases, thus having broader anticancer effects.

It is well established that radiation induces DNA damage and that G2 arrest is a crucial response to DNA damage in most cancer cells [80]. On the basis of the finding that RBX1 silencing triggers DNA double-strand breaks, leading to G2 arrest [70], it is conceivable that knockdown of RBX1 may sensitize otherwise resistant cancer cells to radiotherapy by redistributing them to G2, a more radiosensitive phase of cell cycle. This hypothesis is supported by our finding that RBX1 silencing indeed sensitizes human cancer cells to radiation [81]. The underlying mechanism for RBX1 silencing-mediated radiosensitization is likely attributable to the accumulation of DNA replication licensing proteins CDT1 and ORC1, two known CRL substrates [32], which leads to DNA double-strand breaks, DNA damage response, and G2 arrest, rendering cancer cells more sensitive to radiation [81].

RBX2 is a dual-function protein with CRL-independent antioxidant activity, when acting alone, or CRL-dependent E3 ligase activity, when forming a complex with other CRL components [24,27,82]. Analogous to RBX1, RBX2 silencing also sensitizes other-

wise resistant cancer cells to radiation [71]. However, distinct from RBX1, RBX2 silencing-mediated radiosensitization in human cancer cells appears to be mechanistically linked with accumulation of the proapoptotic protein, NOXA [71]. However, as shown in an *Rbx2* knockout model, complete elimination of *Rbx2* expression sensitized mouse embryonic stem cells to radiation-induced cell killing through mechanisms involving an increase in steady-state levels of intracellular reactive oxygen species because of the abrogation of antioxidant activity of *Rbx2*, as well as the decreased NF- κ B activation associated with accumulation of inhibitor κ B (I κ B) [83]. These findings further support the notion that RBX2 plays a protective role in response to DNA damage and, when absent, sensitizes cells to radiation-induced cell death, suggesting its potential as a novel target for radiosensitization.

Cullins

The cullins are a family of eight members, which do not have intrinsic catalytic activity, when acting alone, but instead are molecular scaffolds that facilitate the assembly of modular CRL complexes and mediate the transfer of ubiquitin from the E2-conjugating enzyme to the substrate proteins [22]. Cullin 1 is overexpressed in 40% of lung cancers [84], whereas cullin 4A expression is elevated in multiple cancer types, such as breast [85–87], hepatocellular [88], and mesothelioma [89]. Overexpression of cullin 4A in MCF10A cells abrogated the G2/M cell cycle checkpoint in response to radiation-induced DNA damage [90]. Because the biologic effects of the cullin proteins are reliant upon their SRSs [F-box, Bric-a-Brac, Tramtrack Broad-complex (BTB), von Hippel-Lindau (VHL) and suppressor of cytokine signaling (SOCS) proteins] and corresponding substrates, cullins themselves are not conventional oncoproteins or tumor suppressors. However, cullin overexpression could increase CRL activity in cancer cells, promoting uncontrolled proliferation. Thus, cullin 1 and cullin 4A are potential anticancer targets that when inhibited may shift cells to a more controlled growth state. Given that CDT1 and WEE1 are the substrates of cullin 1- and cullin 4A-based CRL and their accumulation is responsible for radiosensitization in pancreatic cancer cells [91], targeting either cullin 1 or cullin 4A might be a potential sensitization strategy for radiotherapy.

Substrate-recognition Subunit

SRSs recognize and recruit target substrates to the CRL complexes. Different cullins are known to use distinct classes of SRS, such as F-box proteins for SCF/CRL1, VHL-box for cullin 2, BTB proteins for cullin 3, DCAF proteins for cullin 4A/B, and SOCS-box proteins for cullin 5 [22]. The human genome contains about 69 F-box proteins that provide specificity for the particular substrate to be degraded [34]. Among them, only three are well characterized, namely, oncogenic

SKP2, tumor suppressive FBXW7, and β -TrCP, which is considered either oncogenic or tumor suppressive in a substrate-dependent manner [67,68]. Given that SKP2 and β -TrCP have documented oncogenic activities, we focus on these two F-box proteins as potential therapeutic targets in cancer.

SKP2. SKP2 is the SRS of the SCF^{SKP2} ubiquitin ligase complex and mediates the degradation of several negative cell cycle regulators including p27, p21, p130, and p57, thus, positively regulating the G1/S transition [67]. Extensive studies have defined the oncogenic role of SKP2 in many human cancer types, including gastric [92], colon [93], and breast [94] cancers. Overexpression of SKP2 is associated with decreased p27 levels, which is an indicator of poor prognosis [66]. Elevated expression of SKP2 was shown to promote the radioresistance of esophageal squamous cell carcinoma, which negatively correlated with the survival of patients undergoing radiotherapy [95]. Likewise, depletion of SKP2 through genetic approaches inhibits the growth of many cancer cell lines [9] and also sensitizes esophageal squamous cell carcinoma to radiation-induced cell death [95]. These findings suggest that pharmacological inhibition of the SKP2 pathway may have therapeutic efficacy in cancer. Consistently, using high-throughput screening, Chen et al. recently identified an agent, compound A, which inhibits SCF^{SKP2} by preventing incorporation of SKP2 into the SCF^{SKP2} ligase [96]. Compound A treatment caused accumulation of SCF^{SKP2} substrates (e.g., p27) and consequently induced G1 cell cycle arrest as well as SCF^{SKP2}- and p27-dependent cell killing [96]. It is conceivable that compound A in combination with radiation may have a therapeutic benefit, although it remains to be determined experimentally.

