Commentary

Refining Radiation for the Next Century

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Wilhelm Röentgen's report more than a century ago describing X-ray imaging fostered in the application of ionizing radiation (IR) for cancer therapy. Today, about half of all the patients with cancer are treated with radiotherapy and it remains one of our most effective treatment modalities, much to the surprise of many of my Cancer Biology friends. The use of altered fractionation schedules and image-guided radiotherapy has significantly improved patient outcomes. The concomitant administration of radiation with chemotherapy or targeted agents has been shown to sensitize malignant cells or in some cases protect nonmalignant cells. We now know that the stress and damage caused by IR to nonmalignant and malignant cells evokes adaptive changes that can also be targeted for treatment strategies. Nonetheless, many patients with cancer fail to benefit from these advances in IR treatment protocols and, therefore, efforts to refine further the potential of radiotherapy continue.

Induction of DNA double-strand breaks (DSB) is one of the fundamental mechanisms by which IR kills tumor cells. Cells, however, have evolved elaborate lesion recognition, signal transmission, cell-cycle synchronization, and damage restoration networks that reduce the potential lethal effects of IR and other stresses. In eukaryotic cells, there are two major mechanisms of DSB repair: homologous recombination and nonhomologous end joining (NHEJ). NHEJ operates throughout the cell cycle, works in a template-independent manner, and repairs as much as 85% of IR-induced DSBs in the mammalian cells.

Our emerging understanding of the prosurvival pathways that malignant cells maintain and the mechanisms by which nonmalignant and malignant cells respond to IR damage have stimulated thoughtful searches for new molecular therapeutic strategies that would enhance IR. In this issue of *Molecular Cancer Therapeutics*, there are nine illustrations of how our emerging knowledgebase is being exploited to advance the use of IR for the treatment of glioblastoma, non–small cell lung cancer, head and neck squamous cell carcinoma, melanoma, and prostate cancer. Collectively, these articles highlight the frontiers of modern molecular cancer radiotherapy and are likely to help ensure that IR remains a central pillar for cancer therapy in this century.

Several articles in this issue focus on enhancing the efficacy of IR toward glioblastoma, which remains a poorly responsive lethal disease. The TCGA database reveals that >80% of all glioblastoma have genetic alterations in the PI3K and p53 pathways, which are heavily involved in genotoxic stress and cell fate decisions. Alterations in the PI3K pathway cause the constitutive activation of AKT, which is both progrowth and prosurvival. Conversely, p53 activity is suppressed in most glioblastomas, predominantly from inactivating mutations or increased activity of the E3 ubiquitin-protein ligase MDM2. Suppression of p53 activity alters DNA damage repair, apoptosis, and genetic stability. Palanichamy and colleagues (1) used a phosphatidylinositol ether lipid analogue (PIA), which blocks AKT activity by binding to the PIP_3 site, to define the role of AKT and p53 in glioblastoma. They observed the p53-mt, but not the p53 wild-type glioblastoma cell lines, were sensitized to IR when pretreated with PIA two hours prior to radiation exposure in culture. The radiation sensitization corresponded with an increase in DNA damage and a decrease in the levels of DNA-dependent protein kinase catalytic subunits (DNA-PKcs), which are involved in the fast component of the radiation-induced DNA DSB repair pathway involving NHEJ. Radiation sensitization was not observed in normal human astrocytes. The authors concluded that the increased DNA damage in glioblastoma cell lines with functionally mutant or null p53 results from the down-regulation of DNA-PKcs and propose inhibition of AKT might be a therapeutic strategy for *TP53*-mutant glioblastomas (1). It should be noted, however, that the glioblastoma cells used in the study had hundreds of mutations in *TP53*, which raises the question of whether the type of p53 mutation matters for the synthetic lethality. It is also unclear how selective an AKT inhibitor needs to be, as the allosteric AKT

inhibitor MK2206 produced sensitization to IR in glioblastoma cells that was independent of the p53 status. IR sensitization of glioblastoma was also observed with an inhibitor of the MAPK pathway, MEK162 or binimetinib, which downregulated and dephosphorylated the cell-cycle checkpoint proteins CDK1/CDK2/WEE1 and DNA damage response proteins phosphorylated ATM or CHK2 (2). When combined with IR, the MEK1/2 inhibitor binimetinib prolonged DNA damage and increased apoptosis probably by abrogating the G_2–M checkpoint. When binimetinib was combined with IR treatment in an orthotopic glioblastoma model, synergistic tumor growth reduction was seen with the combination treatment and a significantly longer median survival time compared with vehicle control.

