

## The Inhibition of DNA Damage Response Contributes to Cancer Radiotherapy

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**Conflict-of-interest statement:** The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper.

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Received: January 3, 2018

Revised: February 1, 2017

Accepted: February 3, 2018

Published online: April 16, 2018

### ABSTRACT

The DNA damage response (DDR) is essential for maintaining the genomic integrity of the cell, and its disruption is one of the hallmarks of cancer. More than 50 % of cancer patients will receive some kinds of radiotherapy during their treatment process, either alone or in combination with other treatment modalities. Targeted therapy based on inhibiting the DDR in cancers offers a greater treatment pathway to patients with tumors by impeding DSBs repair. These have led to the development of radiotherapy combined with pharmacological interventions, which are more specifically targeting tumor, leading to the improvements in cancer therapeutic efficacy. This review highlights the different target DDR proteins' inhibitors in cancer and how this can provide significant opportunities for DDR-based sensitivity of radiotherapies in the future.

**Key words:** Cancer radiotherapy; DDR; DDR inhibitors

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Sun M, Chen Y, Hu B. The Inhibition of DNA Damage Response Contributes to Cancer Radiotherapy. *International Journal of Radiology* 2018; 5(1): 149-156 Available from: URL: <http://www.ghrnet.org/index.php/ijr/article/view/2113>

### INTRODUCTION

Radiotherapy (RT) is one of the standard treatment options for some solid malignant cancers. More than 50 % of cancer patients will receive some kinds of RT during their treatment process, either alone or in combination with other treatment modalities<sup>[1]</sup>. For early stage larynx cancer and non-small cell lung cancer, survival rates after RT are high, unfortunately, the therapeutic outcomes in many cases such as glioblastomas, sarcomas and advanced non-small cell lung cancer are not so satisfactory<sup>[2]</sup>. Ionizing radiation (IR) usually triggers proapoptotic signals in cells with irreparable DNA damage or active DNA repair of survived cells, whereas tumor radioresistance leads to a reduction in the efficiency of RT or adaptation to the frequent DNA

damage caused by repeated irradiation with corresponding tumor recurrence and metastasis and thus the treatment failure in many patients after RT<sup>[3]</sup>. Therefore, it is important to investigate the cellular mechanisms leading to this loss of radiosensitivity and to find potential therapy measure which might significantly improve the efficacy of RT. Radioresistance has been shown to be associated with increased cellular functions such as stress responses and DNA repair<sup>[4]</sup>. The process of DNA repair is closely coupled with the DNA damage response (DDR), which involves a cellular mechanism that protects against DNA damage induced by endogenous and exogenous factors. In recent years, the DDR has been recognized as an important innate tumor suppressor pathway<sup>[5-7]</sup>. Inhibition of the DNA damage repair pathway may represent a valid therapeutic approach to fight cancers. Thus, DDR inhibitors should increase the cell killing effects of radiation on cancer cells by increasing the cells' radiosensitivity, without toxicity to the normal cells or tissue meanwhile. It is the ideal thought to use the DDR inhibitor as an anticancer auxiliary strategy in radiotherapy.

## DDR IS AN ANTICANCER BARRIER

The DDR pathway, a cooperation of complex DNA repair and cell cycle control pathways, has evolved to help cells manage DNA damage burden<sup>[8]</sup>. IR can produce DNA double-strand breaks (DSBs), which are considered to be lethal DNA lesions within the cells. Misrepaired DSBs can lead to chromosomal rearrangements such as translocations and deletions, resulting in oncogenic transformation or cell death<sup>[9]</sup>. For DSBs repair, higher eukaryotes possess two principal mechanisms: non-homologous end-joining (NHEJ) and homologous recombination (HR)<sup>[10]</sup>. HR uses sister-chromatid sequences as the template to mediate faithful repair of DNA DSBs, whereas NHEJ utilizes limited or no homology for end joining. HR is generally restricted to S and G2 phases of the cell cycle and plays a major role in the repair of DSBs. In contrast, NHEJ can operate throughout the cell cycle in higher eukaryotes<sup>[11, 12]</sup>. When a cell encounters DNA damage that is more difficult to repair, the DDR machinery delays cell-cycle checkpoints to provide more time for the repair of the lesions<sup>[5]</sup>. Thus, the DDR machinery of tumor cells emerge as an anti-cancer barrier and undermine the effectiveness of therapy.

