Targeting E3 ubiquitin ligases to sensitize cancer radiation therapy

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Abstract
Radiotherapy is an effective treatment for many cancer patients to eliminate malignant cells and increase survival rate. However, cancer cells can develop resistance in response to radiation through activation of signaling pathways that promote cell cycle progression, DNA damage response, cell survival, and inflammation. Various combination therapies are developed to sensitize radiotherapy by targeting key signaling proteins involved in radioresponse. The past decade has seen significant advances in the knowledge of ubiquitin signaling in cancer biology. Developing E3 ubiquitin ligase-related molecules as novel strategies to cure cancer has become an emerging field. This review briefly discusses the potential of targeting diverse E3 ubiquitin ligases as promising strategies for radiosensitization in cancer.

KEYWORDS

cancer, E3 ligase, inhibitor, radiotherapy, ubiquitin

1 | INTRODUCTION

Cancer radiotherapy is widely used as a standard first-line treatment for numerous types of cancer.1 The purpose of radiotherapy is to kill cancer cells or inhibit their growth through high-energy radiation. Radiation generates ions that pass through cells, damaging the genomic DNA of cells, and thus blocking their ability to proliferate and causing cell death.2 Although radiation could impair both normal and cancer cells, normal cells usually more readily recover than cancer cells, because cancer cells are defective in DNA repair machinery.2,3 Radiotherapy, combined with surgery and chemotherapy, has significantly improved the survival rates of many cancer patients.4,5 However, the therapeutic effect of radiation still remains unsatisfactory in many cases where tumor cells develop radioresistance and normal tissues suffer from severe adverse reactions to irradiation.3,4 Recent advances in our understanding of the cellular pathways in response to radiotherapy have opened up possibilities to develop new therapies to achieve radiosensitization. Radiation triggers various pathways and key signaling molecules that regulate DNA repair, cell cycle control, apoptosis, autophagy and inflammation, and ubiquitination signaling functions as fundamental machinery underlying these cellular processes.3,4 In the present review, we briefly summarize the ubiquitin E3 ligases that are potential targets to sensitize cancer radiation therapy.

The ubiquitination system involves E1-activating enzymes, E2 conjugation enzymes, and E3 ligases working in unison to covalently attach ubiquitin to the lysines of substrate proteins.7 The ubiquitin–proteasome system is the major protein degradation machinery in the cell to define the proteome at the post-translational level, which is dependent on Lysine-48 (K48) polyubiquitin chains that mark substrate proteins for proteasomal degradation.7–9 The indication of targeting ubiquitin signaling to sensitize cancer radiation therapy is supported by the fact that bortezomib, a clinically approved proteasome inhibitor to treat several cancers, has been shown to be a tumor radiosensitizer.10–13 However, such proteasome inhibitors are problematic, in that they non-selectively block protein degradation, thus impacting too many pathways and leading to severe toxicity. The new direction of therapeutic development is focused on targeting individual or subtypes of over 600 ubiquitin E3 ligases, which confer specificity of substrates.7,14 Other than ubiquitin–proteasome system, there are various other types of polyubiquitination or monoubiquitination that serve as non-proteolytic signals in DNA repair, subcellular...
localization, and other signal transduction pathways. There are three types of E3 ligases, including RING E3s, HECT E3s, and RING-between-RING (RBR) E3s. In the present review, we discuss the indication of members in these three major E3 families as targets for radiosensitization.

2 | RING E3 LIGASES

RING-type E3 ligases are specified by >600 human genes, comprising >90% of E3 ligases. Distinct from HECT and RBR E3 ligases, which contain catalytic cysteine, forming a thioester bond with ubiquitin, and directly transferring ubiquitin to substrate, RING E3s catalyze the ubiquitination reaction by bringing E2–Ub and substrate in proximity, and facilitate the transfer of ubiquitin to substrate proteins. Because of this, RING E3s are different from classical enzymes in that they do not contain catalytic residues. The ubiquitin ligase activity of RING E3s is dependent on protein–protein interactions, which makes them much less "druggable" considering the difficulties of developing molecules that interfere with the protein–protein interactions. Despite such facts, the past decade has seen significant progress in understanding the biochemistry, structure, and regulation of many RING E3 family members, which has led to exciting new directions that can be applied to cancer radiosensitization.

