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

Dongping Wei, Meredith A. Morgan and Yi Sun

Division of Radiation and Cancer Biology, Department of Radiation Oncology, University of Michigan, Ann Arbor, MI 48109

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.

Address all correspondence to: Yi Sun, MD, PhD, Division of Radiation and Cancer Biology, Department of Radiation Oncology, University of Michigan, 4424B Medical Science-I, 1301 Catherine Street, Ann Arbor, MI 48109. E-mail: sunyi@umich.edu
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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-κB) 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 carfizomib, 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-κB [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
UPS inhibitors				
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]
CRL inhibitor				
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 otherwise 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-κB activation associated with accumulation of inhibitor κB (IκB) [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 $SCF^{β\text{-}TrCP}$ 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., IκB, 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-κB activity and chemoresistance, whereas β-TrCP1 knockdown decreased NF-κB activity and chemoresistance in pancreatic cancer cells [100] and sensitized cervical cancer cells to apoptosis [101]. Given the important role of NF-κB 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 specific 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, IκBα, 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 NAEβ [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.

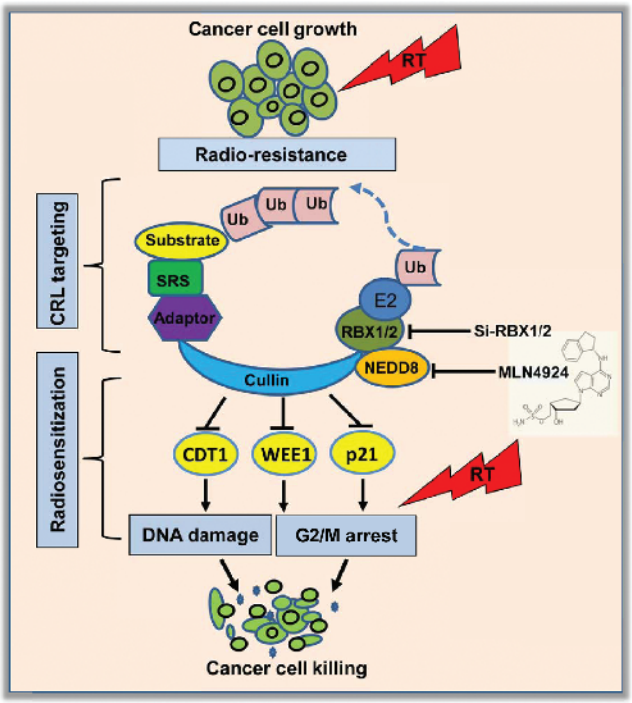

Figure 1. Cancer cell radiosensitization by inactivation of CRL E3: Inactivation of CRL ligase activity by siRNA silencing of its components (e.g., RBX1/RBX2) or by the small-molecule MLN4924 which inhibits cullin neddylation, causes accumulation of CRL substrates. Accumulation of some of these substrates, 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.

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