## THE DDR INHIBITORS AS ANTICANCER DRUGS

DDR mechanisms are also relevant to the effectiveness of standard cancer treatments including RT. RT treatments rely on the induction of DNA damage, which is particularly cytotoxic for proliferating cells and, hence, very effective in targeting highly proliferative cancer cells. Cancer cells can, however, resist the lethal effects of genotoxic cancer therapy by activating the DDR. Regulators of the DDR have therefore become attractive targets for cancer therapy. In fact, an active DDR machinery is essential for the physiology of the cell, ensuring its survival, and is an important mechanism of resistance to cytotoxic approaches. Accordingly, the inhibition of the DDR in tumor cells provides an excellent therapeutic opportunity<sup>[13]</sup>. Inhibitors of the DDR machinery have been used successfully against tumor cells in mono- or combination therapy in order to sensitize tumor cells to the cytotoxic activity. Thus, using DDR inhibitors to increase the effectiveness of standard genotoxic treatments and to help prevent or overcome the development of resistance is an important approach.

## INHIBITION OF DDR USING SMALL MOLECULES INCREASE THE TUMOR CELLULAR RADIOSENSITIVITY

### 1 Inhibition of ATM/Chk2 or ATR/Chk1 pathway

The checkpoint response of the DDR relies on two members of the PIKK family of protein kinases: ataxia-telangiectasia-mutated (ATM) and ataxia telangiectasia and Rad3-related (ATR)<sup>[14]</sup>. ATM and ATR proteins are key regulators of the DDR, and maintain genome integrity in eukaryotic cells<sup>[15]</sup>. ATM and ATR share sequence homology, substrates, and functions, but which phosphorylate only protein substrates. Key among these substrates are the serine-threonine checkpoint effector kinases, Chk2 and Chk1, which are selectively phosphorylated and activated by ATM and ATR respectively, to trigger a wide range of distinct downstream responses<sup>[16]</sup>. ATM is activated by DNA double-strand breaks (DSBs) induced by IR or others, whereas ATR is activated by single-stranded DNA breaks (SSBs) coated with replication protein A (RPA)<sup>[17]</sup>. Chk2 is a stable protein expressed throughout the cell cycle<sup>[18]</sup>, it appears to be largely inactive in the absence of DNA damage and its activation involves dimerization and autophosphorylation. In contrast, the labile Chk1 protein is largely restricted to S and G2 phases<sup>[18]</sup>, it is active even in unperturbed cell cycles<sup>[19]</sup> and although it is further activated in response to DNA damage or stalled replication, this may not require Chk1 dimerization or autophosphorylation. Loss of ATM or ATR activity causes similar cellular phenotypes, including loss of cell cycle checkpoints and increased sensitivity to DNA damage<sup>[20]</sup>. The effects of inhibiting ATR/Chk1 or ATM/Chk2 on DNA damage and replication checkpoint have been widely explored by using chemical inhibition approaches.

Powell *et al.* found that a methyl xanthine named caffeine can inhibit the function of both ATM and ATR in defective p53 cancer cells to the lethal effects of genotoxic modalities, particularly caused by IR<sup>[21-23]</sup>. But the clinically useful radiosensitizing agent about caffeine was limiting to the systemic toxicity at the doses required for radiosensitization and the low serum levels that can be achieved in patients<sup>[24]</sup>. Starting out from the chemical structure of the phosphatidylinositol 3-kinase (PI3K) inhibitor LY294002, the first potent and selective ATM inhibitor, KU-55933 was developed<sup>[25]</sup>. The related molecule KU-58050, which has a piperidine replacement to the morpholine moiety of KU-55933 was subsequently developed. The IC<sub>50</sub> for ATM is ~230 times higher than that for KU-55933 and thus serves as a useful negative control compound for the cellular studies<sup>[26]</sup>. It was shown to be more effective at blocking radiation-induced phosphorylation of ATM downstream targets than KU-55933 and to possess greater potency as a radiosensitizer<sup>[27]</sup>. Even though KU-60019 showed better solubility in aqueous solutions than KU-55933, bio-availability was still poor, limiting utility for *in vivo* studies. Recently, another ATM inhibitor named KU-559403 has been described. Comparing with KU-60019, KU-559403 exhibits improved solubility and bio-availability. In subcutaneous tumor xenografts, concentrations above those required for *in vitro* activity were reached and maintained for at least 4 h, making this the first reported *in vivo* active ATM inhibitor<sup>[28]</sup>.