The PI3K and AKT pathways are also activated by the tyrosine kinase receptors MET, AXL, and FGFR. All three receptors have been associated with mechanisms of resistance to conventional and targeted therapies. S49076 is an oral ATP-competitive inhibitor of MET, AXL, and FGFR1–3 receptors that is currently in phase I/II clinical trials. As with many tyrosine kinase inhibitors, other molecular targets can emerge with more detailed studies and the examination of higher drug concentrations. Clémenson and colleagues (3) found that S49076 at low concentrations, as predicted, caused MET-dependent cytotoxicity through MET inhibition but at higher (but still clinically relevant) concentrations inhibited the growth of MET-independent cells by targeting Aurora B. S49076 improved the antitumor efficacy of IR in both MET-dependent and MET-independent cell lines *in vitro* and in subcutaneous and orthotopic tumor models *in vivo*. Thus, future studies may wish to evaluate S49076 with IR in clinical trials without patient selection based on the tumor MET dependency status.

Radiotherapy, like others forms of stress, is known to activate prosurvival pathways, such as AKT and mTORC, which limit the efficacy of systemic treatments including cytotoxic and targeted therapies. Eke and colleagues (4) explored this adaptive response and found IR increased the AKT and mTOR phosphorylation and the constellation of protein with which they interact in 3D prostate cancer models and tumor xenografts. They documented that pharmacologic inhibitors of AKT (GDC-0068) and mTOR signaling (INK128) were more effective against prostate cancer cells when given after multifractionated IR treatment compared with before IR. These studies are an important proof-of-principle because they highlight the dynamic nature of the survival pathways in tumors after IR exposure. Simply looking at the genomic composition of the tumor prior to treatment without taking into account that the efficiency of targeted drugs can also be modulated by stress-induced protein modifications may be misleading. It may be possible to use IR to prime tumors for enhanced drug-induced killing. It remains unanswered, however, whether this concept will be useful *in vivo* for disseminated tumors or just for local prostate cancer.

Protein proteolysis occurs via the 26S proteasome using E3 ligases and substrate recognition subunits, which confer specificity for the targeted polyubiquitylated substrates. Cullin RING E3 ubiquitin Ligase 4 (CRL4) with the substrate recognition subunit CDT2 is a master regulator of genome stability and the cell cycle, primarily through the targeted proteolysis of the replication licensing protein CDT1, the CDK inhibitor p21, and the histone methyltransferase SET8 during S-phase of the cell cycle. CDT2 is activated, and its substrates are degraded, following IR-induced DNA damage and this is important for DNA repair and IR-induced early G_2–M checkpoint. CDT2-mediated ubiquitylation of CDT1, SET8 and p21 in S-phase of unperturbed cells prevents reinitiation of DNA replication, a phenomenon termed DNA rereplication. Rereplication is deleterious to cells and, in some cases, induces cellular senescence or apoptosis due to the accumulation of toxic replication intermediates and replication fork stalling. Previous studies found that the neddylation inhibitor pevonedistat or MLN4924 inhibits cancer cell lines and tumors and is associated with significant induction of DNA rereplication. Vanderdys and colleagues (5) demonstrated that induction of rereplication genetically or pharmacologically induced robust IR sensitization in head and neck squamous cell carcinoma cells and tumors. Specifically, they found treating mice with penvonedistat 2 hours prior to IR caused significantly greater suppression of the growth of head and neck squamous cell carcinoma xenografts compared with either modality alone. They suggest that pevonedistat may be a possible adjuvant for IR-based treatments.

Groselj and colleagues (6) employed an innovative *in vivo* technique to evaluate the IR sensitization by the pan-histone deacetylase inhibitor panobinostat using subcutaneous xenografts in athymic nude mice. Panobinostat did not add to the acute intestinal toxicity caused by 10–14 Gy IR nor did it increase the delayed IR intestinal and bladder toxicity. The combination of panobinostat with IR was superior to IR alone in reducing bladder cancer xenograft growth but overall survival was not measured. IR sensitization by panobinostat was associated with class I histone deacetylase inhibition and protein downregulation of HDAC2 and MRE11.

It has been known for decades that tumor cells are highly sensitive to arginine deficiency. A proof-of-principle *in vitro* arginine deprivation therapy study was performed using recombinant human arginase or arginine-free diets. Significant IR sensitization was detected and it was more pronounced in 2D and 3D glioblastoma cell

models with cells lacking functional p53 compared with their p53-wild-type counterparts (7). Further work will be needed to define the underlying mechanism responsible for this sensitization and whether or not it can be seen in preclinical mouse models.

The popularity of synthetic lethal strategies has stimulated several groups to seek new IR sensitizers using high-throughput screening methods focused on DNA repair. In some cases, investigators have explored drug repurposing as a faster, more efficient, and economical route for new IR sensitizers. One example is the lipophilic statin, pitavastatin, which was identified as a potent inhibitor of DSB repair in breast and melanoma models both *in vitro* and *in vivo* (8). When combined with IR, pitavastatin increased persistent DSBs, induced senescence, and enhanced acute effects of IR on IR-resistant melanoma tumors. shRNA knockdown studies implicated inhibition of protein prenylation rather than cholesterol biosynthesis for the sensitization. The authors conclude that we should consider repurposing pitavastatin or other lipophilic statins as possible IR sensitizers.

