

Chapter 7

Aiming the Immune System to Improve the Antitumor Efficacy of Radiation Therapy

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Abstract Radiation therapy (RT) has historically been the most common approach used to achieve local tumor control in cancer patients. However, emerging evidences over the last decades suggest an important role for RT in modulating or amplifying the antitumor immune response upon induction of cancer cell death through its direct cytotoxic effect. RT alters multiple components of the tumor microenvironment which affect both the immune cell phenotype and function as well as the interactions between tumor and the immune system. Despite the documented immunostimulatory effects, RT alone rarely induces effective antitumor immunity resulting in systemic tumor rejection. RT can also reinforce immunosuppressive mechanisms within the tumor microenvironment, which negatively impacts on the tumor response to RT. Preclinical and clinical data show that combination RT and immunotherapy can elicit powerful antitumor efficacy through either strengthening the immune activation or counteracting immune suppression. In this review, we summarize the immunological changes in the tumor microenvironment upon exposure to radiation. We also highlight radiation triggered molecular and cellular pathways that may contribute to immune evasion and tumor recurrence. Rational and optimized combination of RT and immunotherapy to achieve synergistic antitumor activities for systemic eradication of cancer cells and development of durable antitumor immunity will also be discussed.

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7.1 Introduction

Radiation therapy (RT) is a well-established conventional cancer treatment modality that is administrated up to 50 % of the cancer patients [1]. Tumoricidal effect of RT lies in its ability to cause DNA damage in irradiated cancer cells. RT is frequently used to achieve local or regional control of cancers either alone or in combination with other treatments, e.g., surgery or chemotherapy. Although RT has been well recognized for its direct cytotoxic and cytostatic effect on neoplastic cells, it is increasingly clear that the immune system also has a major role in the long-term control of tumor growth by RT [2, 3]. This may provide an immunological basis for the abscopal effect of RT [4]. The mechanisms underlying RT-induced antitumor immune responses are complex and involve an active interaction between irradiated cancer cells and the tumor stroma. Historically the tumor cell itself has been the focus to improve the outcomes of RT, while the interplay between the tumor cells and tumor microenvironment (TME) were largely ignored.

The immune compartment within the tumor stroma is primarily constituted of resident or recruited leukocytes with both lymphoid and myeloid origins. Depending on their phenotype and activation state, these immune cells can either promote or suppress tumor progression as well as modulate the therapeutic response to anticancer treatments, including RT [5–7]. Although RT transiently depletes resident leukocytes via direct cytotoxic activity, the rebound effects of immune cells following RT are known to impact on tumor response. In this review, we describe immunological changes in the TME following RT and discuss how this immune profile alteration may promote radio-resistance and tumor recurrence. We will reveal the capability of RT to provoke or modulate an immune response and the immune system's role in regulating the local or regional effects of RT implicate the potential rationale of combination RT with immunotherapy. Lastly, we will discuss how RT may be exploited to counteract tumor-mediated immune evasion and how immunotherapy can be integrated into RT regimen to achieve improved treatment outcome by promoting immune activation and/or overcoming tumor-associated immune suppression.

7.2 Immune Stimulatory Effects of RT

7.2.1 *Induction of Immune Stimulatory Factors by RT*

RT can directly stimulate production of immunostimulatory cytokines and chemokines from both tumor cells and tumor stroma. Tumor necrosis factor- α (TNF- α), which can enhance the radiation lethality of tumor cells upon treatment with X-rays

in human sarcoma cells [8]. Irradiation of B16 mouse melanoma tumors induces production of interferon- γ (IFN- γ), which can act directly on cancer cells to induce upregulation of surface the major histocompatibility complex class I (MHC-I), an antigen-presenting molecule critical for T cell mediated recognition and elimination of cancer cells [9, 10]. Clinically, serum IFN- γ levels were found to increase in a dose-dependent fashion in the 56 of 63 patients with esophageal squamous cell carcinoma who were treated with RT alone. The remaining 7 patients whose IFN- γ levels remained unchanged in response to RT developed local recurrence despite radiation [11]. Type I interferons (IFN- α and IFN- β) not only play important roles in the immune responses to viral infection, but are also actively involved in anti-tumor immunity [12]. RT can induce production of Type I interferons through activation of intracellular DNA sensors, such as the STING-dependent pathway [13, 14]. Induction of type I interferon within the TME is required for generation of type I interferon-dependent innate and adaptive antitumor immunity by potentiating the cross-priming capacity of tumor-infiltrating dendritic cells (DCs) as well as recruitment and effector function of CD8⁺ T effector cells [15, 16]. It is demonstrated that RT substantially enhances the secretion of the chemokine (C-X-C motif) ligand 16 (CXCL16) by mouse and human breast cancer cells. Upon binding to its receptor CXCR6 on T helper cells (Th1 cells) or activated CD8⁺ effector T cells, CXCL16 plays an important role in recruiting antitumor immune effector cells [17]. Therefore, it is suggested that in addition to the direct cytotoxic effects, RT can exert its immune stimulating effects through triggering the production of immune activating cytokines or chemokines, which might be additive to radiation lethality through autocrine and paracrine mechanisms.