β -TrCP. β -TrCP, with two family members of β -TrCP1 and β -TrCP2 (also known as HOS), acts as the SRS of the SCF ^{β -TrCP} complex and promotes ubiquitination and degradation of various cellular proteins [66,67]. However, whether β -TrCP is an oncoprotein or a tumor suppressor seems to be substrate-dependent. In some tissues, β -TrCP acts as an oncoprotein for proteasomal degradation of tumor suppressors (e.g., I κ B, PDCD4, and BimEL1) [9]. Thus, it is anticipated that, in transgenic mice, overexpression of β -TrCP1 in mammary gland, intestine, liver, and kidney would stimulate tumor formation [97,98]. Consistent with its role in promoting tumorigenesis, β -TrCP1 overexpression was found in human breast cancers and β -TrCP1 inhibition sensitizes breast cancer cells to chemotherapy [99]. Similarly, up-regulation of β -TrCP1 increased NF- κ B activity and chemoresistance, whereas β -TrCP1 knockdown decreased NF- κ B activity and chemoresistance in pancreatic cancer cells [100] and sensitized cervical cancer cells to apoptosis [101]. Given the important role of NF- κ B in mediating tumor radioresistance [102], targeting β -TrCP might represent an effective strategy for radiosensitization. Indeed, inhibition of β -TrCP2 was found to sensitize human melanoma cells to apoptosis induced by various anticancer agents, including ionizing radiation [103]. However, the development of inhibitors that selectively disrupt the binding between β -TrCP and tumor suppressive substrates, but not oncogenic substrates, is likely to be a challenge.

Targeting the CRL and Radiosensitization

Underscoring the importance of CRL E3 ligases as potential therapeutic targets, abnormal activation of CRL E3 ligases has been demonstrated in many types of cancer, resulting in the aberrant turnover of numerous cancer-related proteins [10,66]. Efforts to identify spe-

cific small-molecule inhibitors of CRL E3 ligases are well underway and three such inhibitors have recently been reported [96,104,105], although the anticancer properties of these newly identified inhibitors remain to be determined [104,105]. Importantly, the discovery of MLN4924 as a small-molecule inhibitor of NAE, capable of inactivating CRL through blocking cullin neddylation [21], has opened up an alternative strategy for targeting CRL activity. Mechanistically, MLN4924 binds to NAE to form a tight-binding NEDD8-MLN4924 adduct, which resembles the first intermediate of the reaction catalyzed by the NAE, but cannot be further used in subsequent intraenzyme reactions, thus inhibiting the activity of the NEDD8 E1 enzyme [106]. In contrast to bortezomib, MLN4924 appears to be more specific because it does not inhibit bulk proteasomal degradation [21]. In preclinical studies, MLN4924, by inactivating CRL E3 ubiquitin ligase, causes the accumulation of several SCF E3 substrates to induce apoptosis [21,107–110] and senescence [111–113], thus inhibiting growth of a variety of human cancer cells both *in vitro* and *in vivo*. Importantly, MLN4924 was found to inhibit cancer cell growth but was well tolerated under various dose levels and treatment regimens in several mouse xenograft models [21,91], suggesting cancer cell selectivity. MLN4924 is currently being evaluated in a number of phase I clinical trials against some solid tumors and hematologic malignancies [114,115]. Most recently released trial results on cancer patients with metastatic melanoma and other solid tumors showed that MLN4924 indeed targets CRL ligases and leads to disease stabilization with mostly grade 1 or 2 adverse effects, including fatigue, diarrhea, nausea, vomiting, and anemia (http://www.takeda.com/press/article_41890.html).

Given that general proteasome inhibition using bortezomib has been demonstrated to sensitize tumor cells to radiation [58,59,116–118], whether MLN4924 can radiosensitize in a tumor cell-selective manner is an important question. Our recent studies showed that knockdown of RBX1/RBX2, which mimics inhibition of CRL activity, induced tumor cell radiosensitization [71,81], thus suggesting that MLN4924 may act as a radiosensitizing agent. We, therefore, tested this hypothesis and found that indeed MLN4924 possesses potent radiosensitizing activity in pancreatic, lung, and breast cancer cells but, importantly, not in normal lung fibroblasts [91,119], demonstrating the tumor cell selectivity of MLN4924-mediated radiosensitization. The radiosensitizing mechanisms of MLN4924 are causally related to the accumulation of a subset of CRL substrates within cells [91,119]. In pancreatic cancer cells, MLN4924 treatment caused the accumulation of several CRL substrates, including CDT1, WEE1, and NOXA, in parallel with an enhancement of radiation-induced DNA damage, aneuploidy, G2/M phase cell cycle arrest, and apoptosis [91]. Knockdown of CDT1 and WEE1 partially rescued MLN4924-induced aneuploidy, G2/M arrest, and radiosensitization, indicating a causal role of CDT1 and WEE1 accumulation in MLN4924-mediated radiosensitization. Similarly, MLN4924 displayed potent radiosensitizing activity in a human pancreatic tumor xenograft model with minimal toxicity [91]. However, the radiosensitization effect of MLN4924 on breast cancer cells appears to mainly depend on the accumulation of p21, a well-known CRL substrate associated with cell growth arrest, apoptosis, and DNA damage response. This is supported by the finding that p21 accumulates in response to combined MLN4924 and radiation treatment and that transient silencing of p21 partially rescues MLN4924-induced G2/M arrest and radiosensitization [119]. Taken together, these findings suggest that the mechanisms of MLN4924-mediated radiosensitization may be dependent on specific tumor cell types.

Conclusions and Perspectives

The data summarized in this review clearly show that blockage of global protein degradation by general proteasome inhibitors (such as bortezomib) or inactivation of CRL E3s by siRNA silencing of CRL components or small-molecule inhibitors (e.g., MLN4924) can achieve radiosensitization in various human cancer cells (Figure 1). Although MLN4924 should be less toxic than bortezomib because of its selective inactivation of one type of E3 ligase rather than general inhibition of proteolysis, for the future development of MLN4924 as an anticancer or radiosensitizing agent, some intrinsic specificity issues are worth considering. First, MLN4924 is an NAE inhibitor and would likely inhibit, in addition to cullins, other cellular neddylation reactions [37,38], although cullins are the only known physiological substrates [36,38]. Second, in addition to causing accumulation of some tumor suppressors (e.g., p21, p27, I κ B α , DEPTOR, NOXA, or PDCD4), our unpublished data also showed that MLN4924 could cause accumulation of some oncogenic proteins (e.g., c-Jun, cyclin D1, c-Myc, or Notch1), all of which are known CRL substrates [66,120] in a cell line-dependent manner. Thus, the net outcome of MLN4924 action will depend on the interaction of these substrates in a cell context, temporal, and spatial dependent manner. Third, two recent studies showed that cancer cells could develop resistance to MLN4924 by selecting rare clones with heterozygous mutations in the targeting enzyme NAE β [121,122]. Nevertheless, given the fact that human cancer harbors multiple mutations with alterations in multiple signaling pathways [123,124], it is unlikely that drugs that target a