Another innovative screening approach looked for NHEJ inhibitors by using the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system and high-resolution melting analysis (9). The assay relied on the error-prone nature of the NHEJ-mediated ligation of the site-specific DSB induced by Cas9 nuclease to cause the mutation of the targeted sequence as detected by high-resolution melting analysis. Armed with this assay, the authors probed a 1,540-compound drug library and identified the cardiac glycoside ouabain and the first-generation antipsychotic penfluridol inhibited NHEJ activity and sensitized HeLa cells to IR. Ouabin and penfluridol have a number of other pharmacologic effects and have not been evaluated with IR *in vivo* so further screening and optimization are likely to be required. Nonetheless, this is an interesting cell-based screening platform for NHEJ inhibitors, which could assist in the discovery of novel sensitizers of IR.

Both the bone marrow and the gastrointestinal tract are highly sensitivity to IR toxicity. Morita and colleagues (10) found that 5-chloro-8-quinolinol (5CHQ), albeit at high concentrations, shifted p53 transactivation activity from proapoptotic to antiapoptotic by enhancing p21 induction and suppressing PUMA induction. This p53-modulating effect seemed to be attributable to the sequence-specific alteration of p53 DNA-binding, as evaluated by chromatin immunoprecipitation and electrophoretic mobility shift assays. The authors found 5CHQ protected the hematopoietic and gastrointestinal system in mouse irradiation models with dose reduction factors in total body and abdominally irradiated mice of about 1.2 and 1.3, respectively. 5CHQ effectively protected mouse epithelial stem cells from a lethal dose of abdominal irradiation. These results provide initial evidence that the pharmacologic upregulation of IR-protective p53-target genes as a potential strategy for addressing the gastrointestinal syndrome.

Radiotherapy continues to evolve. The articles in this issue demonstrate the multidisciplinary muscle that is required for therapeutic advancement and the value of combining the unique skills found in the field of Radiation Biology, namely quantitative analytics of DNA damage and repair, biophysics, and replication death measurements, with those of other fields, such as signal transduction, bioinformatics, gene expression, genomics, proteomics and pharmacology. There is fertile soil for the growth of new IR strategies for cancer treatments even after a century of the discovery of X-rays.

See all articles in this *MCT Focus* section, "Developmental Therapeutics in Radiation Oncology."

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References

1. Palanichamy K, Patel D, Jacob JR, Litzenberg KT, Gordon N, Acus K, et al. Lack of constitutively active DNA repair sensitizes glioblastomas to Akt inhibition and induces synthetic lethality with radiation treatment in a p53-dependent manner. Mol Cancer Ther 2018;17:336–46.
2. Narayan RS, Gasol A, Slangen PLG, Cornelissen FMG, Lagerweij T, Veldman HYYE, et al. Identification of MEK162 as a radiosensitizer for the treatment of glioblastoma. Mol Cancer Ther 2018;17:347–54.
3. Clémenson C, Chargari C, Liu W, Mondini M, Ferté C, Burbridge MF, et al. The MET/AXL/FGFR inhibitor S49076 impairs Aurora B activity and improves the antitumor efficacy of radiotherapy. Mol Cancer Ther 2017;16:2107–19.
4. Eke I, Makinde AY, Aryankalayil MJ, Sandfort V, Palayoor ST, Rath BH, et al. Exploiting radiation-induced signaling to increase the susceptibility of resistant cancer cells to targeted drugs: AKT and mTOR inhibitors as an example. Mol Cancer Ther 2018;17:355–67.
5. Vanderdys V, Allak A, Guessous F, Benamar M, Read PW, Jameson MJ, et al. The neddylation inhibitor pevonedistat (MLN4924) suppresses and radiosensitizes head and neck squamous carcinoma cells and tumors. Mol Cancer Ther 2018;17:368–80.
6. Groselj B, Ruan J-L, Scott H, Gorrill J, Nicholson J, Kelly J, et al. Radiosensitization *in vivo* by histone deacetylase inhibition with no increase in early normal tissue radiation toxicity. Mol Cancer Ther 2018;17:381–92.

7. Hinrichs CN, Ingargiola M, Käubler T, Löck S, Temme A, Köhn-Luque A, et al. Arginine deprivation therapy: putative strategy to eradicate glioblastoma cells by radiosensitization. Mol Cancer Ther 2018;17:393–406.
8. Efimova EV, Ricco N, Labay E, Mauceri H, Flor AC, Ramamurthy A, et al. HMG-CoA reductase inhibition delays DNA repair and promotes senescence after tumor irradiation. Mol Cancer Ther 2018;17:407–18.
9. Du J, Shang J, Chen F, Zhang Y, Yin N, Xie T, et al. A CRISPR/Cas9-based screening for non-homologous end joining inhibitors reveals ouabain and penfluridol as radiosensitizers. Mol Cancer Ther 2018;17:419–31.
10. Morita A, Takahashi I, Sasatani M, Aoki S, Wang B, Ariyasu S, et al. A chemical modulator of p53 transactivation that acts as a radioprotective agonist. Mol Cancer Ther 2018;17:432–42.

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