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Review

Immune modulation by hypofractionated stereotactic radiation therapy: Therapeutic implications

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ABSTRACT

Stereotactic body radiation therapy (SBRT) has become an attractive treatment modality and a safe, non-invasive alternative to surgery to control primary or secondary malignant tumors. While emphasis has been on the local tumor control as a treatment objective for SBRT, the rare but intriguing observations of abscopal (or out-of-field) effects have pointed to the exciting possibility of activating anti-tumor immunity by using high-dose radiation. This review summarizes the available evidence supporting immune modulation by SBRT alone, as well as its potential combination with immunotherapy. Promising preclinical research has revealed an array of immune changes following SBRT, which could affect the balance between anti-tumor immunity and tumor-promoting immunosuppression. However, shifting this balance in the clinical setting to obtain survival benefits has rarely been achieved so far, emphasizing the need for a better understanding of the interactions between high-dose radiotherapy and immunity or immunotherapy. Nevertheless, the combination of SBRT with immunotherapy, particularly with immune checkpoint blockers, has the clear potential to substantially increase the rate of abscopal effects. This warrants further research in this area, both in mechanistic preclinical studies and in clinical trials incorporating correlative studies.

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Radiation therapy is the mainstay of treatment in many oncologic diseases. This local treatment modality may improve the survival and quality of life of cancer patients, even in late stages of their disease. On the other hand, with the advent of the immune checkpoint blockers, immunotherapy has recently emerged as a potentially curative systemic therapy for several cancers. The interaction between these potentially curative treatment modalities remains to be completely characterized. For example, some studies refer to the immunosuppressive effects of radiation therapy [1], while others indicate that radiation therapy may in fact enhance anti-tumor immunity [2]. Emerging evidence suggests that high-dose radiotherapy – clinically used in hypofractionated regimens or in ablative stereotactic body radiotherapy (SBRT) and radiosurgery (SRS) – may elicit a pronounced anti-tumor immune effect [3–9]. It remains unclear whether this effect is stronger than when using conventionally fractionated radiation therapy, however the current data are intriguing and warrant further investigation. In this article, we review the available evidence for immune stimulation induced by radiotherapy, with focus on the existing literature on the local and general immune reactions after SBRT, and its combination with immunotherapy.

Stereotactic body radiation therapy (SBRT)

SBRT is a technique often described as an extracranial extension of the successfully implemented SRS of intracranial tumors. Similar to the latter, SBRT is based on a great accuracy of tumor targeting and improved protection of normal surrounding tissues, and allows use of high-doses in a hypofractionated manner. Its development opened an array of treatment possibilities, which have already incorporated in the management of isolated targets in organs such as lung, liver, kidney, prostate or spine [10–15].

SBRT is indicated for either early-stage tumors, which have not yet metastasized, or for oligometastatic cancers with controlled primary lesions [16]. The fractionation determines a dose-dependent high tumor control rate, usually with acceptable acute toxicity profiles [10,17]. Several studies suggested a significant survival improvement in oligometastatic cancer patients treated with SBRT compared to historical controls [11,12]. Unfortunately, patients with macroscopic oligometastases often also have occult microscopic tumor cell deposits at other sites [18]. This explains

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the high risk of developing new distant metastases after SBRT (60–80%) [11,12].

In this context, an intriguing yet rare occurrence noted with SBRT is the abscopal effect (from the Latin *ab scopus*, away from the target), originally defined by Mole in 1953 [19]. The term describes the additional regression of tumor burden in non-irradiated sites after local radiation therapy, analog to a distant bystander effect [20,21]. In this era of major progresses in immunotherapy, this potential effect of radiation therapy—and in particular of hypofractionated ablative irradiation—is widely seen as a potentially important therapeutic opportunity. However, much remains to be understood in this regard to successfully integrate radiation with novel immunotherapies.

Preclinical evidence for immune modulation by SBRT

During progression, tumors recruit stromal elements from the neighboring tissues or blood circulation, such as fibroblasts, immune cells, vascular and lymphatic endothelial cells, pericytes and adipocytes [22]. This microenvironment created by the proliferating cancer cells is highly abnormal, and characterized by immunosuppression and evasion of immune surveillance. Hypofractionated radiation therapy may induce complex changes in the immune microenvironment of tumors, as summarized in Table 1 and Fig. 1 and discussed below.

Tumor cell death induced by radiation appears to be differential depending on the applied dose. For example, lower doses (used in conventional fractionation regimens) can provoke mitotic catastrophe, DNA-damage-induced apoptosis and autophagy, while higher doses (ablative or hypofractionated) can induce necrosis/necroptosis and senescence [23–26]. The latter are considered "pro-inflammatory" death types, as they lead to an increased secretion of damage-associated molecules, and thus are more likely to generate a shift of the tumor microenvironment toward anti-cancer immunity [27–30]. This occurs through liberation of cellular stres s/danger/"eat-me" signals, which trigger the innate immunity, as well as through the presentation of tumor antigens in the tumor microenvironment, which activates the body's adaptive immunity [20,28,29].

