Is tumor (R)ejection by the immune system the “5th R” of radiobiology?

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Introduction

Early studies have implicated DNA as the primary target for radiation-induced cell death. However, additional pathways have recently been shown to be important for the clinical response to local radiotherapy, including an active cross-talk between the tumor microenvironment and the immune system.

Summary of Existing Evidence

Radiation-mediated immunogenic cell death

Three distinct immunogenic components of cell death, namely, the exposure of calreticulin on the cell surface and the release of ATP and high mobility group box 1 (HMGB1), appear to be required for dendritic cell (DC) activation and immune priming against malignant cells succumbing to anticancer therapy (Fig. 1).1 We recently examined these 3 hallmarks of immunogenic cell death (ICD) and demonstrated that radiation therapy induces each of them in a dose-dependent fashion.2 Thus, radiation-induced tumor cell death may lead to improved immunologic responses ab initio.

Pro-immunogenic effects of ionizing radiation on the tumor microenvironment

Innate and adaptive immune responses are potentiated by the cellular damage elicited by radiation therapy upon the release of pro-inflammatory cytokines like interleukin-1β (IL-1β), tumor necrosis factor α (TNFα), and chemokine (C-X-C motif) ligand 16 (CXCL16) as well as the upregulation of MHC molecules, co-stimulatory molecules, adhesion molecules, and death receptors on irradiated cancer cells, surrounding stroma, and the vascular endothelium.3 Furthermore, radiotherapy has recently been shown to reprogram macrophages toward a iNOS+/M1 phenotype, endowing them with the ability to recruit tumor-specific T cells that promote tumor rejection and hence improving the survival of otherwise immunotherapy-refractory tumor-bearing hosts.4

Successful combinations of radiation and immunotherapy

Cytotoxic T lymphocyte-associated protein 4 (CTLA4) is a prototypical and targetable immune checkpoint regulator that limits the activation and proliferation of effector T cells to prevent autoimmunity. Chronic tumor antigen exposure leads to T-cell exhaustion, a phenomenon that is associated with the upregulation of CTLA4, thereby promoting immunological tolerance. CTLA4-blocking agents (e.g., the monoclonal antibodies ipilimumab and tremelimumab) have been designed to overcome this type of tolerance. However, the standalone blockade of CTLA4 is insufficient to elicit antitumor immune responses in some oncological settings.5 Several reports have shown that CTLA4-blocking agents combined with local radiotherapy can achieve synergistic antitumor effects, lending support to the notion that local radiotherapy may contribute to the establishment of a pro-immunogenic milieu.5-8 Moreover, the individual responses to CTLA4 blockade may depend on the immunological status of the patient prior to therapy. For instance, individuals bearing low levels of CD14+HLA-DRneg/low myeloid-derived suppressor cells (MDSCs) in the peripheral blood turned out to be more likely to respond to ipilimumab than subjects in which the circulating amount of these cells were high.9 Thus, it is plausible that radiotherapy may provide the otherwise missing signals necessary to evoke a clinically meaningful immune response.

Productive immune synapses have been documented to form upon the interaction between members of the radiation-induced retinoic acid early transcript 1 (RAET1) protein family and killer cell lectin-like...
receptor subfamily K, member 1 (KLRK1, best known as NKG2D), a cell surface glycoprotein expressed by natural killer (NK) and CD8⁺ T cells (Fig. 1). In this setting, the effect of radiation on the immune synapse was required for the local therapeutic response to CTLA-4 blockade. Moreover, not only did cytotoxic CD8⁺ T cells contribute to the therapeutic response observed within the irradiated field, but they also inhibited the growth of distant, non-irradiated metastases, a phenomenon known as abscopal effect (from the Latin “ab scopus,” away from the target) (Fig. 1).