7.2.2 RT-Increased Antigen Presentation Within the TME

Activation of naïve T cells requires both the recognition of antigen-MHC complexes by the T cell antigen receptor and additional costimulatory signals, including B7 molecules (CD80 and CD86) on the antigen-presenting cells (APCs) [18, 19]. Killing of cancer cells and the subsequent inflammatory responses can make the tumors visible to the immune system if released tumor antigens are taken up by DCs and presented to T cells along with effective co-stimulation signals [20]. RT can induce extensive immunogenic alterations of dying and surviving tumor cells within the TME. The resulting stress and death of tumor cells could activate tumor-specific immune responses through the liberation of damage-associated molecular patterns (DAMPs) upon binding to their corresponding pattern recognition receptors (PRRs), such as toll-like receptors (TLRs) on APCs [21]. It was recently reported that RT triggered immunogenic cell death (ICD), which is defined by translocation of stress protein calreticulin from the endoplasmic reticulum (ER) to the tumor cell-surface or extracellular release of the nuclear high-mobility group protein-1 (HMGB1) and adenosine triphosphate (ATP) [22–25]. Although TLRs in the mammalian immune system was first described as innate receptors recognizing pathogen-associated

molecules, there is growing evidence that the TLRs also sense and respond to DAMPs, endogenous molecules or signals associated with cellular stress and tissue injury [26]. In breast cancer patients with loss-of-function alleles in TLR4, which mediates a signaling response to the HMGB1 stimulation, relapse occurs more quickly after chemoradiation compared with patients with wild-type alleles, indicating that the mode of host response to cancer cell death can affect clinical outcomes of cancer therapy [27].

As opposed to normal self-antigens, immune responses can be biased against the tumor-specific antigens that the immune system has been tolerated without resulting in side effects associated with standard cytotoxic therapies [24, 27]. Studies in mouse models revealed that the antigenic repertoire of tumor cells was substantially altered following RT. Radiation-induced exposure of antigenic peptides have been identified as a mechanism underlying RT-elicited antitumor immune response [28]. The ‘danger’ signals, including immunostimulatory cytokines, generated by radiation within the TME can activate DCs phenotypically and functionally for effective cross-presentation of tumor antigens [29]. Intratumoral injection of DCs alone does not show evident antitumor effects in mice with squamous cell carcinoma; however, significant tumor regression were observed when combined with chemoradiation, suggesting that the immune environment conditioned by the RT favors DC activation and fosters generation of antitumor immunity [30]. In both mice and humans, activation of tumor antigen-specific T cell immunity following RT requires TLR4 on DCs. Efficient processing and cross-presentation of antigens from dying tumor cells by DCs during RT are dependent on signaling through TLR4 and its adaptor MyD88 [27]. Similarly, local high-dose irradiation of B16 tumors results in activation of tumor-associated DCs as well as the consequent mobilization of tumor-reactive CD8⁺ T cells [31]. Ablative RT can dramatically improve the cross-priming capacity of tumor-infiltrating DCs. The autocrine effect of type I IFNs is required for the enhanced cross-priming ability of DCs after their infiltration into the irradiated tumor tissues [15]. A recent study demonstrated that adaptor protein STING in DCs and downstream type I IFN signaling are essential for RT-induced adaptive immune responses [13]. In addition, the cytokine secretory profile and its relevance to DC function upon direct radiation exposure have been noted. DCs show enhanced expression of IL-2, IL-12 and IFN- γ after exposure to low dose irradiation, which is positively correlated with their enhanced capability to prime T cells compared with non-irradiated DCs [32].

7.2.3 Activation and Recruitment of T Cells by RT

In addition to direct damage to the tumor cell DNA, accumulating data supports the notion that T cell recruitment and activation represent important mechanisms mediating the antitumor effect of RT. Stone et al. provided the first evidence supporting T cell repertoire dependent tumor response to RT by comparing the efficacy of RT in immunocompetent and T cell deficient mice [33]. RT can also

remodel the abnormal tumor vessels and facilitate efficient tumor infiltration of anti-tumor T cells in a transgenic mouse model of insulinoma with multiple carcinogenesis. The remodeling of the tumor vasculature directly affects lymphocyte extravasation and effector function [34]. Up-regulation of vascular cell adhesion molecule (VCAM)-1 after RT promotes T cell infiltration into mouse B16 melanomas [10, 35]. Recruitment of CD8⁺ cytotoxic T lymphocytes (CTLs) into 4 T1 mammary tumors was found to depend on RT-induced CXCL16 release from tumor cells [17]. Indeed, the chemokine CXCL16 has been identified as a prognostic factor that correlates with improved survival and increased numbers of tumor-infiltrating lymphocytes in colorectal cancer and renal cell carcinoma [36, 37]. Prostate cancer patients developed detectable tumor-specific CD4⁺ and CD8⁺ T cells responses following RT that were undetectable prior to the treatment [38]. RT has been reported to dramatically increase the T-cell priming in lymphoid tissues or tumor tissue. The efficacy of RT can be abolished upon depletion of CD8⁺ T cells through administration of anti-CD8 monoclonal antibodies [39, 40]. Combination of RT with Th1 cell therapy augments the generation of tumor-infiltrating CTLs, resulting in complete regression of mouse EG7 lymphomas, which suggests that CD4⁺ T cells are also critically involved in RT-induced CTL response and tumor eradication [40].