single mutated gene product/single pathway would be effective. Because MLN4924 targets multiple signaling pathways by inactivating CRL E3s, it would be likely more efficacious as a single anticancer or radiosensitizing agent. Finally, quantitative proteomic analysis at the unbiased global level in a variety of MLN4924-treated cancer cells [125–128], when performed in combination with radiation, would likely identify potential targets as well as biomarkers for radiosensitization. Thus, future mechanistic characterization of MLN4924 or other CRL E3 inhibitors and development of these inhibitors as a novel class of radiosensitizers would eventually benefit cancer patients by enhancing the efficacy of radiotherapy.

References

- [1] Begg AC, Stewart FA, and Vens C (2011). Strategies to improve radiotherapy with targeted drugs. *Nat Rev Cancer* **11**, 239–253.
- [2] Delaney G, Jacob S, Featherstone C, and Barton M (2005). The role of radiotherapy in cancer treatment: estimating optimal utilization from a review of evidence-based clinical guidelines. *Cancer* **104**, 1129–1137.
- [3] Bhide SA and Nutting CM (2010). Recent advances in radiotherapy. *BMC Med* **8**, 25.
- [4] Hershko A and Ciechanover A (1998). The ubiquitin system. *Annu Rev Biochem* **67**, 425–479.
- [5] Keating SE and Bowie AG (2009). Role of non-degradative ubiquitination in interleukin-1 and toll-like receptor signaling. *J Biol Chem* **284**, 8211–8215.
- [6] Edelmann MJ, Nicholson B, and Kessler BM (2011). Pharmacological targets in the ubiquitin system offer new ways of treating cancer, neurodegenerative disorders and infectious diseases. *Expert Rev Mol Med* **13**, e35.
- [7] Strieter ER and Korasick DA (2012). Unraveling the complexity of ubiquitin signaling. *ACS Chem Biol* **7**, 52–63.
- [8] Deshaies RJ and Joazeiro CA (2009). RING domain E3 ubiquitin ligases. *Annu Rev Biochem* **78**, 399–434.
- [9] Jia L and Sun Y (2011). SCF E3 ubiquitin ligases as anticancer targets. *Curr Cancer Drug Targets* **11**, 347–356.
- [10] Sun Y (2006). E3 ubiquitin ligases as cancer targets and biomarkers. *Neoplasia* **8**, 645–654.
- [11] Kang C and Yi GS (2011). Identification of ubiquitin/ubiquitin-like protein modification from tandem mass spectra with various PTMs. *BMC Bioinformatics* **12**(suppl 14), S8.
- [12] Kerscher O, Felberbaum R, and Hochstrasser M (2006). Modification of proteins by ubiquitin and ubiquitin-like proteins. *Annu Rev Cell Dev Biol* **22**, 159–180.
- [13] Pickart CM (2001). Ubiquitin enters the new millennium. *Mol Cell* **8**, 499–504.
- [14] Chen ZJ and Sun LJ (2009). Nonproteolytic functions of ubiquitin in cell signaling. *Mol Cell* **33**, 275–286.
- [15] Skaug B, Jiang X, and Chen ZJ (2009). The role of ubiquitin in NF- κ B regulatory pathways. *Annu Rev Biochem* **78**, 769–796.
- [16] Bennett EJ and Harper JW (2008). DNA damage: ubiquitin marks the spot. *Nat Struct Mol Biol* **15**, 20–22.
- [17] Ulrich HD and Walden H (2010). Ubiquitin signalling in DNA replication and repair. *Nat Rev Mol Cell Biol* **11**, 479–489.
- [18] Acconcia F, Sigismund S, and Polo S (2009). Ubiquitin in trafficking: the network at work. *Exp Cell Res* **315**, 1610–1618.
- [19] Ye Y and Rape M (2009). Building ubiquitin chains: E2 enzymes at work. *Nat Rev Mol Cell Biol* **10**, 755–764.
- [20] Bernassola F, Karin M, Ciechanover A, and Melino G (2008). The HECT family of E3 ubiquitin ligases: multiple players in cancer development. *Cancer Cell* **14**, 10–21.
- [21] Soucy TA, Smith PG, Milhollen MA, Berger AJ, Gavin JM, Adhikari S, Brownell JE, Burke KE, Cardin DP, Critchley S, et al. (2009). An inhibitor of NEDD8-activating enzyme as a new approach to treat cancer. *Nature* **458**, 732–736.
- [22] Sarikas A, Hartmann T, and Pan ZQ (2011). The cullin protein family. *Genome Biol* **12**, 220.
- [23] Duan H, Wang Y, Aviram M, Swaroop M, Loo JA, Bian J, Tian Y, Mueller T, Bisgaier CL, and Sun Y (1999). SAG, a novel zinc RING finger protein that protects cells from apoptosis induced by redox agents. *Mol Cell Biol* **19**, 3145–3155.
- [24] Sun Y, Tan M, Duan H, and Swaroop M (2001). SAG/ROC/Rbx/Hrt, a zinc RING finger gene family: molecular cloning, biochemical properties, and biological functions. *Antioxid Redox Signal* **3**, 635–650.