Nishida *et al.* first reported that Schisandrin B, a naturally-occurring dibenzocyclooctadiene lignan found in the medicinal herb *Schisandra chinensis*, was a selective inhibitor of ATR in 2009<sup>[29]</sup>. However, the inhibitory potency against ATR was weak and required the use of high drug concentrations<sup>[29]</sup>. Recently, the first series of potent and selective ATR inhibitors were discovered. NU6027 is a

more potent ATR inhibitor, was reported in 2011 and demonstrated to sensitize several breast and ovarian cancer cell lines to IR<sup>[30]</sup>. However, this compound was originally developed as a CDK2 inhibitor and is not selective for ATR. VE-821 and VX-970 are worthy of consideration for clinical trials in combination with Top1 inhibitors<sup>[31]</sup>. Moreover, VX-970 was the first selective ATR inhibitor to enter clinical development. Importantly, VX-970 was well tolerated in mice and did not enhance toxicity in normal cells and tissues<sup>[32]</sup>. Another ATR inhibitor AZD6738 is currently being investigated in clinical trials<sup>[33]</sup>. A phase I clinical trial to assess the safety of AZD6738 alone and in combination with RT in patients with solid tumors is currently recruiting (ClinicalTrials.gov: NCT02223923).

AZD7762, SCH900766 and UCN-01 were currently in Phase I clinical trials as Chk1 inhibitors. In preclinical studies AZD7762 has been shown to synergize with IR in a variety of tumor cell lines and xenografts with evidence of greater potency in p53-deficient cells<sup>[34,35]</sup>. Inhibition of Chk1 and Chk2 by AZD7762 was more effective in p53-null Em-myc cells<sup>[36]</sup>. SCH-900766 has been shown to abrogate both the intra-S and G2 checkpoints and is a selective Chk1 inhibitor in combination with IR for the treatment of tumor cells<sup>[35]</sup>. Treatment of Em-myc cells with the selective Chk1 inhibitor PF-0477736 induced an increase in the level of DNA damage<sup>[36]</sup>. UCN-01 was the first Chk1 inhibitor approved for clinical trials, however, undesirable pharmacological characteristics combined with lack of selectivity limited its utility and led to the development of several new and more selective agents.

Small-molecule inhibitors of Chk2 have been evaluated in clinical trials in combination with other therapies. However, the outcomes have been contrasting<sup>[37]</sup>. Conversely, it has been shown that Chk2 inhibition can provide protection from RT or chemotherapy. It is encouraging that Chk2 suppression could sensitize tumors with a p53-deficient background to DNA-damaging therapies<sup>[38]</sup>.

## 2 Inhibition of DNA-PKcs and Ku70/80

DNA-PK catalytic subunit (DNA-PKcs) is the catalytic subunit of DNA-dependent protein kinase (DNA-PK), a PI3K-related kinase similar to ATM and ATR. The NHEJ pathway requires the activity of DNA-PK. After the induction of a DSB, the Ku heterodimer [composed of polypeptides of about 70 (Ku70) and 80 kDa (Ku80)] binds DNA break ends and recruits the DNA-PKcs. DNA-PKcs induces recruitment of repair proteins to DSBs and activation of checkpoints, which leads to the formation of the DNA-PK holo-enzyme<sup>[39,40]</sup>. DNA-PK then forms a functional complex with Artemis, which provides nucleolytic processing activity required to prepare DNA ends for ligation<sup>[41]</sup>. Many studies showed that cells deficient in DNA-PK exhibit hypersensitivity to IR<sup>[40]</sup>. Radiosensitization has been observed with wortmannin<sup>[42]</sup> which is the first identified DNA-PK inhibitor. However, lacking of specificity and *in vivo* toxicity precluded its clinical use<sup>[43]</sup>. Another radiosensitizer named LY294002 is a reversible kinase domain inhibitor with non-selective *in vivo* toxicity<sup>[42]</sup>. NU7026 and NU7441 are selective ATP-competitive inhibitors of DNA-PK undergoing preclinical development. It is reported that the G2 block induced by IR was prolonged and the cells with unrepaired DNA persisted longer in the group treated with NU7441<sup>[44]</sup>. However, the poor pharmacokinetics of NU7026 and NU7441 precluded them from further clinical development<sup>[43]</sup>. NVP-BE235, a PI3K/mTOR inhibitor already in clinical trials, has been reported that can potently inhibit the DNA repair enzymes, DNA-PKcs and ATM, *in vivo*, resulting in abrogation of DNA repair and striking radiosensitization of subcutaneous and orthotopic brain tumors<sup>[45]</sup>. Other DNA-PK inhibitors, such as CC-115 (a DNA-PKcs/mTOR inhibitor), CC-

122 (a DNA-PK inhibitor), ZSTK474 (an ATP-competitive inhibitor of PI3K, also inhibits DNA-PK) and MSC2490484 are currently in preclinical development.