7.2.4 Other Immune Cells Activated by RT

Natural killer (NK) cells are considered to be innate lineage cells based on their characteristic that there are no specific antigen receptors on their surface unlike T and B cells. NK cells play an important role in antitumor immunity by directly targeting tumor cells through cytotoxicity or the secretion of soluble immune mediators [41]. Ionizing radiation can increase the expression of natural-killer group 2 member D (NKG2D) ligands in human cancer cell lines, including KM12, NCI-H23, HeLa and A375. This makes the irradiated cancer cells more susceptible to NK cell-mediated cytotoxicity via the activating receptors NKG2D [42, 43].

7.3 Immune Suppressive Mechanisms Engaged by RT

7.3.1 Immunosuppressive Factors Induced by RT

Although RT can induce immune responses to tumor antigens, it is not sufficient to prime T cells specific for endogenous antigens that can efficiently reject the poorly immunogenic tumors. This may be attributed to the preexisting immune suppression in the TME and the immunosuppressive factors induced by RT. Activation of TGF- β is an early as well as a persistent event in tumors exposed to RT [44, 45]. Serum levels of TGF- β during the course of RT was evaluated in patients with

non-small-cell lung cancer to adaptively deliver higher doses of radiation [46]. TGF- β plays a dual role both limiting tumor growth and stimulating tumor cells progression. Although TGF- β seems to be an antitumor factor at the early stages of cancer progression, it eventually becomes protumorigenic [47, 48]. In the mouse mammary tumor virus-polyoma virus middle T antigen (MMTV-PyVmT) transgenic model of metastatic breast cancer, RT significantly increases the circulating TGF- β and lung metastases, which can be suppressed by the deficiency of type II TGF- β receptor. This implicates RT induced TGF- β as a pro-metastatic signal for tumor cells [49]. TGF- β neutralization in mice bearing 4 T1 mammary tumors enhances radiation sensitivity and significantly delays tumor growth [50]. A recent study reported that TGF- β activity is a major obstacle that hinders the ability of RT to induce antigen-specific anti-tumor immunity. Neutralization of TGF- β by antibody injection during RT effectively rescues a CD8⁺ T-cell response against poorly immunogenic mouse carcinomas [51].

Hypoxia-inducible factors (HIFs) are the main molecular transcriptional factors in the hypoxia response [52, 53]. HIF-1 is highly induced in the irradiated tumors and high HIF-1 activity is often used as an independent predictor of poor prognosis after RT [54–56]. Expression of HIF-1 and HIF-2 is strongly associated with RT failure in patients with head and neck squamous cell carcinoma [57]. HIF-1 can stimulate the production of stromal-derived factor-1 (SDF-1), a chemokine that recruit tumor-promoting and immunosuppressive myeloid cells through the chemokine receptor CXCR4 [58, 59].

7.3.2 *Tumor-Associated Macrophages*

Tumor-associated macrophages (TAMs), derived from circulating monocytes, make up a critical component of immune cells in solid tumors [60, 61]. Although a few studies showed that RT enhances the anti-tumor properties of TAMs, including enhanced cytolytic activity and increased secretion of IL-12 and IL-18 [62, 63], there exists extensive literature indicating that TAMs enhance resistance to RT. CD11b⁺ myeloid cells, including TAMs, are believed to be the major source of pro-tumor growth factors that support angiogenic programs during tumor progression, e.g., vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9) [58]. Murine tumors are more sensitive to RT when are transplanted in CD18 hypomorphic or CD11b knockout mice. Resistance of tumors to RT is partially restored by rescue of CD18 hypomorphism with the reconstitution of wild-type bone marrow [64].

Depletion of TAMs by injection of liposomal clodronate prior to RT enhances tumor control, emphasizing an important role of TAM for modulating tumor response to RT. Radiation exposure upregulates VEGF expression in macrophages and VEGF-neutralization subcutaneously improves the antitumor potency of RT

[65]. Recently, it was revealed that CD11b⁺ monocytes/macrophages restored the damaged vasculature by promoting vasculogenesis and growth of surviving cancer cells following RT in a human glioblastoma xenograft model. Blocking the influx of CD11b⁺ monocytes/macrophages by pharmacologic inhibition of HIF-1 or SDF-1-CXCR4 pathway can prevent tumor recurrence [66]. Similar observation was made using human breast and lung carcinoma xenografts, further supporting a critical role of myeloid cells, primarily macrophages, in promoting tumor regrowth after RT. It is proposed that TAMs facilitate tumor recurrence by promoting the survival of endothelial cells (ECs) and tumor revascularization [67]. Studies of three murine tumors (TRAMP-C1 prostate adenocarcinoma, ALTS1C1 astrocytoma, and GL261 glioma) demonstrate that CD11b^{low}/F4/80⁺ macrophages locate at the junctions between central necrotic and surrounding hypoxic regions in the irradiated tumors. Hypoxia-aggregated TAMs are more polarized toward an immunosuppressive and pro-angiogenic M2 phenotype, indicated by the higher expression of arginase I [68]. Thus, despite a potential stimulatory effect of radiation on cytolytic activity of macrophages, the recruitment and alternative activation of macrophages in the TME shifts the balance toward immunosuppression and pro-angiogenesis that benefits tumor recurrence.