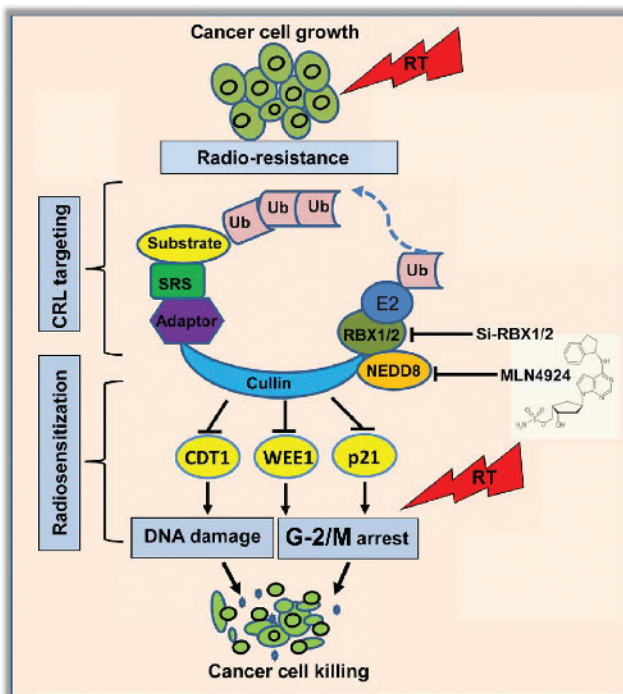


Figure 1. Cancer cell radiosensitization by inactivation of CRL E3: Inactivation of CRL ligase activity by siRNA silencing of its components (e.g., RBX1/RBX2) or by the small-molecule MLN4924 which inhibits cullin neddylation, causes accumulation of CRL substrates. Accumulation of some of these substrates, such as CDT1, WEE1, and p21, leading to altered DNA damage response and G2/M arrest, was found to be causally related to MLN4924-mediated radiosensitization in a cancer cell type-dependent manner.