The Ku 70/80 heterodimer protein serves as the central regulating factor during repair of DSBs<sup>[46]</sup>. Initiation of the NHEJ pathway is highly dependent on the Ku70/80 heterodimer<sup>[47]</sup>, making these proteins the most logical choice for inhibition of the entire NHEJ process. Ku70- or 80-depletion sensitized pancreatic cells to IR, suggesting that the idea of developing inhibitors which target the Ku70/80-DNA interaction is acceptable in cancer therapy in the future<sup>[48]</sup>. Inhibition of Ku70/80 can be achieved by direct or indirect mechanisms. Indirect inhibitors lead to the downregulation of Ku 70/80 expression. For example, upon treatment with the HADC inhibitor TSA, Ku70 was acetylated and releases Bax, which then translocates to mitochondria and triggers cytochrome c release, resulting in caspase-dependent death<sup>[49]</sup>. HADC inhibitors have also been reported to induce Ku70 acetylation, thereby diminishing the ability of Ku70 to repair DNA damage<sup>[50]</sup>. Recently, a novel putative small molecule was identified by computational screening. This compound synergistically sensitized human cell lines to radiation treatment, indicating a clear potential to diminish DSBs repair<sup>[51]</sup>. Though the process of searching for Ku70/80 inhibitors, the specific clinic trial inhibitors have not been published.

## 3 Inhibition of PARP

Poly-(ADP-ribose) polymerase (PARP) bind the catalytic domain of the PARP protein, mostly as antagonists of the PARP cofactor  $\beta$ -NAD<sup>+</sup><sup>[52]</sup>. Because of the binding of the inhibitors, the PARP enzymes could be inhibited in the catalytic activity, with the final result of converting SSBs into DSBs and determining cell death in DSB repair deficient cells<sup>[53]</sup>. The PARP inhibitors are selectively lethal to HR-deficient cells<sup>[54]</sup>. The clinical utility of PARP inhibitors as monotherapy is based on the concept of synthetic lethality with loss of BRCA1 or BRCA2 function, where neither PARP inhibition alone nor BRCA deficiency alone is not lethal but the combination is mortal<sup>[55]</sup>. For example, ovarian cancers with impaired HR show increased sensitivity to PARP inhibitors. All clinical PARP inhibitors inhibit both PARP1 and PARP2, highlighting the difference between pharmacological inhibition and a deficiency of both proteins during embryonic development<sup>[56]</sup>. The many *in vitro* experiments demonstrating that PARP-1 inhibitors potentiate the cytotoxicity of IR, and the fact that, *in vivo*, PARP-1 knock out mice show increased sensitivity to these DDR inhibited agents, has stimulated the development of specific PARP-1 inhibitors as potential radiosensitizers<sup>[57]</sup>. A number of PARP inhibitors are in clinical development. AG0146999 was the first used in phase I trial<sup>[55,57]</sup>. For the treatment of patients with BRCA1- and BRCA2- associated cancers, tumor cells lack wild-type BRCA1 or BRCA2 potentially providing a large therapeutic window. In a phase I trial, preliminary observations in patients carrying BRCA mutations with ovarian cancer suggest low toxicities, with some promising indicators of responses measured radiologically and using tumor markers. E7016 is used for oral administration and now being tested in Phase I toxicity studies in combined RT<sup>[58]</sup>. Olaparib (also known as AZD2281) has been approved for clinical use<sup>[59]</sup> in 2014 as a PARP inhibitor for use in women with heavily pretreated ovarian cancers that are associated with defective BRCA genes or as a maintenance therapy in BRCAmutated (germline or somatic) platinum-sensitive, advanced ovarian, fallopian tube or primary peritoneal cancers. Similar antitumor activity to olaparib, including niraparib, rucaparib, and talazoparib, has been documented with other inhibitors that trap PARP<sup>[60]</sup>.

