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Radiosensitization by inhibiting DNA repair – turning the spotlight on homologous recombination

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Hepatocellular carcinoma (HCC) is the fifth most common cancer in men and ninth most common cancer in women with an estimated incidence of 782,000 new cases worldwide in 2012. It is the second most common cause of death from cancer with an overall mortality to incidence ratio of 0.95.¹ In patients with disease localized to the liver that is not amenable to surgical resection or transplantation, the liver-directed therapies frequently utilized in clinical practice are radiofrequency ablation, transarterial chemoembolization and radiation (selective internal radiation therapy with ⁹⁰Y microspheres or external beam radiation therapy). Distinct advantages of external radiation therapy (RT) with photons or charged particles are the non-invasive nature of treatment, the ability to treat multiple adjacent lesions that can be encompassed within a treatment field, the ability to treat tumors in close proximity to the vasculature, the diaphragm, and biliary ductal structures, and the potential to extend treatment to patients with medical comorbidities. Yet the outcomes after focal RT are suboptimal with failures within the treatment field and within the liver outside the treatment field. Attempts to improve outcomes by escalating the radiation dose are hindered by the decreased functional reserve of liver that is already compromised by cirrhosis in many instances and the fear of triggering radiation-induced liver disease.² As noted earlier by the authors of the accompanying article, sublethal doses of radiation may in fact enhance migration and invasion of HCC cells via convergence of PI3K/Akt and MAPK/ERK pathways on NF-κB and MMP-9.³ This is akin to the adage “what doesn’t kill you, makes you stronger”. One potential way to circumvent the challenge of limited tolerance of normal liver to radiation and the consequent inability to administer a tumoricidal dose of radiation is to use radiosensitizing drugs that increase the efficacy of RT without any undue damage to the surrounding liver parenchyma.^{4, 5} An enticing strategy is to amplify the putative dominant mechanism of action of RT, i.e., DNA damage.

Among the different types of DNA damage triggered by radiation, double strand breaks (DSBs) are the most lethal because incorrect repair of these defects can result in cell death or carcinogenesis. Compounding this damage via inhibition of cellular response to DSBs is, therefore, a radiosensitization strategy that strikes at the heart of radiation’s dominant biologic effect. DSB repair is mediated by homologous recombination (HR) or non-homologous end joining (NHEJ). Given the predominance of NHEJ in all cell cycle phases, understandably a lot of attention is focused on inhibition of this pathway. However, the authors of the accompanying article show that HR is also an important player in DSB repair.

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The authors describe a novel mechanism of HCC radiosensitization by inactivating histone deacetylases (HDACs), either pharmacologically by using a pan-HDAC inhibitor (panobinostat) or via RNA interference using shRNA to HDAC4 (hereafter referred to as HDAC inactivation), leading to impairment of DNA damage repair. Targeting HDACs by either strategy resulted in enhanced radiosensitivity *in vitro* and *in vivo*. This radiosensitization was associated with an increase in the number of gamma H2AX foci suggestive of persistence of unrepaired DNA damage, where the evolutionarily conserved gamma H2AX performs its canonical role as a chromatin modifier that recruits DSB repair proteins. Interestingly, the DNA damage repair protein that was found to be critically impacted was Rad51, a pivotal mediator of the HR pathway that searches for a homologous strand on a sister chromatid for pairing and exchange. The authors did not address whether the NHEJ pathway was impacted or not, probably because a number of prior studies have correlated HDAC activity with HR inhibition via transcriptional or translational regulation.⁶⁻⁸ The authors of the accompanying article had previously reported, however, that a phenylbutyrate-derived HDAC sensitizes HCC to RT via inhibition of the DNA end-binding activity of Ku70, a critical component of the NHEJ repair pathway.⁹ The apparent dichotomy in HDACs radiosensitizing via HR inhibition in one case and NHEJ inhibition in another case may possibly be related to the fatty acid structure of phenylbutyrate restricting its activity to inhibition of class I and II HDACs while the hydroxamic acid structure of panobinostat inhibits class I, II and IV HDACs. Nonetheless, the authors show that whereas radiation resulted in increased translocation of HDAC4 and Rad51 from the cytoplasm to the nucleus, HDAC inactivation blocked this translocation and resulted in reduced number and size of Rad51 foci but not 53BP1 foci. This hints at the selectivity of HDAC targeting strategies for inhibition of HR but not NHEJ since 53BP1 antagonizes DNA end resection which is a pre-requisite for HR during the G1 phase of the cell cycle when a sister chromatid is unavailable in close proximity to serve as a template for DNA repair by HR. Conversely, during late S and G2 phases of the cell cycle, the antagonism of DNA end resection by 53BP1 is counteracted by other factors to now allow HR to compete with NHEJ for DSB repair.¹⁰ Parallel experiments noting no radiosensitization by HDAC inactivation during the G1 phase of the cell cycle and more pronounced radiosensitization by HDAC inactivation when cells are synchronized in the late S and G2 phases of the cell cycle may have cemented the authors' conclusions on selective abrogation of HR repair mechanisms. Nonetheless, the authors demonstrate convincingly that HDAC inhibition results in histone hyperacetylation as well as acetylation of Ubc9 which no longer shuttles from the cytoplasm to the nucleus (similar to HDAC4 and Rad51) to colocalize as the protein complex that binds cleaved DNA at the end of DSB and facilitates resection and creation of single stranded DNA filaments. Consequently, HDAC inactivation counteracts the radiation-induced translocation of HDAC4 and Ubc9 to the nucleus to assist in propagating the DSB repair that was initiated. Collectively, these investigations advance a role for HR as an attractive target for radiosensitization of HCC via HDAC inactivation.

The focus of this study on HR as a target for radiosensitization strategies is a slight departure from the general notion that NHEJ is the predominant mechanism of DNA repair employed by cells inflicted with the genotoxic insult of a DNA DSB secondary to radiation. Indeed, early studies of small molecule inhibitors of NHEJ repair proteins had shown promise but

progress to clinical translation was stymied by the lack of specificity for the protein of interest, the lack of preferential toxicity for cancer cells, the incomplete understanding of mechanisms of action, and the limited exploration of clinical trial design strategies that allowed enrichment for patients with tumors that may succumb to synthetically lethal combination therapy. Nonetheless, in recent years, novel small molecule inhibitors are increasingly being deployed in clinical trials for the treatment of a variety of cancers. The approach utilized by the authors of this study is to effect an epigenetic change in contrast to targeting genetic mutations in a cancer and thereby change the acetylation status of chromatin and non-histone proteins culminating in alteration in gene expression, induction of apoptosis, cell cycle arrest, inhibition of angiogenesis, and reduction of metastatic burden. Given that vorinostat, panobinostat, belinostat and romidepsin are already approved by FDA for use in various hematological malignancies, the findings of this study raise the tantalizing possibility that repurposing an approved drug as a radiosensitizer could advance rapid clinical translation with relative ease and at a lower cost than de novo development of a drug as a radiosensitizer. This is especially pertinent for HCC patients where improvements in the therapeutic ratio will require biological approaches to be coupled with technological advancement in the quality and delivery of RT that have made big strides in recent years to improve anti-tumor efficacy by escalating the physical radiation dose to the tumor and sparing adjacent non-tumor-bearing liver.

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