Cullin-RING E3 ubiquitin ligases (CRLs) represent the largest class of E3 ligases, constituting major cellular machinery for re-modeling the proteome, and can account for up to 20% of ubiquitin-dependent protein turnover in the cell. CRLs are modular assemblies with three main elements: a cullin protein as the scaffold, a RING finger protein (RBX1 or RBX2) that recruits ubiquitin-charged E2 enzymes, and a substrate receptor that docks substrate protein in proximity to E2 to facilitate ubiquitin transfer. There are six major cullins (CUL1, CUL2, CUL3, CUL4A, CUL4B, and CUL5), each assembling with a distinct set of >200 substrate receptor modules. CRLs and related enzymes constitute a very dynamic system. Catalyzed by CAND1/2, CRLs undergo active exchange of substrate receptors in response to particular cellular events to turn over specific substrates. Regulation of CRL enzymatic activity also involves dynamic activation/deactivation cycles: conjugation of NEDD8 (neddylation) to cullin activates CRLs, and the COP9 signalosome complex (CSN), which deneddylates cullin, puts CRLs into an inactive state. Such detailed illustration of the regulation of the CRL system enabled discoveries of several targeted therapies for different CRL components, which have been tested for radiosensitization.

Early studies using RNAi to silence RBX1/2, the catalytic subunit of CRLs, confirmed that crude inhibition of CRL activity could sensitize cancer cells to radiation. The role of NEDD8 conjugation on cullins as an essential activating modification makes the neddylation enzymes promising targets to modulate CRL activities and enhance radiation therapeutic efficacy. This is supported by Wang et. al. that silencing of UBC12, the NEDD8 E2 enzyme, sensitizes prostate cancer cells to radiation. In 2009, a small-molecule inhibitor called MLN4924 was developed to specifically target the neddylation E1 enzyme, NAE, potently blocking cullin neddylation and activation. MLN4924 treatment leads to deneddylation within 1 h, followed by accumulation of numerous CRL substrates impacting multiple facets of biology. Indeed, MLN4924 has been shown to exert a radiosensitization effect in many cancer types, including pancreatic, lung, breast, neck-and-oral, prostate, and blood tumors. In these studies, it is well established that MLN4924 sensitizes cancer radiotherapy through inducing G2/M cell cycle arrest and DNA damage response. The underlying mechanism is that MLN4924 treatment results in an accumulation of CRL substrates, such as Weel, p21, p27, and Cdt1, regulators of cell cycle and DNA damage response. Knocking down p21, Weel, or Cdt1 could attenuate the radiosensitizing effect of MLN4924. Although most studies attribute the mechanism of MLN4924 to the proteins and pathways mentioned above, it is proposed that MLN4924 mediates radiosensitization in different cancer types through different mechanisms. Considering the diversity of the CRL pool and their substrates, it is plausible that the repertoire of active CRLs in different cancer types could be variable, and they might respond differently to MLN4924. It is also a major concern that MLN4924 as a non-selective inhibitor of CRL system could cause toxicity in normal tissues. Therefore, the efforts to develop inhibitors targeting other CRL components is desired to further explore the therapeutic potential of the CRL system in radiation oncology.

Recently, Scott et. al. developed a set of specific and potent inhibitors targeting DCN1, a subunit of the NEDD8 E3. Compared with MLN4924, these compounds also block cullin neddylation effectively, but only dramatically inhibit certain types of cancer cells with DCN1 amplification. Other than the neddylation enzymes, Schnierf et al. tried to target CSN, which is the deneddylation machinery in the CRL system, by developing a new inhibitor called CSN5i-3 to inhibit CSN5, the proteolytic subunit of CSN. Previous biochemical characterization of the CRL system has clearly shown the importance of active cycles of neddylation/deneddylation together with CAND1/2-mediated substrate receptor exchange to maintain normal function of CRLs. Inhibition of deneddylation could profoundly alter the activity of CRLs. CSN5i-3 treatment could dramatically increase cullin neddylation levels, and lead to inactivation of a subset of CRLs and accumulation of their substrates. Phenotypically, CSN5i-3 induces a differentiating impact on cancer cells and inhibits cancer cell growth. Another very exciting direction of harnessing the CRL system is CUL1-TrCP-, CUL2-VHL-, and CUL4-CRBN-based ProTAC (proteolysis targeting chimera). This new technology utilizes bivalent molecules that bring E3 ligases and another targeted protein in proximity, inducing selective degradation. ProTAC opens up a new avenue to pharmacologically target many undruggable proteins for cancer radiation therapies. It remains to be investigated whether these new CRL-related inhibitors can be applied to sensitize cancer radiotherapy.