#### 4 Inhibition of MRN

Mre11, Rad50 and Nbs1 form a tight complex (MRN) which is distributed throughout the nuclei of mammalian cells. MRN plays essential functions in the cellular response to DSBs and is involved in virtually all aspects of DNA end metabolism including DSBs repair, DNA damage signaling and genome stability<sup>[61]</sup>. Although the importance of the ATM signaling pathway in DNA repair and cell cycle checkpoints has been established, the MRN complex has emerged as an essential factor in ATM activation<sup>[62]</sup>. After irradiation, the MRN complex rapidly migrates to the sites of DSBs, forming foci which remains until DSBs repair is completed<sup>[63]</sup>. Mirin inhibited MRN complex-dependent ATM activation and Mre11-associated exonuclease activity, leading to abolishment of the G2/M checkpoint and impairment of HR repair<sup>[64]</sup>. Considering the importance of the MRN complex in DNA repair and cell cycle checkpoints, MRN complex inhibitors appear to be very promising as radiosensitizers.

#### 5 Inhibition of Wee1

Wee1 silencing with siRNA or inhibition of Wee1 by small molecular inhibitors was reported to sensitize cells to DNA damage<sup>[65]</sup>. Modification of the initial hit compounds by leveraging the information on structure-activity relationships led to the identification of a potent and selective small molecule inhibitor of Wee1 kinase, AZD1775 (previously MK-1775). AZD1775 is a first-in-class, pyrazolo-pyrimidine derivative with high potency, selectivity, and oral bioavailability in preclinical animal models<sup>[66]</sup>. AZD1775 abrogates DNA damage checkpoint, leading to apoptosis in combination with several DNA damaging agents selectively in p53-deficient tumor cell lines<sup>[67]</sup>. A phase I study, which used single agent AZD1775 to treat refractory solid tumors, had activities in patients with a BRCA mutation<sup>[68]</sup>. In established glioblastoma cell lines, AZD-1775 showed a dose dependent attenuation of the radiation-induced G2 checkpoint arrest and increase in radiosensitization<sup>[66,69]</sup>. Clinical trials combining AZD1775 and RT in various cancer types are underway.

#### 6 Inhibition of RAD51

Rad51 plays an important role in maintaining genome stability through the HR pathway in response to DNA damage. This is highlighted by the fact that Rad51 knockout mice show early embryonic lethality<sup>[70]</sup>. RAD51 mRNA levels are elevated by approximately 4-6 fold and the activity increased at least 840-fold in cancer cells compared to the normal cells<sup>[71]</sup>. As a RAD51 inhibitor, T0070907 was reported to increase tumor response to RT. T0070907 can efficiently decrease the levels of RAD51 protein in cervical cancer cells, even overcoming the upregulation of RAD51 induced by radiation<sup>[72]</sup>. Treatment with imatinib had a sensitizing effect on tumor cells that was not replicated in normal fibroblasts. Decreased clonogenic survival was observed in tumor cells exposed to IR following treatment with imatinib. Treatment with imatinib in combination with RT resulted in significantly delayed tumor growth compared to RT alone, which could be attributed partly to reduced RAD51 expression levels<sup>[73]</sup>. Inhibitors of RAD51 are now in early drug discovery and novel agents targeting RAD51 are now emerging in the clinical field. MP470 is an oral but nonselective multitargeted tyrosine kinase inhibitor that also suppresses RAD51 protein and has synergistic activity with RT in glioblastoma cells and the first-in-human safety study has recently been completed (ClinicalTrials.gov NCT00894894)<sup>[74]</sup>. More specific RAD51 inhibitors including halenaquinone, B02, RI-1 and IBR2 are now being discovered through high-throughput screening of small molecule libraries<sup>[75]</sup>.