7.3.3 Myeloid-Derived Suppressor Cells

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of myeloid cells, comprised of myeloid progenitor cells and immature myeloid cells [69, 70]. MDSCs are often expanded in tumor-bearing hosts and have been well documented to act as a suppressor of antitumor immunity [71–73]. MDSCs are believed to be one of the mechanisms by which cancers escape from immune surveillance or resist immunotherapy [71–73]. MDSCs are characterized as CD11b⁺Gr-1⁺ cells in mice [69, 70] and CD11b⁺CD14⁻CD33⁺ in human [74]. Two distinct subsets of MDSCs have been identified in mice, i.e., monocytic MDSCs (M-MDSCs; CD11b⁺Ly6G⁻Ly6C^{high}) and granulocytic MDSCs (G-MDSCs; CD11b⁺Ly6G⁺Ly6C^{int}), both characterized by the expression of Gr-1 on the cell surface [75]. CSF signaling has been documented to expand and recruit myeloid cells or MDSCs to the tumor sites [76, 77]. Use of selective inhibitor of colony-stimulating factor 1 receptor (CSF1R) can suppress tumor growth more effectively when combined with RT, highlighting the significance of CSF1/CSF1R signaling in the recruitment of myeloid cells (e.g., MDSCs) that limit the efficacy of RT [78]. The role of MDSCs in limiting the efficacy of RT has also been demonstrated in RT in combination with Sunitinib, an angiogenesis inhibitor [79, 80]. A recent study found that Sunitinib treatment decreased M-MDSC levels and enhanced T-cell proliferative activity in cancer patients with oligometastases [81]. Moreover, the synergistic effect of Sunitinib and stereotactic body radiotherapy (SBRT) was only seen in the responders whose CD11b⁺CD33⁺ myeloid cell populations were reduced by Sunitinib [81].

7.3.4 Regulatory T Cells

FoxP3⁺ regulatory T (Treg) cells are suppressive immune cells that promote tumor progression through suppressing anti-tumor immune responses [82–84]. Treg cell ablation in a polyoma middle-T antigen-driven tumor model significantly reduces tumor burden and improves overall survival following RT. Combining Treg cell ablation with RT could provide beneficial effects for the poorly immunogenic malignancies [85]. Epidermal mononuclear phagocytes Langerhans cells (LCs) are resistant to the depletion by high dose irradiation. Upon exposure to RT, LCs upregulates MHCII molecule and induces the expansion of Treg cells that can dampen anti-tumor immunity [86, 87].

7.4 Combining RT with Immunotherapy to Improve Therapeutic Index

RT alone is often insufficient to achieve a permanent cure in many clinical scenarios. This is primarily a result of insufficient radiation doses to control tumor without resulting in unacceptable toxicity related to normal tissues. This also suggests that despite the numerous pro-immunogenic or immunostimulating effects, RT as a sole modality fails in shifting the immunosuppressive TME. Systemic antitumor responses following local RT or abscopal responses are also extremely rare in clinical practice. However, RT-induced systemic abscopal response through development of effective and durable antitumor immunity can be promoted by additional immune manipulation. Therefore, it provides a scientific rationale for integrating RT with immunotherapy to amplify the systemic antitumor immunity and to improve overall therapeutic outcomes.

Irradiated tumor cells have been shown to be a source of tumor-associated antigens which can elicit anti-tumor T cell responses after capture and presentation by DCs [88]. Combination of RT and concurrent administration of DCs may result in *in situ* vaccination against tumors. Injection of unpulsed autologous DCs directly into irradiated D5 melanoma or MCA 205 sarcoma tumors was shown to activate tumor-specific reactive T cells and generate a potent systemic antitumor response causing regression of established tumors [89]. In a recent phase I clinical trial of combining external beam RT and intraprostatic DC injection, patients with high-risk prostate cancer showed increased tumor-infiltrating CD8⁺ T-cells as well as prostate specific CD8⁺ T-cells in the peripheral blood [90]. In patients with high-risk soft tissue sarcoma that received this combinational therapy, 9 of 17 patients developed tumor-specific immune responses and 12 patients remained free of progression 1 year after treatment [91]. Another recent trial was conducted in 40 patients with recurrent, metastatic, or locally advanced tumors [92]. Patients were treated with conformal RT and autologous DCs pulsed with autologous tumor cell lysates or tumor-specific peptides. Of 9 patients with evaluable tumor response outside the

RT target site, 22% had a partial response and 33% had stable disease, indicating that the combination of RT and DC-based vaccination induces measurable clinical responses [92].