- [25] Ohta T, Michel JJ, and Xiong Y (1999). Association with cullin partners protects ROC proteins from proteasome-dependent degradation. *Oncogene* **18**, 6758–6766.
- [26] Swaroop M, Wang Y, Miller P, Duan H, Jatkoa T, Madore S, and Sun Y (2000). Yeast homolog of human SAG/ROC2/Rbx2/Hrt2 is essential for cell growth, but not for germination: chip profiling implicates its role in cell cycle regulation. *Oncogene* **19**, 2855–2866.
- [27] Wei D and Sun Y (2010). Small RING finger proteins RBX1 and RBX2 of SCF E3 ubiquitin ligases: the role in cancer and as cancer targets. *Genes Cancer* **1**, 700–707.
- [28] Kamura T, Maenaka K, Kotoshiba S, Matsumoto M, Kohda D, Conaway RC, Conaway JW, and Nakayama KI (2004). VHL-box and SOCS-box domains determine binding specificity for Cul2-Rbx1 and Cul5-Rbx2 modules of ubiquitin ligases. *Genes Dev* **18**, 3055–3065.
- [29] Deshaies RJ (1999). SCF and cullin/Ring H2-based ubiquitin ligases. *Annu Rev Cell Dev Biol* **15**, 435–467.
- [30] Feldman RM, Correll CC, Kaplan KB, and Deshaies RJ (1997). A complex of Cdc4p, Skp1p, and Cdc53p/cullin catalyzes ubiquitination of the phosphorylated CDK inhibitor Sic1p. *Cell* **91**, 221–230.
- [31] Skowyra D, Craig KL, Tyers M, Elledge SJ, and Harper JW (1997). F-box proteins are receptors that recruit phosphorylated substrates to the SCF ubiquitin-ligase complex. *Cell* **91**, 209–219.
- [32] Petroski MD and Deshaies RJ (2005). Function and regulation of cullin-RING ubiquitin ligases. *Nat Rev Mol Cell Biol* **6**, 9–20.
- [33] Zheng N, Schulman BA, Song L, Miller JJ, Jeffrey PD, Wang P, Chu C, Koepf DM, Elledge SJ, Pagano M, et al. (2002). Structure of the Cul1–Rbx1–Skp1–F box^{Skp2} SCF ubiquitin ligase complex. *Nature* **416**, 703–709.
- [34] Jin J, Cardozo T, Lovering RC, Elledge SJ, Pagano M, and Harper JW (2004). Systematic analysis and nomenclature of mammalian F-box proteins. *Genes Dev* **18**, 2573–2580.
- [35] Pan ZQ, Kentsis A, Dias DC, Yamoah K, and Wu K (2004). Nedd8 on cullin: building an expressway to protein destruction. *Oncogene* **23**, 1985–1997.
- [36] Rabut G and Peter M (2008). Function and regulation of protein neddylation. ‘Protein modifications: beyond the usual suspects’ review series. *EMBO Rep* **9**, 969–976.
- [37] Xirodimas DP (2008). Novel substrates and functions for the ubiquitin-like molecule NEDD8. *Biochem Soc Trans* **36**, 802–806.
- [38] Deshaies RJ, Emberley ED, and Saha A (2010). Control of cullin-RING ubiquitin ligase activity by Nedd8. In *Conjugation and Deconjugation of Ubiquitin Family Modifiers: Subcellular Biochemistry*. M Groettrup (Ed). Springer, NY. Vol. 54. pp. 41–56.
- [39] Merlet J, Burger J, Gomes JE, and Pintard L (2009). Regulation of cullin-RING E3 ubiquitin-ligases by neddylation and dimerization. *Cell Mol Life Sci* **66**, 1924–1938.
- [40] Gong L and Yeh ET (1999). Identification of the activating and conjugating enzymes of the NEDD8 conjugation pathway. *J Biol Chem* **274**, 12036–12042.
- [41] Liakopoulos D, Doenges G, Matuschewski K, and Jentsch S (1998). A novel protein modification pathway related to the ubiquitin system. *EMBO J* **17**, 2208–2214.
- [42] Osaka F, Kawasaki H, Aida N, Saeki M, Chiba T, Kawashima S, Tanaka K, and Kato S (1998). A new NEDD8-ligating system for cullin-4A. *Genes Dev* **12**, 2263–2268.
- [43] Huang DT, Ayrault O, Hunt HW, Taherhoy AM, Duda DM, Scott DC, Borg LA, Neale G, Murray PJ, Roussel MF, et al. (2009). E2-RING expansion of the NEDD8 cascade confers specificity to cullin modification. *Mol Cell* **33**, 483–495.
- [44] Kamura T, Conrad MN, Yan Q, Conaway RC, and Conaway JW (1999). The Rbx1 subunit of SCF and VHL E3 ubiquitin ligase activates Rub1 modification of cullins Cdc53 and Cul2. *Genes Dev* **13**, 2928–2933.
- [45] Morimoto M, Nishida T, Nagayama Y, and Yasuda H (2003). Nedd8-modification of Cul1 is promoted by Roc1 as a Nedd8-E3 ligase and regulates its stability. *Biochem Biophys Res Commun* **301**, 392–398.
- [46] Yang X, Zhou J, Sun L, Wei Z, Gao J, Gong W, Xu RM, Rao Z, and Liu Y (2007). Structural basis for the function of DCN-1 in protein neddylation. *J Biol Chem* **282**, 24490–24494.
- [47] Broemer M, Tenev T, Rigbolt KT, Hempel S, Blagoev B, Silke J, Ditzel M, and Meier P (2010). Systematic *in vivo* RNAi analysis identifies IAPs as NEDD8-E3 ligases. *Mol Cell* **40**, 810–822.
- [48] Nalepa G, Rolfe M, and Harper JW (2006). Drug discovery in the ubiquitin-proteasome system. *Nat Rev Drug Discov* **5**, 596–613.
- [49] Chen D, Frezza M, Schmitt S, Kanwar J, and Dou QP (2011). Bortezomib as the first proteasome inhibitor anticancer drug: current status and future perspectives. *Curr Cancer Drug Targets* **11**, 239–253.
- [50] Kane RC, Bross PF, Farrell AT, and Pazdur R (2003). Velcade: U.S. FDA approval for the treatment of multiple myeloma progressing on prior therapy. *Oncologist* **8**, 508–513.
- [51] Bedford L, Lowe J, Dick LR, Mayer RJ, and Brownell JE (2011). Ubiquitin-like protein conjugation and the ubiquitin-proteasome system as drug targets. *Nat Rev Drug Discov* **10**, 29–46.
- [52] Orłowski RZ and Kuhn DJ (2008). Proteasome inhibitors in cancer therapy: lessons from the first decade. *Clin Cancer Res* **14**, 1649–1657.
- [53] Richardson PG, Mitsiades C, Hideshima T, and Anderson KC (2006). Bortezomib: proteasome inhibition as an effective anticancer therapy. *Annu Rev Med* **57**, 33–47.
- [54] Shah MA, Power DG, Kindler HL, Holen KD, Kemeny MM, Ilson DH, Tang L, Capanu M, Wright JJ, and Kelsen DP (2011). A multicenter, phase II study of bortezomib (PS-341) in patients with unresectable or metastatic gastric and gastroesophageal junction adenocarcinoma. *Invest New Drugs* **29**, 1475–1481.
- [55] Pajonk F, Pajonk K, and McBride WH (2000). Apoptosis and radiosensitization of Hodgkin cells by proteasome inhibition. *Int J Radiat Oncol Biol Phys* **47**, 1025–1032.
- [56] Russo SM, Tepper JE, Baldwin AS Jr, Liu R, Adams J, Elliott P, and Cusack JC Jr (2001). Enhancement of radiosensitivity by proteasome inhibition: implications for a role of NF- κ B. *Int J Radiat Oncol Biol Phys* **50**, 183–193.
- [57] Motegi A, Murakawa Y, and Takeda S (2009). The vital link between the ubiquitin-proteasome pathway and DNA repair: impact on cancer therapy. *Cancer Lett* **283**, 1–9.
- [58] Lioni M, Noma K, Snyder A, Klein-Szanto A, Diehl JA, Rustgi AK, Herlyn M, and Smalley KS (2008). Bortezomib induces apoptosis in esophageal squamous cell carcinoma cells through activation of the p38 mitogen-activated protein kinase pathway. *Mol Cancer Ther* **7**, 2866–2875.
- [59] Kamer S, Ren Q, and Dicker AP (2009). Differential radiation sensitization of human cervical cancer cell lines by the proteasome inhibitor velcade (bortezomib, PS-341). *Arch Gynecol Obstet* **279**, 41–46.
- [60] Huang CY, Wei CC, Chen KC, Chen HJ, Cheng AL, and Chen KF (2012). Bortezomib enhances radiation-induced apoptosis in solid tumors by inhibiting CIP2A. *Cancer Lett* **317**, 9–15.
- [61] Grimes KR, Daosukho C, Zhao Y, Meigooni A, and St Clair W (2005). Proteasome inhibition improves fractionated radiation treatment against non-small cell lung cancer: an antioxidant connection. *Int J Oncol* **27**, 1047–1052.
- [62] Pajonk F, van Ophoven A, Weissenberger C, and McBride WH (2005). The proteasome inhibitor MG-132 sensitizes PC-3 prostate cancer cells to ionizing radiation by a DNA-PK-independent mechanism. *BMC Cancer* **5**, 76.
- [63] Vlashi E, Mattes M, Lagadec C, Donna LD, Phillips TM, Nikolay P, McBride WH, and Pajonk F (2010). Differential effects of the proteasome inhibitor NPI-0052 against glioma cells. *Transl Oncol* **3**, 50–55.
- [64] O’Neil BH, Raftery L, Calvo BF, Chakravarthy AB, Ivanova A, Myers MO, Kim HJ, Chan E, Wise PE, Caskey LS, et al. (2010). A phase I study of bortezomib in combination with standard 5-fluorouracil and external-beam radiation therapy for the treatment of locally advanced or metastatic rectal cancer. *Clin Colorectal Cancer* **9**, 119–125.
- [65] Edelman MJ, Burrows W, Krasna MJ, Bedor M, Smith R, and Suntharalingam M (2010). Phase I trial of carboplatin/paclitaxel/bortezomib and concurrent radiotherapy followed by surgical resection in stage III non-small cell lung cancer. *Lung Cancer* **68**, 84–88.
- [66] Nakayama KI and Nakayama K (2006). Ubiquitin ligases: cell-cycle control and cancer. *Nat Rev Cancer* **6**, 369–381.
- [67] Frescas D and Pagano M (2008). Deregulated proteolysis by the F-box proteins SKP2 and β -TrCP: tipping the scales of cancer. *Nat Rev Cancer* **8**, 438–449.
- [68] Welcker M and Clurman BE (2008). FBW7 ubiquitin ligase: a tumour suppressor at the crossroads of cell division, growth and differentiation. *Nat Rev Cancer* **8**, 83–93.
- [69] Huang Y, Duan H, and Sun Y (2001). Elevated expression of SAG/ROC2/Rbx2/Hrt2 in human colon carcinomas: SAG does not induce neoplastic transformation, but antisense transfection inhibits tumor cell growth. *Mol Carcinog* **30**, 62–70.
- [70] Jia L, Soengas MS, and Sun Y (2009). ROC1/RBX1 E3 ubiquitin ligase silencing suppresses tumor cell growth via sequential induction of G2-M arrest, apoptosis, and senescence. *Cancer Res* **69**, 4974–4982.

