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Review Article

Effects of radiation on T regulatory cells in normal states and cancer: mechanisms and clinical implications

Shu Liu[1*], Xiangdong Sun[2*], Jinhua Luo[3*], Hongcheng Zhu[1], Xi Yang[1], Qing Guo[4], Yaqi Song[5], Xinchen Sun[1]

Departments of [1]Radiation Oncology, [3]Thoracic Surgery, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China; [2]Department of Radiotherapy, The 81st Hospital of PLA, Nanjing 210002, China; [4]Department of Oncology, Taizhou People's Hospital, Taizhou 225300, China; [5]Department of Radiation Oncology, Huai'an First People's Hospital, Nanjing Medical University, Nanjing 223300, Jiangsu, China. [*]Equal contributors.

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Abstract: Radiation remains an important component of cancer treatment. In addition to inducing tumor cell death through direct cytotoxic effects, radiation can also promote the regression of tumor via augment of immune response. Regulatory T cells (Tregs) are a unique subpopulation of CD4 positive cells, which are characterized by expression of the forkhead box P3 (Foxp3) transcription factor and high levels of CD25. Mounting evidence has shown that Tregs are implicated in the development and progression of various types of cancer, which makes Tregs an important target in cancer therapeutics. Generally, lymphocytes are regarded as radiosensitive. However, Tregs have been demonstrated to be relatively resistant to radiotherapy, which is partly mediated by downregulation of pro-apoptotic proteins and upregulation of anti-apoptotic proteins. Moreover, radiotherapy can increase the production of Tregs and the recruitment of Tregs to local tumor microenvironment. Tregs can attenuate radiation-induced tumor death, which cause the resistance of tumor to radiotherapy. Recent experimental studies and clinical trails have demonstrated that the combination of radiation with medications that target Tregs is promising in the treatment of several types of neoplasms. In this review, we discussed the effect of radiation on Tregs in physiological states and cancer. Further, we presented an overview of therapies that target Tregs to enhance the efficacy of radiation in cancer therapeutics.

Keywords: Regulatory T cell, T regulatory cells, Tregs, radiation, radiotherapy, cancer, immune response

Introduction

Radiotherapy constitutes a mainstay in cancer therapy. Although radiation has been thought to induce tumor cell death via direct cytotoxic effects, various studies has shown that radiation can lead to the regression of tumor by enhancing immune response [1]. For instance, radiotherapy is associated with the spontaneous regression of metastatic tumor that is distant from the irradiated location [2]. This phenomenon, which is known as the "abscopal effect", is demonstrated to be at least partially mediated by activation of immune system [1, 2]. The titer of antibody that is against specific tumor antigen was markedly elevated in the serum of the patient after completing radiotherapy, which was also correlated with a therapeutic response [2].

Multiple mechanisms have been unraveled to be involved in the radiation-mediated immune response. Tumor cells often escape immune surveillance by loss of expression of tumor specific antigens. Radiation can cause the release of tumor specific antigens that are subsequently presented by dendritic cells to initiate a T-cell dependent immune response. Radiation can also promote immune recognition by increasing the expression of major histocompatibility complex-I molecules (MHC-I), which are downregulated by tumor cells. The increased expression of MHC-I molecules can facilitate the presentation of new peptides generated by tumor cells, further augmenting anti-tumor response. Aside from effects on tumor antigen presentation, radiation can upregulate the expression of Fas antigen, a molecule that is actively involved in lymphocyte-mediated cytotoxicity, thus sensi-

tizing tumor cells to T-cell mediated cytotoxicity [3]. Additionally, the expression of NKG2D ligands, which can be induced under cellular stress, is increased by radiation through activation of ATM pathway [4]. Ligation of NKG2D receptor that is expressed on activated T cells and NK cells by NKG2D ligand results in increased production of cytokine and cytotoxicity in both $CD8^+$ T cells and natural killer (NK) cells [5, 6].

In addition to direct effects on tumor cells, radiotherapy is capable of enhancing immune response by modulating the tumor microenvironment. The tumor microenvironment is composed of stromal cells that consist of fibroblasts, endothelial cells as well as various leukocytes, cytokines and the extracellular matrix [7]. Radiation can promote leukocyte adhesion and migration by up-regulating the expression of adhesion molecules including vascular cell adhesion protein 1 (VCAM-1), E-selectin, and intercellular adhesion molecule 1 (ICAM-1) on endothelial cells. Further, radiation can increase vascular permeability and chemokine expression, resulting in more recruitment of T cells into the tumor microenvironment. The detailed mechanisms by which radiation modulates the tumor microenvironment are out of the scope of this review, which has been extensively reviewed elsewhere [7].