In addition to the promising results of CRLs, there are other members in the RING E3 family indicated as putative targets for cancer radiosensitization. Anaphase promoting complex (APC/C) is a...
central cell cycle regulator. It is shown that silencing of anaphase promoting complex enhances radiosensitivity of nasopharyngeal cancer cells through blocking G2/M-cell cycle progression. Similar to anaphase promoting complex, knocking down of the E3 ligase C-terminus of Hsc70-interacting protein leads to radiosensitization of lung cancer cells by interrupting cell cycle progression. C-terminus of Hsc70-interacting protein directly ubiquitinates p21 and C-terminus of Hsc70-interacting protein depletion caused p21 accumulation after radiation. XIAP or RNF2 silencing could sensitize esophageal cancers to radiotherapy by promoting G1/S phase cell cycle arrest and apoptosis. RNF8 depletion inhibits DNA damage response and radioresistance of bladder cancer cells. RNF138 can target rpS3, a ribosome subunit, which has been shown to interact with nuclear factor-κB in the nucleus, mediating radioresistance of glioblastoma cells. Silencing of RNF138 enhances radiotherapy of glioblastoma. These studies showed the radiosensitization effect of targeting these RING E3s, and there are probably many more RING E3 ligases involved in radioreponse signaling. Developing specific inhibitors for these RING E3s is a challenging, but exciting, direction in the future.

3 | HECT AND RBR E3 LIGASES

As mentioned above, HECT and RBR E3 ligases contain a catalytic cysteine that receives ubiquitin from E2, forming an E3–Ub thioester, and then transfers the ubiquitin to substrate proteins. There are much fewer members in the HECT and RBR families, and studies directly investigating the radiosensitizing effect of them are generally lacking. However, recent progress in the mechanistic insights into the autoregulatory mechanisms of these enzymes has led to the discovery of novel inhibitors, which showed promising antitumor activity. Considering the vital roles these E3s are playing in controlling numerous cellular functions in cancer cells, it is conceivable that therapies targeting some members in the HECT and RBR families will greatly improve radiotherapy.

There are 32 HECT E3s in the human genome, which are featured by comprising a HECT catalytic domain. Many HECT members are shown to ubiquitinate critical signaling protein molecules in the pathways responding to radiation-induced DNA damage response and signal transduction, making them attractive targets for developing radiosensitizers.

p53 is an important tumor suppressor protein that is posttranscriptionally activated upon ionizing radiation induced DNA damage. p53 is the most frequently mutated gene in cancer, and its downregulation protects tumor cells from apoptotic cell death. Reactivation of p53 by targeting p53 degrading E3 ligases might be an appealing strategy to sensitize radiotherapy. In mucosal human papillomavirus-induced epithelial tumors, including cervical carcinoma and some head-and-neck cancers, E6AP can be hijacked by human papillomavirus oncoprotein E6 to ubiquitinate and degrade p53. Other HECT E3s, such as HUWE1 and WWP1, have also been shown to regulate p53 levels. In a syngeneic tumor mouse model, HUWE1 inactivation inhibits lung cancer tumorigenesis in a p53-dependent manner. Deletion of HUWE1 leads to p53 protein accumulation, growth arrest, and apoptosis in MYC-driven B-cell lymphomas. Knocking down WWPP1 leads to p53 accumulation and HCC cell apoptosis. Interestingly, instead of degrading p53, WWP1 stabilizes p53, leading to its cytoplasmic accumulation and reduction of transcriptional activity.