- [71] Jia L, Yang J, Hao X, Zheng M, He H, Xiong X, Xu L, and Sun Y (2010). Validation of SAG/RBX2/ROC2 E3 ubiquitin ligase as an anticancer and radiosensitizing target. *Clin Cancer Res* **16**, 814–824.
- [72] Sun Y (1999). Alterations of SAG mRNA in human cancer cell lines: requirement for the RING finger domain for apoptosis protection. *Carcinogenesis* **20**, 1899–1903.
- [73] Yang ES and Park JW (2006). Regulation of nitric oxide-induced apoptosis by sensitive to apoptosis gene protein. *Free Radic Res* **40**, 279–284.
- [74] Kim SY, Kim MY, Mo JS, Park JW, and Park HS (2007). SAG protects human neuroblastoma SH-SY5Y cells against 1-methyl-4-phenylpyridinium ion (MPP⁺)-induced cytotoxicity via the downregulation of ROS generation and JNK signaling. *Neurosci Lett* **413**, 132–136.
- [75] Lee SJ, Yang ES, Kim SY, Shin SW, and Park JW (2008). Regulation of heat shock-induced apoptosis by sensitive to apoptosis gene protein. *Free Radic Biol Med* **45**, 167–176.
- [76] He H, Gu Q, Zheng M, Normolle D, and Sun Y (2008). SAG/ROC2/RBX2 E3 ligase promotes UVB-induced skin hyperplasia, but not skin tumors, by simultaneously targeting c-Jun/AP-1 and p27. *Carcinogenesis* **29**, 858–865.
- [77] Chalaris A, Sun Y, Latchman DS, and Stephanou A (2003). SAG attenuates apoptotic cell death caused by simulated ischaemia/reoxygenation in rat cardiomyocytes. *J Mol Cell Cardiol* **35**, 257–264.
- [78] Kim DW, Lee SH, Jeong MS, Sohn EJ, Kim MJ, Jeong HJ, An JJ, Jang SH, Won MH, Hwang IK, et al. (2010). Transduced Tat-SAG fusion protein protects against oxidative stress and brain ischemic insult. *Free Radic Biol Med* **48**, 969–977.
- [79] Yang GY, Pang L, Ge HL, Tan M, Ye W, Liu XH, Huang FP, Wu DC, Che XM, Song Y, et al. (2001). Attenuation of ischemia-induced mouse brain injury by SAG, a redox-inducible antioxidant protein. *J Cereb Blood Flow Metab* **21**, 722–733.
- [80] Wilson GD (2004). Radiation and the cell cycle, revisited. *Cancer Metastasis Rev* **23**, 209–225.
- [81] Jia L, Bickel JS, Wu J, Morgan MA, Li H, Yang J, Yu X, Chan RC, and Sun Y (2011). RBX1 (RING box protein 1) E3 ubiquitin ligase is required for genomic integrity by modulating DNA replication licensing proteins. *J Biol Chem* **286**, 3379–3386.
- [82] Swaroop M, Bian J, Aviram M, Duan H, Bisgaier CL, Loo JA, and Sun Y (1999). Expression, purification, and biochemical characterization of SAG, a RING finger redox-sensitive protein. *Free Radic Biol Med* **27**, 193–202.
- [83] Tan M, Zhu Y, Kovacec J, Zhao Y, Pan ZQ, Spitz DR, and Sun Y (2010). Disruption of Sag/Rbx2/Roc2 induces radiosensitization by increasing ROS levels and blocking NF- κ B activation in mouse embryonic stem cells. *Free Radic Biol Med* **49**, 976–983.
- [84] Salon C, Brambilla E, Brambilla C, Lantuejoul S, Gazzeri S, and Eymin B (2007). Altered pattern of Cul-1 protein expression and neddylation in human lung tumours: relationships with CAND1 and cyclin E protein levels. *J Pathol* **213**, 303–310.
- [85] Chen LC, Manjeshwar S, Lu Y, Moore D, Ljung BM, Kuo WL, Dairkee SH, Wernick M, Collins C, and Smith HS (1998). The human homologue for the *Caenorhabditis elegans cul-4* gene is amplified and overexpressed in primary breast cancers. *Cancer Res* **58**, 3677–3683.
- [86] Melchor L, Saucedo-Cuevas LP, Munoz-Repeto I, Rodriguez-Pinilla SM, Honrado E, Campoverde A, Palacios J, Nathanson KL, Garcia MJ, and Benitez J (2009). Comprehensive characterization of the DNA amplification at 13q34 in human breast cancer reveals TFDP1 and CUL4A as likely candidate target genes. *Breast Cancer Res* **11**, R86.
- [87] Schindl M, Gnant M, Schoppmann SF, Horvat R, and Birner P (2007). Overexpression of the human homologue for *Caenorhabditis elegans cul-4* gene is associated with poor outcome in node-negative breast cancer. *Anticancer Res* **27**, 949–952.
- [88] Yasui K, Arai S, Zhao C, Imoto I, Ueda M, Nagai H, Emi M, and Inazawa J (2002). TFDP1, CUL4A, and CDC16 identified as targets for amplification at 13q34 in hepatocellular carcinomas. *Hepatology* **35**, 1476–1484.
- [89] Hung MS, Mao JH, Xu Z, Yang CT, Yu JS, Harvard C, Lin YC, Bravo DT, Jablons DM, and You L (2011). Cul4A is an oncogene in malignant pleural mesothelioma. *J Cell Mol Med* **15**, 350–358.
- [90] Gupta A, Yang LX, and Chen L (2002). Study of the G2/M cell cycle checkpoint in irradiated mammary epithelial cells overexpressing Cul-4A gene. *Int J Radiat Oncol Biol Phys* **52**, 822–830.
- [91] Wei D, Li H, Yu J, Sebolt JT, Zhao L, Lawrence TS, Smith PG, Morgan MA, and Sun Y (2012). Radiosensitization of human pancreatic cancer cells by MLN4924, an investigational NEDD8-activating enzyme inhibitor. *Cancer Res* **72**, 282–293.