#### 7 Inhibition of p53

The central role of wild-type p53 for DNA repair after exposure to IR has led to considerable interest in development of strategies to restore normal p53 function in tumors with defective p53-dependent signaling. Most of the clinically used cytotoxic agents will activate p53 and induce p53-mediated DNA damage responses in cancer cells expressing wild-type p53. A number of promising discoveries, based on p53 degradation by MDM2 or p53 family proteins, provide a foundation for future drug design. In these models, either target genes of p53 were deleted or the p53 gene itself mutated to impair some or all of its transcriptional activities. Pifithrin- $\alpha$ , a small molecule inhibitor of p53, has been shown to protect mice from a lethal dose of IR<sup>[75]</sup> and protect normal tissues while maximizing tumor cell death. Though clinical trials indicated ONYX-015 (a genetically modified adenovirus) as a single agent produced marginal benefit, its administration in combination with radiation produced more significant impact<sup>[76,77]</sup>. PRIMA-1, or p53 reactivation and induction of massive apoptosis, was identified from NCI chemical library screening<sup>[78]</sup>. Studies showed that PRIMA-1(met), an analog of PRIMA-1 with improved efficacy, radiosensitizes prostate cancer cells<sup>[79]</sup>. CDB3 is a p53 molecular inhibitor which sensitized cancer cells that carried wild-type p53 to IR-induced apoptosis<sup>[80]</sup>. As the first class of small molecule inhibitors of MDM2, nutlins were shown to radiosensitize lung cancer cells or prostate cancer cells with wild-type p53<sup>[81, 82]</sup>. 37AA is a 37 amino acid peptide derived from the DNA-binding domain of wild-type p53. In combination with radiation therapy, 37AA would produce synergistic effects in p53-mutant or p53-null settings<sup>[83]</sup>.

## CONCLUSION AND OUTLOOK

The DDR represents a complex network of multiple signaling pathways involving cell cycle checkpoints, DNA repair, transcriptional programs and apoptosis, through which cells maintain genomic integrity following various endogenous (metabolic) or environmental stresses. Radioresistance is a serious obstacle to effective killing cancer cells during RT. The main mechanism of RT is to kill cells through inducing DNA damage directly. Cancer cells can also utilize the DNA repair machinery to process DNA lesions induced by IR in order to maintain cellular survival, which is therefore also an important mechanism of therapeutic resistance. Therefore, the use of DDR inhibitors, either singly or in combinations, is a mode with a great deal of potential. In general, the RT clinical development of ATM, ATR, Chk1, Chk2, DNA-PKcs, Ku70/80, PARP, MRN, Wee1, RAD51 and p53 inhibitors are being pursued actively. From directly inhibiting the repair processes, by abrogating cell cycle checkpoints, specific small molecule inhibitors have emerged from both academic and industrial groups. Targeting DNA repair proteins has significantly increased over the past decade. The impact of DNA repair on resistance to RT is well documented in numerous cancers.

Moreover, many lessons have been learnt about the exploitation of synthetic lethality. In order to translate these results into innovative trials, assimilating preclinical information on the complex nature of DDR and the tumor characteristics that may predict for increased sensitivity to DDR inhibition is needed. Radiosensitization with DDR inhibitors is promising preclinical. Synthetic lethality is a more exciting approach in patients with DDR defects. Clinical proof-of-principle data that this pattern can work are provided by the BRCA-defective or p53-defective cancers. In addition to the BRCA- and p53- lesion system, deficiencies of other DNA damage repair pathways play important roles in tumorigenesis. Thus, both mutational and epigenetic landscapes are necessary to stratify the tumor patients,

especially for genes in DNA damage repair pathways.

Inhibitors that can act via modulating DNA repair to facilitate the death of cancer cells have been developed. In principle, the success of inhibition of DDR in cancer therapy depends on the selection of the protein target. In general, an inhibitor target would be a repair protein that is directly involved in oncogenesis or displays synthetic lethal interactions with the affected repair protein. Repair proteins that might be directly involved in tumorigenesis would be either overexpressed or hyperactive or possess aberrant activity because of mutations.

Our previous work demonstrated that a natural compound named coroglaucigenin (CGN) isolated from *Calotropis gigantea* can not only enhance DNA damage but also arrest the cell cycle in G2/M phase in cancer cells, which may in part account for the effect of CGN on the enhancement of radiosensitivity of A549 cells<sup>[84]</sup>. In our undergoing work, two molecules obtained from a medical plant showed significant inhibition against Cdc25A/B. Docking study showed these two molecules might act by occupying the active site of enzymes, which provides a clue to obtain and further optimize the inhibitors of DNA damage repair from natural products.

In summary, the field of targeted radiosensitization of tumors is developing rapidly and drawing much attention. DNA repair targeted agents represent an exciting group of emerging therapeutics with potential to improve outcomes across a variety of cancer types.

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