An alternative approach that combined local RT and concomitant expansion of DCs *in vivo* through systemic administration of fms-like tyrosine kinase-3 ligand (Flt3L) was also shown to improve survival of animals bearing Lewis lung carcinoma by generating a long-term tumor-specific immune response [93]. The use of Flt3L was also shown to facilitate abscopal effect of RT, indicated by inhibition of both the irradiated breast tumor and the contralateral untreated tumor [94].

Manipulation of the TLR signaling can increase the functional activation of APCs and provide co-stimulation signals to T cells, thereby facilitating an effective adaptive antitumor immunity after RT [95, 96]. A phase II trial conducted in patients with recurrent anaplastic glioma showed that combined RT and intramuscular injection of poly-ICLC, a TLR3 agonist, improved 1-year overall survival compared to RT alone [97]. The TLR7 agonist imiquimod has been approved for the treatment of basal cell skin carcinomas and melanomas. Topical imiquimod can synergize with RT to inhibit tumor growth in a mouse model of skin-involving breast cancer, which is associated with increased number of tumor-infiltrating CD11c⁺, CD4⁺ and CD8⁺ cells [98]. Based on a recent clinical study demonstrating imiquimod-induced immune rejection of skin metastases in breast cancer patients [99], a trial is ongoing to test combination of imiquimod and RT for improving therapeutic outcomes in brain cancer (ClinicalTrials.gov: #NCT01400672).

Our studies have recently identified scavenger receptor A (SRA) or CD204, a pattern recognition innate receptor, as an immunosuppressive molecule expressed on DCs that dampens DC function and T cell activation against several cancers by suppressing DC-intrinsic TLR signaling [100–102]. Our subsequent work revealed that absence of SRA/CD204 significantly increased the immunogenicity of ionizing radiation-treated mouse prostate cancer cells [103], which provides a scientific rationale for combining RT with *in situ* vaccination using SRA/CD204-downregulated DCs. We showed that intratumoral administration of SRA/CD204-silenced DCs, not DC counterparts without genetic modification, profoundly enhanced the control of RT-treated mouse prostate tumor as well as its metastases, which was mainly mediated by IFN- γ -producing CTLs [104]. These preclinical evidence supports the further development of TLR or SRA-targeting strategies for combinational use with RT to convert the tumor into an effective individualized vaccine.

Adoptive cell therapy (ACT) is a passive cancer immunotherapy by transferring of tumor-specific T cells that have been expanded *ex vivo* to cancer patients [105, 106]. Local irradiation of mouse MC38 colon tumors causes up-regulation of Fas on tumor cells and potentiates tumor eradication by adoptively transferred antigen-specific CTLs [107]. Local tumor irradiation combined with intratumoral DC vaccination regimens significantly enhances the therapeutic efficacy of ACT in a mouse liver cancer model, evidenced by reduced local tumor size, decreased metastasis, and prolonged survival. The enhanced antitumor activity is correlated

with the activation of endogenous CD4⁺ T cells [108]. Therapeutic vaccination represents an active immunotherapy that aims to stimulate T cell response against specific tumor antigens. The synergistic antitumor effect of RT and therapeutic immunization is supported by a preclinical study in colon tumor-bearing mice that received RT plus recombinant vaccinia encoding carcinoembryonic antigen [109]. In a randomized phase II trial patients received RT alone or RT plus a viral vaccine targeting tumor antigen PSA and co-stimulatory B7.1. The results showed an elevated PSA-specific T cell response in the combination group compared to the RT alone arm [110].

Immune modulators that target immunosuppressive signaling in T cells to overcome immune suppression and restore and/or sustain antitumor function of T cells for tumor eradication have shown promise in cancer patients [111]. Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death 1 (PD-1) are two primary immune checkpoint molecules that inhibits T cell activation upon binding to their ligands, B-7 molecules and PD-L1, respectively [20, 111, 112]. As T cell activation relies on the engagement of both antigen receptor and the costimulatory molecule CD28 [113], it is conceivable that using RT to increase antigen availability and APC activation in conjunction with anti-CTLA-4 or anti-PD-L1 therapy will further augment antitumor immune responses.

Anti-CTLA-4 antibody, ipilimumab, has been approved by FDA in the treatment of patients with metastatic melanoma [114]. Combination of local RT of 4T1 mouse mammary carcinoma with the anti-CTLA-4 monoclonal antibodies 9H10 significantly elicited an anti-tumor immunity that inhibited metastases [115]. In another preclinical study administration of 9H10 was shown to optimize tumor response to fractionated RT by inducing an abscopal effect involving activation of CD8⁺ T cells [116]. The abscopal effect was also reported in a patient with metastatic melanoma following treatment with RT plus ipilimumab. Clinical observation obtained several months after last dose of RT revealed that tumor masses in the spleen and hilar lymph nodes eventually reached the point of stable minimal disease [4]. Complete response in both the primary tumor and the metastatic lesions was also achieved in another patient with asymptomatic melanoma treated with ipilimumab and concurrent RT [117]. A recent case report described that a patient with non-small-cell lung carcinoma also showed abscopal response upon the combination therapy [118]. A phase III trial that evaluated RT combined with ipilimumab therapy in metastatic castration-resistant prostate cancer (mCRPC) was recently completed [119]. The trial did not meet its primary endpoint, however, there was an improvement in overall survival of patients treated with RT plus and ipilimumab compared to RT plus placebo arm (11.2 months vs. 10 months; $p=0.053$) [119]. Currently, more than ten phase I/II clinical trials that are testing the combination of RT and ipilimumab for treatment of multiple cancer types including melanoma, head and neck cancer, and cervical cancer are ongoing.