- [92] Masuda TA, Inoue H, Sonoda H, Mine S, Yoshikawa Y, Nakayama K, and Mori M (2002). Clinical and biological significance of S-phase kinase-associated protein 2 (Skp2) gene expression in gastric carcinoma: modulation of malignant phenotype by Skp2 overexpression, possibly via p27 proteolysis. *Cancer Res* **62**, 3819–3825.
- [93] Shapira M, Ben-Izhak O, Linn S, Futerman B, Minkov I, and Hershko DD (2005). The prognostic impact of the ubiquitin ligase subunits Skp2 and Cks1 in colorectal carcinoma. *Cancer* **103**, 1336–1346.
- [94] Signoretti S, Di Marcotullio L, Richardson A, Ramaswamy S, Isaac B, Rue M, Monti F, Loda M, and Pagano M (2002). Oncogenic role of the ubiquitin ligase subunit Skp2 in human breast cancer. *J Clin Invest* **110**, 633–641.
- [95] Wang XC, Tian LL, Tian J, and Jiang XY (2012). Overexpression of SKP2 promotes the radiation resistance of esophageal squamous cell carcinoma. *Radiat Res* **177**, 52–58.
- [96] Chen Q, Xie W, Kuhn DJ, Voorhees PM, Lopez-Girona A, Mendy D, Corral LG, Krenitsky VP, Xu W, Moutouh-de Parseval L, et al. (2008). Targeting the p27 E3 ligase SCF(Skp2) results in p27- and Skp2-mediated cell-cycle arrest and activation of autophagy. *Blood* **111**, 4690–4699.
- [97] Kudo Y, Guardavaccaro D, Santamaria PG, Koyama-Nasu R, Latres E, Bronson R, Yamasaki L, and Pagano M (2004). Role of F-box protein betaTrcp1 in mammary gland development and tumorigenesis. *Mol Cell Biol* **24**, 8184–8194.
- [98] Belaidouni N, Peuchmaur M, Perret C, Florentin A, Benarous R, and Besnard-Guerin C (2005). Overexpression of human β TrCP1 deleted of its F box induces tumorigenesis in transgenic mice. *Oncogene* **24**, 2271–2276.
- [99] Tang W, Li Y, Yu D, Thomas-Tikhonenko A, Spiegelman VS, and Fuchs SY (2005). Targeting β -transducin repeat-containing protein E3 ubiquitin ligase augments the effects of antitumor drugs on breast cancer cells. *Cancer Res* **65**, 1904–1908.
- [100] Muerkoster S, Arlt A, Sipos B, Witt M, Grossmann M, Kloppel G, Kalthoff H, Folsch UR, and Schafer H (2005). Increased expression of the E3-ubiquitin ligase receptor subunit β TRCP1 relates to constitutive nuclear factor- κ B activation and chemoresistance in pancreatic carcinoma cells. *Cancer Res* **65**, 1316–1324.
- [101] Tan M, Gallegos JR, Gu Q, Huang Y, Li J, Jin Y, Lu H, and Sun Y (2006). SAG/ROC-SCF ^{β -TrCP} E3 ubiquitin ligase promotes pro-caspase-3 degradation as a mechanism of apoptosis protection. *Neoplasia* **8**, 1042–1054.
- [102] Deorukhkar A and Krishnan S (2010). Targeting inflammatory pathways for tumor radiosensitization. *Biochem Pharmacol* **80**, 1904–1914.
- [103] Soldatenkov VA, Dritschilo A, Ronai Z, and Fuchs SY (1999). Inhibition of homologue of Slimb (HOS) function sensitizes human melanoma cells for apoptosis. *Cancer Res* **59**, 5085–5088.
- [104] Aghajan M, Jonai N, Flick K, Fu F, Luo M, Cai X, Ouni I, Pierce N, Tang X, Lomenick B, et al. (2010). Chemical genetics screen for enhancers of rapamycin identifies a specific inhibitor of an SCF family E3 ubiquitin ligase. *Nat Biotechnol* **28**, 738–742.
- [105] Orlicky S, Tang X, Neduva V, Elowe N, Brown ED, Sicheri F, and Tyers M (2010). An allosteric inhibitor of substrate recognition by the SCF(Cdc4) ubiquitin ligase. *Nat Biotechnol* **28**, 733–737.
- [106] Brownell JE, Sintchak MD, Gavin JM, Liao H, Bruzese FJ, Bump NJ, Soucy TA, Milhollen MA, Yang X, Burkhardt AL, et al. (2010). Substrate-assisted inhibition of ubiquitin-like protein-activating enzymes: the NEDD8 E1 inhibitor MLN4924 forms a NEDD8-AMP mimetic *in situ*. *Mol Cell* **37**, 102–111.
- [107] Milhollen MA, Traore T, Adams-Duffy J, Thomas MP, Berger AJ, Dang L, Dick LR, Garnsey JJ, Koenig E, Langston SP, et al. (2010). MLN4924, a NEDD8-activating enzyme inhibitor, is active in diffuse large B-cell lymphoma models: rationale for treatment of NF- κ B-dependent lymphoma. *Blood* **116**, 1515–1523.
- [108] Swords RT, Kelly KR, Smith PG, Garnsey JJ, Mahalingam D, Medina E, Oberheu K, Padmanabhan S, O'Dwyer M, Nawrocki ST, et al. (2010). Inhibition of NEDD8-activating enzyme: a novel approach for the treatment of acute myeloid leukemia. *Blood* **115**, 3796–3800.
- [109] Tan M, Li Y, Yang R, Xi N, and Sun Y (2011). Inactivation of SAG E3 ubiquitin ligase blocks embryonic stem cell differentiation and sensitizes leukemia cells to retinoic acid. *PLoS One* **6**, e27726.
- [110] Milhollen MA, Narayanan U, Soucy TA, Veiby PO, Smith PG, and Amidon B (2011). Inhibition of NEDD8-activating enzyme induces rereplication and apoptosis in human tumor cells consistent with deregulating CDT1 turnover. *Cancer Res* **71**, 3042–3051.

