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## COMMENTARY

# New Translational Possibilities for Microenvironmental Modulation of Radiosensitivity

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### INTRODUCTION

Reduced sensitivity to radiation therapy is a clinical phenomenon often observed in solid tumors. It has been attributed to different factors, including tumor hypoxia, aberrant DNA repair or changes in the signaling pathways involved in cell survival or death. These topics were the focus of a recent NCI workshop [(1); available online at <http://dx.doi.org/10.1667/RR0L02.1>]. Here we present a brief review of factors in the tumor microenvironment that can impact on tumor DNA repair and survival after radiation treatment and how they might influence clinical results.

### TUMOR HYPOXIA

Accumulating evidence has established that hypoxia can affect tumor biology in many ways. Although hypoxia was initially considered to be nondamaging to DNA due to the presumed lack of reactive oxygen molecules, much research has shown that hypoxia is actually a driver of genetic instability and mutagenesis and can influence DNA metabolism. Mechanistically, while hypoxia does not directly cause detectable DNA damage by standard assays for strand breaks, it does trigger  $\gamma$ -H2AX phosphorylation (2, 3). ATM and ATR are activated, but without focus formation, consistent with the absence of strand breaks. Studies have demonstrated that hypoxia causes S-phase arrest and hinders the ability to restart replication in S-phase cells after prolonged hypoxia (2, 3). Hypoxia also leads to substantially reduced dNTP pools via ribonucleotide reductase inhibition, accounting for the stalled replication (2, 3). DNA repair pathways that are downregu-

lated in hypoxia include DNA mismatch repair (MMR) and homology-directed repair (HDR) via specific transcriptional and post-transcriptional mechanisms (4, 5). In therapeutic terms, the downregulation of HDR (specifically, BRCA1 and RAD51) in hypoxic cancer cells led to the hypothesis that hypoxic cells would be sensitive to a new class of cancer therapy agents, PARP inhibitors, and this has now been demonstrated experimentally (6). Furthermore, experimental evidence suggests that modulation of HDR in response to hypoxia may render posthypoxic, reoxygenated cells more sensitive to radiation than previously suspected (7), perhaps providing a new explanation for the value of fractionated therapy and the impact of reoxygenation.

Hypoxia itself is complex and is manifest as acute and chronic hypoxia as well as cycling hypoxia. Cycling hypoxia strongly induces HIF-1, increases glucose uptake and drives the Warburg effect (8). This is due in part to reoxygenation posthypoxia increasing free radicals and thereby increasing HIF-1. One strategy that exploits the effects of hypoxia is the use of drugs that will inhibit glucose consumption by hypoxic tumor cells. This can be accomplished via HIF-1 inhibitors or by inhibition of MCT1 to force aerobic tumor cells to consume more glucose and less lactate and reduce glucose availability to the less well-perfused hypoxic cells (9).

Although it is well known that hypoxia decreases radiation sensitivity in cancer cells, successful strategies targeting hypoxia during radiotherapy in the clinic have remained elusive, at least in head and neck cancer (HNC) (10). What is emerging is that there is a need to distinguish chronic from cycling hypoxia and that the production of reactive oxygen species and reactive nitrogen species that accrue from cycling hypoxia can influence cancer cell biology in multiple ways. There is also a need for clinically validated biomarkers that select for patients who would most benefit from hypoxia-targeting therapy (11).