Hypofractionated radiation therapy may also indirectly promote tumor cell death. Death receptors (e.g., Fas-receptors) can trigger apoptosis upon binding with their ligands and are used by cytotoxic T lymphocytes (CTLs) for killing targets such as tumor cells. However, in tumors, the expression of Fas receptors is often downregulated. After high-dose radiotherapy, Fas-receptors undergo functional up-regulation [2,31]. Intercellular adhesion molecules (ICAM) as well as vascular adhesion molecules (VCAM) are also downregulated. ICAM-1 normally promotes cell adhesion, co-stimulates CTLs and is used by CTLs for binding and killing targets, such as tumor cells, while VCAM-1 attracts CTLs. SBRT up-regulates the expression of ICAM-1 and VCAM-1 by increasing interferon gamma (IFN-γ) expression [2,4]. In addition, the NKG2D transmembrane protein expressed by NK cells, which is mainly responsible for the recognition of signals from CD8 $^{+}$ T cells and for the activation of cytotoxicity, can increasingly recognize cells affected by DNA damage after radiotherapy [32].

Effects of SBRT on immune cells

SBRT leads to increased recruitment of immune cells into the tumor. These cells can mediate both tolerogenic and anti-tumor effects, with preclinical data suggesting a dominance of the anti-tumor immunity.

Myeloid-derived cells are essential components of the tumor microenvironment, as regulators of the anti-tumor immunity. Myeloid cells may be involved in tumor antigen presentation and the initiation of immune responses, or, on the contrary, may promote immune evasion via suppressive effects on lymphocytes. They include activated (M1, anti-tumor) or "alternatively" activated (M2, pro-tumor) tumor-associated macrophages (TAMs), polymorphonuclear neutrophils (PMNs), dendritic cells (DCs) and myeloid-derived suppressor cells (MDSCs) [22]. Myeloid cells are necessary for priming T cells, however in tumor-bearing hosts they often elicit primarily immunosuppressive effects [33]. TAMs mediate both antitumor immunity and immune tumor tolerance [34]. M2-type TAMs and MDSCs overexpress cytokines such as interleukin 10 (IL-10), thus promoting an immunosuppressive environment [35,36]. Multiple reports have shown increased recruitment of TAMs and MDSCs after radiation therapy, and some have shown redistribution of these cells within tumor hypoxic/necrotic areas [37,38].

DCs can mediate both priming of T cells and immune tolerance in the tumor microenvironment. DC recruitment, maturation and presentation of antigens to CTLs may increase after SBRT [3,39,40]. DCs travel to draining lymph nodes and present tumor antigen-derived peptides via major histocompatibility complex (MHC) class I and II proteins to naïve T-cells. MHC class I and II, which normally display epitopes of tumor antigens on the cell surface for recognition by circulating T cells, are down-regulated in tumor-bearing hosts. After SBRT, their expression and cell surface localization has been also shown to increase [2,3].

A study by Lee et al. [3] showed that the therapeutic effect of ablative radiotherapy depends on the increased recruitment and priming of T cells in immunocompetent mice, as the same tumors were largely radio-resistant in immunodeficient (athymic nude) mice. CTLs appeared to be indispensable, as antibody-mediated CD8^{+} T-cell depletion resulted in a significant decrease in tumor growth delay and survival. CD4^{+} T cells also play key roles in anti-tumor immunity. Among them, the regulatory T cells (Tregs) have an important role in promoting immunosuppression by secreting tolerogenic cytokines, such as IL-10 or transforming growth factor beta (TGF-β), and by expressing immune checkpoints, for example the cytotoxic T-lymphocyte antigen 4 (CTLA-4) [41,42]. A marked Treg infiltration of the spleen and of the tumor was observed in mice after ablative radiation therapy [42].

Effects of SBRT on immune cytokines and chemokines

An important role in the initiation and coordination of the cellular immune responses is played by cytokines, chemokines and adhesion molecules, including interferons (IFNs), ILs, colony-stimulating factors (CSF), tumor necrosis factor alpha (TNF-α) and TGF-β. Of the cytokines, type I (IFN-α and -β) and type II (IFN-γ) IFNs promote the recruitment and activation of CTLs, both directly and via upregulation of CTL-attracting CXC chemokines [4,39,43–45]. TNF-α is produced by T cells and contributes to the selective elimination of MDSCs [44]. Multiple other molecules mediate CD4^{+} T cell-antigen-presenting cell (APC) interactions, either by exerting a co-stimulatory effect, such as the TNF receptor family member OX40 [46], or a suppressive one, such as the lymphocyte activation gene-3 (LAG-3) [47]. T cell responses provoked by hypofractionated irradiation also induce an overexpression of molecules with tolerogenic effects. Among them, the B7 family inhibitory ligands – programmed death-ligands PD-L1 and PD-L2 are important proteins promoting self-tolerance [48]. LAG-3 and other immune checkpoint molecules, such as Tim-3, appear to potentiate the effects of the programmed cell death protein 1 (PD-1) in creating dysfunctional CD8^{+} T cells [49]. IL-4 and GM-CSF induce PD-L2 expression, IL-10 and TGF-ß impair effector T-cells and NK cells and convert DCs into regulatory DCs, which can further impair T-cell function, while TGF-ß also stimulates Tregs to suppress antitumor effector T-cells.

The local secretion of molecules such as IFNs, IL-2, IL-1α, IL-1β, IL-12, and TNF-α are involved in the initiation of an adaptive