Constitutively active EGFR is overexpressed in many cancer types and is associated with poor prognosis. Ionizing radiation can activate EGFR and its downstream signaling pathway, and EGFR-targeted therapy has been approved by the FDA for the treatment of head-and-neck cancer in combination with radiation. HECT E3 ligase SMURF2 has been demonstrated as an E3 for EGFR. Intriguingly, SMURF2-catalyzed EGFR ubiquitination is not a proteolytic, but rather, stabilizing signal that protects EGFR from c-Cbl-mediated degradation. Depleting SMURF2 destabilizes EGFR and significantly inhibits head-and-neck squamous cancer cell line UM-SCC74B growth in a nude mice tumor model. EGFR downstream pathways include the pro-proliferative and pro-survival PI3K/PTEN/AKT pathway. Targeting E3 ligases for PTEN might synergize with EGFR blockade to sensitize radiation therapy. Several HECT E3s, including NEDD4-1, WWP2, and WWP1, have been shown to ubiquitinate PTEN. Knocking down WWP1 or WWP2 renders an increase in PTEN protein levels, along with decreased AKT phosphorylation and inhibited tumor growth. However, the underlying mechanisms are distinct among these HECT E3s. WWP1 catalyzes non-degradative K27-linked polyubiquitination of PTEN, which suppresses its dimerization, membrane recruitment, and tumor suppressor functions both in vitro and in vivo. WWP2 polyubiquitinates PTEN through K48-linked ubiquitin chains and leads to its proteasomal degradation. WWP2 is abnormally expressed in various types of cancers, including human oral cancer, endometrial cancer, liver cancer, glioma, and lung cancer.

Recent advances in the structural and biochemical characterization of HECT E3s has revealed molecular details of their autoregulation and catalysis mechanisms. The activity of HECT E3s is tightly regulated through various autoinhibitory mechanisms, such as intramolecular autoinhibitory domains and dimerization, and they can be activated through phosphorylation, binding to ubiquitin at the allosteric site or interacting with other proteins. The biochemical understanding of HECT E3s has assisted identification of novel molecules that can modulate their activity. By using a phage library, Mund et al. identified bicyclic peptides that block the binding of E2 to the HECT domains of SMURF2, NEDD4-1, WWP1, and HUWE1, and further improvement of the most promising peptide gives rise to Heclin (HECT E3 ligase inhibitor), which induces a conformational change that renders the catalytic cysteine residue more susceptible to oxidation. Indole-3-carbinol is a natural compound found in vegetables, such as broccoli, cauliflower, and cabbage, which exerts the ability to inhibit NEDD4-1 and WWP1, and thus activates PTEN in tumor cells. Given the fact that p53 and PTEN are two important players in regulating radiosensitivity and radioresistance, their ubiquitin E3s inhibitors could be potential radiosensitizers, whereas such investigations are yet to be carried out.
4 | CONCLUSION AND FUTURE PERSPECTIVES

Targeting ubiquitin E3 ligases has proved to be a promising direction to improve the efficacy of cancer radiotherapy. In the past decades, numerous seminal breakthroughs have been made in basic science to understand the structure, biochemistry and regulation of various E3 ligases, as well as in translational research, which unraveled the roles of different E3s in cancer, and targeted compounds for them, showing therapeutic potentials. Accumulating evidence from pre-clinical studies using RNAi justified that many E3 ligases are candidate targets for radiosensitizers. However, there is still little known about most members of the large E3 ligase family. Current advanced high-throughput technologies, such as RNA sequencing, CRISPR screening, and proteomics, should be carried out to uncover new E3 ligases with critical functions in cancer and radioresponse. Furthermore, there are dozens of new inhibitors for the ubiquitin system that remain to be tested for radiosensitization, such as DCN1 inhibitors, CSN5i-3, and Heclin. With the enthusiasm of both academia and industry in developing therapies targeting E3s nowadays, it is likely that more and more new compounds will be made available, and it will be exciting to explore their radiosensitizing effect in the future.

CONFLICT OF INTEREST

The authors declare that they had read the article and there are no competing interests.

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