PD-1 receptor is another important immune checkpoint molecule that down-regulates T cell-mediated immune responses. Expression of PD-1 on T cells in

the TME is an indicator of their exhaustion that is often associated with an impaired T cell response. Overexpression of the PD-1 ligand (PD-L1), also known as B7 homolog ligand 1 (B7-HL1), in a variety of malignant cancers such as renal, lung, ovarian, breast, head and neck cancers, represents one of the mechanisms responsible for tumor immune evasion [20, 120]. Low doses of fractionated RT increases the tumor expression of PD-L1 in a number of syngeneic mouse cancer models, which is attributed to IFN- γ produced by CD8⁺ T cells [121]. Upregulation of the PD-L1 on tumor cells were shown to contribute to radio-resistance of cancer and suppress the antitumor function of tumor-infiltrating T cells [121–123]. Recently, antibodies targeting PD-L1 (BMS-936559, MEDI4736, MPDL3280A) and its receptor PD-1 (Nivolumab, Pidilizumab, Lambrolizumab) have been developed to overcome PD-1/PD-L1 signaling-mediated immune suppression. Clinical studies showed that anti-PD-1/PD-L1 antibodies have achieved significantly increased objective response (~20–30 %) in the treatment of several types of cancers including non-small cell lung cancer, melanoma, and renal-cell cancer [124–126]. Studies using pre-clinical models demonstrated the synergistic effects of RT combined with PD-1 checkpoint inhibitors. Adding anti-PD-1 antibody to combination therapy of RT and anti-CD137 therapy resulted in cure of the primary mammary tumors [127]. Treatment with RT in conjunction with anti-PD-1 antibody resulted in synergistic inhibition of mouse glioma, TUBO mammary carcinoma, and MC38 colon adenocarcinoma, probably through increasing the infiltration of IFN- γ - or TNF- α -expressing CTLs while decreasing the accumulation of Treg and MDSCs within the TME that normally suppress T cell function [122, 128]. Despite the impressive clinical responses resulted from immune checkpoint inhibitors, optimization is required to overcome multiple non-redundant mechanism of immune resistance. A recent phase I trial reported that melanoma patients with high expression of PD-L1 did not respond to RT plus anti-CTLA4 therapy [129]. Mouse studies found that this resistance was due to upregulation of PD-L1 on melanoma cells during RT and consequent T-cell exhaustion, which allows tumors to escape anti-CTLA4 therapy. Thus, triple combination of RT, anti-CTLA4 and anti-PD-L1 treatments, which enhances the diversity of the T-cell receptor repertoire of intratumoral T cells, inhibits Treg cells, thereby increasing the ratio of CTL to Treg, and reverses T-cell exhaustion, can achieve maximum antitumor response by engaging distinct mechanisms [129].

In addition to targeting immune checkpoint molecules to rescue and sustain T cell functions in the TME, other approaches directed to promote co-stimulation can also enhance T cell priming and effector function. OX40 signaling is one of the co-stimulatory mechanisms involved in T cell activation [130–132]. Administration of OX40 agonistic antibodies in combination with RT significantly extends the mouse survival in a model of primary sarcoma by augmenting the activity of tumor antigen-specific CTLs following RT [133]. Clinical trials of combining RT and OX40 agonist for treatment of metastatic prostate cancer (ClinicalTrials.gov: #NCT01303705) and breast cancer (ClinicalTrials.gov: #NCT01642290) are ongoing.

7.5 Challenges and Opportunities

Encouraging preclinical results of combining RT and immunotherapy for cancer treatment have stimulated clinical translation of this combinatorial therapeutic modality. However, clinical data from trials that combined RT with other immune modulating agents, e.g., vaccines, immune checkpoint blockade, have only shown a modest promise. While these outcomes provide the rationale for current clinical trials of both treatments, further investigation is required to realize the full potential of the combination.

The immunogenic alterations in the TME induced RT at molecular and cellular levels are just beginning to be elucidated. The immunoregulatory effects of dose and fractionation schedules as well as delivery during RT remain to be further defined. The timing of RT relative to immune manipulation should also be carefully determined in preclinical and clinical studies. Extrapolation of data derived from mouse models may have its own limitation because currently most animal studies involve radiation to tumor xenografts. Use of spontaneous transgenic mouse model instead of transplantation mouse model to address these questions may better guide the optimal design of clinical RT in combination with immunotherapy. Mechanistic understanding of immunological and biological changes in the TME has an important impact on capitalization of tumor destruction capacity of radiation and immune augmentation.