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## TUMOR IMMUNITY

Recent studies have underscored the importance of immune cells in mediating the effectiveness of radiation therapy. As highlighted in the workshop report (1), CD11b<sup>+</sup> bone marrow-derived monocytes, which differentiate into intratumoral macrophages, play a crucial role in mediating tumor regrowth after radiotherapy (12). Similarly, Lee *et al.* showed that large-fraction ablative radiotherapy ( $\geq 15$  Gy) is better than conventionally fractionated radiotherapy in generating adequate CD8<sup>+</sup> T-cell-dependent immunity for subsequent tumor eradication (13). This may partially account for the high local control rate observed in the clinic for stereotactic body radiotherapy (SBRT). Intriguingly, they also demonstrated that such ablative radiation-mediated immunity could be reduced by the administration of conventional chemotherapy, challenging our current concept of combined chemo-radiotherapy. Using an elegant animal model, Takeshima *et al.* demonstrated that tumor-specific cytotoxic T lymphocytes (CTL), which were induced in the draining lymph nodes from radiotherapy, played a crucial role in inhibiting tumor regrowth (14). Depletion of CD8<sup>+</sup> T cells or removal of the draining lymph nodes (by surgical or genetic means) significantly attenuated radiation-mediated tumor response. Similarly, injection of OVA activated T-helper (Th1) cells, which augmented the generation of tumor-specific CTL at the tumor site, significantly enhanced the tumor control rate with local-field radiotherapy. These data suggest that modulation of the immune system may improve the efficacy of radiotherapy, and this concept is currently being tested in human breast cancers (1).

## TUMOR SIGNALING AND DNA REPAIR

Another novel observation is that nuclear EGFR may mediate resistance to radiotherapy in cancer cells. Accumulating evidence over the last decade has revealed the importance of a nuclear EGFR-signaling pathway. This pathway is characterized by EGFR shuttling from the membrane to the nucleus where it functions as a transcriptional co-factor to regulate genes including cyclin D1, iNOS (15) B-myb, Aurora kinase A and COX2 (16). Radiation has been shown to induce EGFR nuclear translocation, which is linked to the activation of DNAPK, resulting in enhanced radiation resistance (17). High nuclear EGFR levels have also been correlated with poor clinical outcome in many solid tumors. These findings suggest that blocking nuclear EGFR function may improve radiation sensitivity. This concept is presently being evaluated in RTOG 0920 ([www.RTOG.org](http://www.RTOG.org)), where patients with high risk HNC postoperatively are being randomized to radiation or radiation + Cetuximab, which is administered both during and up to 4 weeks after the completion of radiotherapy. The adjuvant phase of Cetuximab is meant to minimize the

development of radiation resistance by blocking both membrane and nuclear EGFR function.

## TUMOR SIGNALING AND THE MICROENVIRONMENT

Recent work has shown that signaling originating in tumor cells can impact the microenvironment in ways that can either promote survival or potentiate tumor killing. One example of this is the potential for killed tumor cells to stimulate growth in surviving cells through caspase-activated signaling (18). Inhibiting this signaling could suppress tumor repopulation during fractionated radiotherapy or between courses of chemotherapy. Another aspect of the tumor microenvironment is elevated angiogenic signaling that contributes to poor vascular function in tumors and promotes resistance both through limits in drug delivery and through promoting tumor hypoxia. Normalization of tumor vasculature through modulation of oncogenic or angiogenic signaling could be used to reverse this effect for therapeutic gain (19).

## THE TUMOR MICROENVIRONMENT AND SYNTHETIC LETHALITY IN THE CONTEXT OF RADIOTHERAPY

In certain instances cancer-specific mutations can be exploited to improve the overall outcome after radiotherapy. Synthetic lethality is one means to exploit specific defects in the cancers to improve the therapeutic ratio between normal and cancer cell toxicity. In particular there are numerous mutations in cancer that can be exploited. The best known of these are the BRCA1 and 2 mutations that confer synthetic lethality to PARP inhibition. As mentioned above, hypoxia-mediated downregulation of HDR (specifically, BRCA1 and RAD51) could potentiate sensitivity to PARP inhibitors (6). These inhibitors used in the context of radiotherapy could mitigate the radioprotective effects of hypoxia by potentiating the killing of these cells. Interestingly, PARP inhibitors have also been shown to suppress *BRCA1* and *RAD51* expression, and this downregulation may contribute directly to radiosensitization produced by PARP inhibition (20). Another example of synthetic lethality that is being explored is the targeting of p53 mutant cancers with Chk1 inhibition. P53-mutated cancers often accumulate in the G<sub>2</sub> checkpoint and employ homologous recombination for repair, both processes controlled by the Chk1 protein. Inhibitors of Chk1 have been demonstrated to improve the response to radiotherapy by both abrogating the G<sub>2</sub> checkpoint and inhibiting homologous recombination (21).

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