Clinical translation of the abscopal effect

Abscopal responses and their immunological correlates have recently been documented in melanoma patients treated with local radiotherapy and ipilimumab. Moreover, we have recently reported the first abscopal response and its immunological correlates in a patient with metastatic lung cancer (Fig. 1). The table provided in Figure 1B summarizes these cases based on the linear-quadratic (LQ) model for biological effective dose (BED), a model based on a formula (BED = nd + d²/α/β), where n is the number of fractions and d is the dose per fraction) that enables a comparison across different dose and fractionation regimens by applying a specific coefficient (an α/β value) for each tissue irradiated. Assuming the α/β value of 10 Gy for established metastases, an “ablative” dose of at least 20 Gy per 3 fractions, corresponding to a BED of 180 Gy₁₀ (the subscript 10 refers to the assumed α/β value used to calculate the BED) is required to control clinically detectable metastatic lesions. In contrast, each of the radiation regimens employed in combination with CTLA4-blocking agents, utilized sub-ablative BEDs ranging between 43.2 and 55.6 Gy₁₀, while achieving systemic and local tumor control, a proof of

| Reference | Regimen | BED  |
|-----------|---------|------|
| Stamell et al. | 8 Gy x3 | 43.2 Gy₁₀ |
| Golden et al. | 6 Gy x5 | 48 Gy₁₀ |
| Postow et al. | 9.5 Gy x3 | 55.6 Gy₁₀ |
| “Ablative” dose | 20 Gy x3 | 180 Gy₁₀ |

Figure 1A. Immunological effects of local radiotherapy and CTLA4 blockade. (A) Pre and 1-y post-treatment fluorodeoxyglucose-based positron emission tomography (FDG-PET) scans of a lung cancer patient treated with ipilimumab and radiotherapy for a single intrahepatic metastasis. (B) Abscopal responses of non-irradiated lesions in the liver, lung, and bone. Various case reports of abscopal response and their corresponding radiation regimens and biological effective doses (BEDs) are summarized. (C) Local and abscopal immune responses as mediated by the combination of local radiotherapy and cytotoxic T lymphocyte-associated protein 4 (CTLA4) inhibition. The immunogenic demise of cancer cells as induced by radiotherapy promote the translocation of calreticulin on the cell surface as well as the release of ATP and high mobility group box 1 (HMGB1). These factors promote the uptake of tumor-associated antigens (TAA) by dendritic cells (DCs), followed by their maturation and the consequent cross-priming of TAA-specific CD8⁺ T cells. Irradiated cancer cells also release chemokine (C-X-C motif) ligand 16 (CXCL16) and express increased amounts of retinoic acid early transcript 1 (RAE1) proteins on their surface, which supports the recruitment of CD8⁺ T cells to neoplastic lesions and the formation of productive immunological synapses, respectively. Moreover, TAA-specific CD8⁺ T cells primed by this mechanism can mount effective antitumor immune responses at distant (non-irradiated) tumor sites, hence contribute to clinically observable abscopal responses.
Discussion

During the past decade, the cross-talk between radiation-invoked immune responses and cancer has been evaluated at the cellular and molecular levels. Radiotherapy was traditionally considered an immunosuppressive treatment modality, mainly based on the effects of whole-body myeloablative irradiation regimens as employed prior to adoptive cell transfer or hematopoietic stem cell transplantation. Such an immunosuppressive nature of whole-body radiotherapy was erroneously attributed to local radiotherapy. Rather, local radiotherapy appears to be a powerful tool for converting malignant cells into an anticancer vaccine in situ. The immunostimulatory effects of local radiotherapy are mitigated by the microenvironment of established cancers, which is often (if not always) highly immunosuppressive. For radiotherapy to be maximally efficient, this issue must be circumvented by means of adequate immunotherapeutic interventions. These new discoveries and the emerging clinical applications of radiotherapy as an immunological adjuvant have opened to radiobiologists an additional and impactful area of research. Thus, we propose that the Rejection of neoplastic lesions by the immune system constitutes the “5th R” of radiobiology, a concept that is already under intense preclinical and clinical investigation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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