The immunosuppressive effect of the TME remains a major hurdle for clinical success of combined RT and immunotherapy. The promising results observed in clinical trials with immune checkpoint blockade therapy highlights the therapeutic potential by modification of the TME to overcome immunosuppressive pathways. Exploring and identifying additional and non-redundant immunosuppressive molecules or mechanisms that operate in the TME will provide new therapeutic targets to synergize with RT to mount an effective and durable antitumor immunity.

Personalized medicine has been an important part of therapeutic endeavors in the field of cancer research and treatment. Emerging data suggests that each patient's own immune system have the potential to develop a tailored immune response to the unique clonal populations within the tumors. Thus, immunotherapy is at the forefront of personalize anticancer therapy and precision oncology. With the advantage of its very focused and localized nature, RT is ideal for combination with proper modulations of the immune system and has a great potential to convert the tumor into an individualized vaccine. Ongoing and future randomized trials with RT and immune-based combinations will help determine if such regimens can change the RT paradigm and, more importantly, revolutionize cancer treatment (Table 7.1).

Table 7.1 Ongoing clinical trials of combined radiation therapy with immunotherapy

Description	Cancer type	ClinicalTrials.gov Identifier
Radiation therapy plus dendritic cells vaccination	Metastatic melanoma	NCT01973322
	Pancreatic cancer	NCT00868114
	Glioblastoma	NCT02010606
	Glioblastoma	NCT02366728
Radiation therapy plus toll-like receptor signaling-targeting agents	Soft tissue sarcoma	NCT02180698
	Breast cancer	NCT01421017
	T cell lymphoma	NCT02061449
	B cell lymphoma	NCT02254772
Radiation therapy plus immune checkpoint blockade	Pancreatic cancer	NCT02311361
	Metastatic melanoma	NCT02406183
	Non-small cell lung cancer	NCT02599454
	Non-small cell lung cancer	NCT02434081
Radiation therapy plus adoptive T cell transfer	Merkel cell carcinoma	NCT02584829
	Merkel cell carcinoma	NCT01758458
	Nasopharyngeal carcinoma	NCT01462903
	Hepatocellular carcinoma	NCT01462903
	Breast carcinoma	NCT01462903

7.6 Concluding Remarks

The effort in improving the therapeutic efficacy of RT have focused on the capability of ionizing radiation to kill neoplastic cells while sparing normal healthy tissues. However, accumulating evidence supports the immune modulating effects of RT (Fig. 7.1a). RT-mediated destruction of cancer cells releases tumor antigens along with ‘danger’ signals or PAMPs that defines the immunogenicity of tumor or ICD. These immunostimulatory factors result in recruitment and activation of APCs (e.g., DCs), which facilitates subsequent T cell priming and antitumor immune response. However, it is recognized that multiple mechanisms in the TME, which involve induction of immunosuppressive factors (TGF- β , CTLA-4 and PD-1) and recruitment of immunosuppressive cells (TAM, MDSC, Treg cell), dampen or impair immune effector function and promote immune tolerance. The substantial expansion and recruitment of myeloid cells following RT is known to facilitate tumor revascularization and possibly immune suppression as well. Therefore, it is unlikely RT alone is capable of generating an effective immune response that can eradicate tumors locally and abscopally. Nevertheless, interaction of RT and the immune system offers new opportunity to strengthen and improve antitumor