- [111] Jia L, Li H, and Sun Y (2011). Induction of p21-dependent senescence by an NAE inhibitor, MLN4924, as a mechanism of growth suppression. *Neoplasia* **13**, 561–569.
- [112] Lin HK, Chen Z, Wang G, Nardella C, Lee SW, Chan CH, Yang WL, Wang J, Egia A, Nakayama KI, et al. (2010). *Skp2* targeting suppresses tumorigenesis by Arf-p53-independent cellular senescence. *Nature* **464**, 374–379.
- [113] Lin JJ, Milhollen MA, Smith PG, Narayanan U, and Dutta A (2010). NEDD8-targeting drug MLN4924 elicits DNA rereplication by stabilizing Cdt1 in S phase, triggering checkpoint activation, apoptosis, and senescence in cancer cells. *Cancer Res* **70**, 10310–10320.
- [114] Soucy TA, Dick LR, Smith PG, Milhollen MA, and Brownell JE (2010). The NEDD8 conjugation pathway and its relevance in cancer biology and therapy. *Genes Cancer* **1**, 708–716.
- [115] Soucy TA, Smith PG, and Rolfe M (2009). Targeting NEDD8-activated cullin-RING ligases for the treatment of cancer. *Clin Cancer Res* **15**, 3912–3916.
- [116] Goktas S, Baran Y, Ural AU, Yazici S, Aydur E, Basal S, Avcu F, Pekel A, Dirican B, and Beyzadeoglu M (2010). Proteasome inhibitor bortezomib increases radiation sensitivity in androgen independent human prostate cancer cells. *Urology* **75**, 793–798.
- [117] Fu DX, Tanhehco Y, Chen J, Foss CA, Fox JJ, Chong JM, Hobbs RF, Fukayama M, Sgouros G, Kowalski J, et al. (2008). Bortezomib-induced enzyme-targeted radiation therapy in herpesvirus-associated tumors. *Nat Med* **14**, 1118–1122.
- [118] Kubicek GJ, Werner-Wasik M, Machtay M, Mallon G, Myers T, Ramirez M, Andrews D, Curran WJ Jr, and Dicker AP (2009). Phase I trial using proteasome inhibitor bortezomib and concurrent temozolomide and radiotherapy for central nervous system malignancies. *Int J Radiat Oncol Biol Phys* **74**, 433–439.
- [119] Yang D, Tan M, Wang G, and Sun Y (2012). The p21-dependent radiosensitization of human breast cancer cells by MLN4924, an investigational inhibitor of NEDD8 activating enzyme. *PLoS One* **7**, e34079.
- [120] Skaar JR, D'Angiolella V, Pagan JK, and Pagano M (2009). SnapShot: F box proteins II. *Cell* **137**, 1358.e1–1358.e2.
- [121] Milhollen MA, Thomas MP, Narayanan U, Traore T, Riceberg J, Amidon BS, Bence NF, Bolen JB, Brownell J, Dick LR, et al. (2012). Treatment-emergent mutations in NAE β confer resistance to the NEDD8-activating enzyme inhibitor MLN4924. *Cancer Cell* **21**, 388–401.
- [122] Toth JI, Yang L, Dahl R, and Petroski MD (2012). A gatekeeper residue for NEDD8-activating enzyme inhibition by MLN4924. *Cell Rep* **1**, 309–316.
- [123] Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, et al. (2008). Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* **321**, 1801–1806.
- [124] McLendon R, Friedman A, Bigner D, Van Meir EG, Brat DJ, Mastrogiannis M, Olson JJ, Mikkelsen T, Lehman N, Aldape K, et al. (2008). Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* **455**, 1061–1068.
- [125] Liao H, Liu XJ, Blank JL, Bouck DC, Bernard H, Garcia K, and Lightcap ES (2011). Quantitative proteomic analysis of cellular protein modulation upon inhibition of the NEDD8-activating enzyme by MLN4924. *Mol Cell Proteomics* **10**, M111.009183.
- [126] Emanuele MJ, Elia AE, Xu Q, Thoma CR, Izhar L, Leng Y, Guo A, Chen YN, Rush J, Hsu PW, et al. (2011). Global identification of modular cullin-RING ligase substrates. *Cell* **147**, 459–474.
- [127] Lee JE, Sweredoski MJ, Graham RL, Kolawa NJ, Smith GT, Hess S, and Deshaies RJ (2011). The steady-state repertoire of human SCF ubiquitin ligase complexes does not require ongoing Nedd8 conjugation. *Mol Cell Proteomics* **10**, M110.006460.
- [128] Bennett EJ, Rush J, Gygi SP, and Harper JW (2010). Dynamics of cullin-RING ubiquitin ligase network revealed by systematic quantitative proteomics. *Cell* **143**, 951–965.
- [129] Jung J, Kim EJ, Chung HK, Park HJ, Jeong SY, and Choi EK (2012). c-Myc down-regulation is involved in proteasome inhibitor-mediated enhancement of radiotherapeutic efficacy in non-small cell lung cancer. *Int J Oncol* **40**, 385–390.
- [130] Warren G, Grimes K, Xu Y, Kudrimoti M, and St Clair W (2006). Selectively enhanced radiation sensitivity in prostate cancer cells associated with proteasome inhibition. *Oncol Rep* **15**, 1287–1291.
- [131] Munshi A, Kurland JF, Nishikawa T, Chiao PJ, Andreeff M, and Meyn RE (2004). Inhibition of constitutively activated nuclear factor- κ B radiosensitizes human melanoma cells. *Mol Cancer Ther* **3**, 985–992.