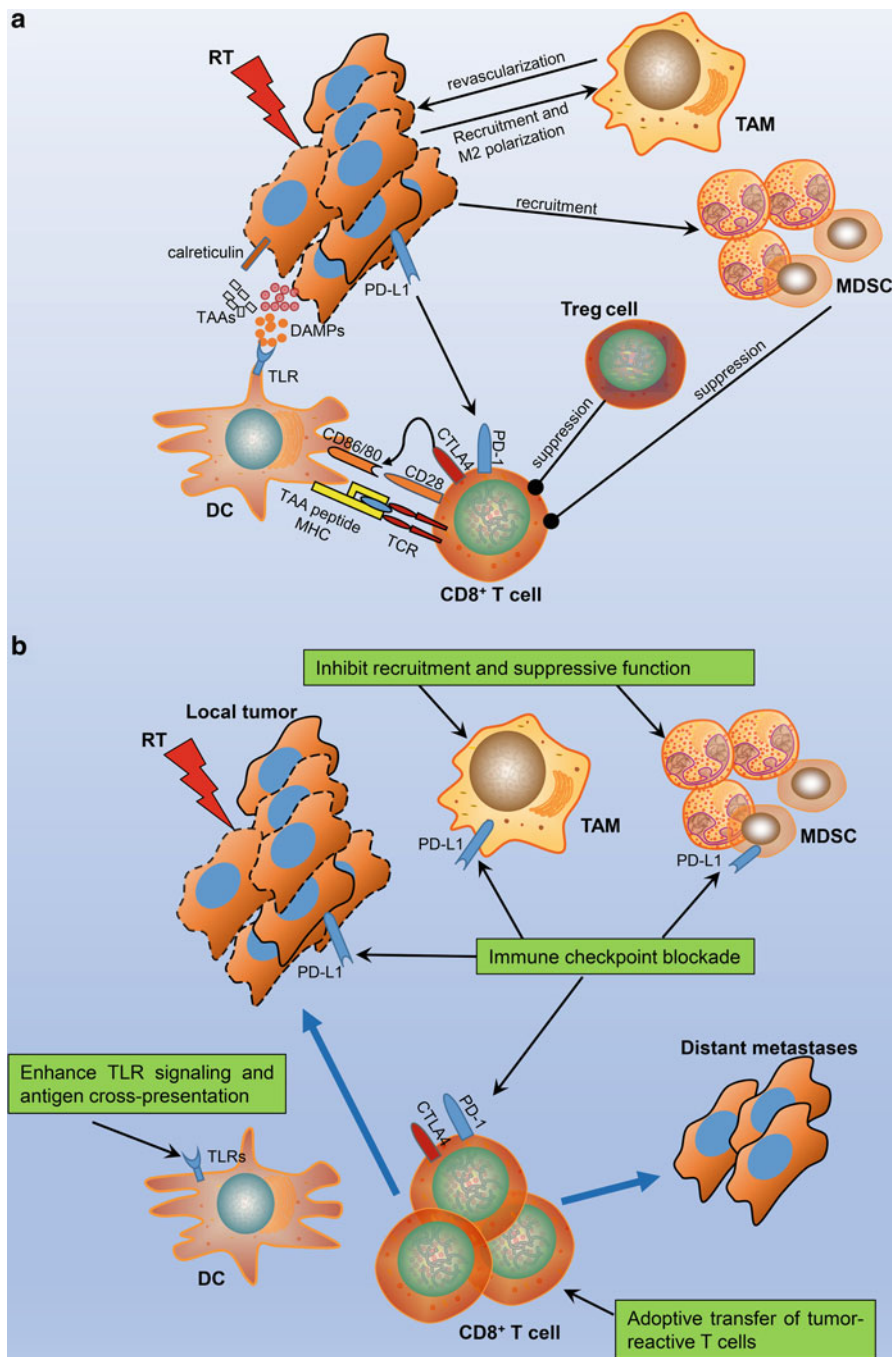


Fig. 7.1 Exploiting the interplay between radiation therapy (RT) and the immune system to improve cancer therapeutic index. (a) RT can induce immunogenic cell death associated with release of tumor-associated antigens (TAA) with damage-associated molecular patterns (DAMPs), which recruit and stimulate dendritic cells (DCs) via toll-like receptor (TLR) for antigen cross-presentation.

response by strategically combining RT and immune interventions or immunotherapy, e.g., in situ DC vaccination, TLR activation, immune checkpoint blockade, immune co-stimulation (Fig. 7.1b). Cancer immunotherapy has emerged as a viable therapeutic option and with multiple agents in clinical development the immunotherapeutic portfolio is expected to expand significantly in the near future. While more research is needed to precisely understand and rationally optimize the protocol of this combinatorial treatment, clinical studies have started to show the promise in improved treatment outcome, which we believe may lead to ultimate elimination of cancers and metastases by amplifying immune-mediated abscopal effects after standard RT.

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Fig. 7.1 (continued) Subsequent priming of CD8⁺ T cells through engaging surface T cell receptor (TCR) contributes to cancer cell killing during RT. However, concurrent increase in the number of pro-angiogenic and immunosuppressive cell types, such as tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Treg), as well as induction of immunosuppressive factors, such as cytotoxic T-lymphocyte-associated protein-4 (CTLA-4), programmed cell death-1 (PD-1) and programmed death-ligand 1 (PD-L1), compromise immune effector function within the tumor microenvironment (TME) and promote cancer escape and recurrence. **(b)** While RT-induced immune response is not adequate to achieve systemic eradication of tumors, integrating RT with immunotherapy could amplify the systemic antitumor immunity and improve overall treatment outcomes. Selective stimulation of TLRs or strategic administration of ex vivo expanded DCs can enhance DC function and antigen cross-presentation by providing additional co-stimulatory signals. Adoptive transfer of tumor-reactive T cells, either ex vivo expanded T cells or genetically modified T cells, can further strengthen the effector arm of antitumor mechanisms since radiation exposure sensitizes cancer cells to the killing of T cells. Additionally, immune checkpoint inhibitors can be used to rescue the effector function of T cells by counteracting the immunosuppressive signaling mediated by CTLA-4 or PD-1/PD-L1. Other approaches directed to block the recruitment and/or to overcome the immunosuppressive activity of myeloid cells (e.g., TAMs, MDSCs) can also boost antitumor immune response. The increased magnitude and duration of systemic activation tumor-specific T cells combined with direct tumoricidal effect of RT could lead to synergistic and optimized destruction of local/regional tumors as well as metastases

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