T Regulatory cells (Tregs) constitute a unique subpopulation of $CD4^+$ cells which constitutionally express the forkhead box P3 (Foxp3) transcription factor and high levels of CD25 on their surface [8]. Tregs account for 5-10% of peripheral $CD4^+$ T cells in physiological condition in rodents and humans, and play a crucial role in mediating immune homeostasis, as well as maintaining self-tolerance [9]. Moreover, Tregs are engaged in the suppression of tumor-induced immune responses, which makes them one of the attractive targets of cancer therapeutics. In this review, we will discuss the effect of radiation on Tregs in physiological conditions and pathological states. Further, we will present an overview of therapies that target Tregs to enhance the efficacy of radiation in cancer therapy.

Physiology of Tregs

Tregs are characterized by expression of transcription factor Foxp3, a member of the forkhead transcription factor family, which is specific for distinguishing Tregs from other T helper subsets. Expression of Foxp3 is central to the development, maintenance and function of Tregs [10]. Mice with loss-of-function Foxp3 mutation lack functional Tregs, and eventually succumb to lymphoproliferative diseases. Similarly, humans with hypomorphic Foxp3 mutation present with immunodysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX) [11]. Accordingly, changed prevalences and functional deficits of Tregs are associated with a multitude of autoimmune diseases [12]. Persistent Foxp3 expression is indispensable for Tregs to maintain their suppressive functions. Deletion of Foxp3 in differentiated mature Tregs leads to the dysregulation of its target genes and the loss of suppressive function [13].

Two major subsets of Tregs have been defined in humans: natural Tregs (nTregs) and inducible or adaptive Tregs (iTregs). NTregs, which are derived from the thymus, constitute the majority of Tregs for maintaining peripheral tolerance. Many co-stimulatory signals have been involved in the development and lineage commitment of nTregs including CD28 ligation by CD80/CD86, interlukin-2 receptor (IL2R), thymic stromal-derived lymphopoietin receptor (TSLPR), CD154, glucocorticoid-induced tumor necrosis factor receptor (GITR), and signal transducer and activator of transcription 5 (STAT5) signaling [14]. NTregs express various molecules which mediate tissue and microenvironment homing. After exiting the thymus, nTregs migrate to the inflammation sites and suppress various effector lymphocytes, especially helper T (Th) cells including Th1, Th2, Th17, and follicular Th (Tfh) cells [15]. NTregs mediate immune suppression that requires cell-to-cell contact mechanisms including the granzyme B/perforin or Fas/FasL pathways [16, 17]. ITregs are different from nTregs in their generation, cell fate and functional stability. ITregs originate in the periphery under a variety of conditions, which include not only antigen presentation under subimmunogenic or noninflammatory conditions, but also inflammation and infections [18]. It has been demonstrated that $Foxp3^+$ iTreg cell development requires at least T cell receptor (TCR) stimulation and the cytokines transforming growth factor beta (TGF-β) and interlukin-2 (IL-2) [19]. However,

unlike nTregs, iTregs do not need costimulation. ITregs suppress antigen-specific T cell response through a cytokine-dependent mechanism through releasing TGF-β, interlukin-10 (IL-10) as well as other immunosuppressive factors [20].

Tregs in cancer

Tregs associate with different prognosis in cancer

In spite of the well-established role of Tregs in the maintenance of normal immune status, increasing evidences have revealed that Tregs are involved in the development and progression of some human malignancies. $Foxp3^+$ Tregs has been shown to be present at high frequencies in tumor tissues of various types of cancer such as breast, lung, liver, pancreatic and gastrointestinal cancers and malignant melanoma [21]. In addition, the frequency of Tregs was also increased in the peripheral blood lymphocyte of patients with head and neck squamous cell carcinoma (HNSCC) [22]. Furthermore, accumulation of Tregs within tumor samples predicted for worse survival in patients with ovarian cancer, breast cancer, and gastric cancer [23]. And another study also showed that a high regulatory/$CD8^+$ T cell ratio was predictive of unfavorable prognosis in ovarian cancer [24]. It could be expected since Tregs-mediated suppression eliminates anti-tumor function of effector cells, thus favoring tumor growth. However, evidence indeed exists that enrichment of $Foxp3^+$ Tregs, as determined by immunohistochemistry (IHC), does not always indicate a poor prognosis. Studies have identified Tregs infiltration as a favorable prognostic factor in colon cancer and ovrian cancer patients, indicating a more complicated role for Tregs in the prognosis of cancer patients [25-27]. There are several reasons for this discrepancy. It is known that Tregs exert inhibitory functions in some contexts but not others. Besides, in some cancers, Tregs are positively correlated with tumor-infiltrating $CD8^+$ T cells, T helper 17 (Th17) cells or other effectors [28], all of which are usually predictive of better prognosis. As a result, in this context, Tregs being identified as a favorable predictor may be probably due to its association with the count of tumor-infiltrating cells. Furthermore, other studies have suggested that, in gastric and colorectal cancers, Tregs may suppress tumor-promoting inflammatory response to microbes, accounting for their association with better prognosis in these cancers [29].

Tregs are involved in the pathogenesis and progression in cancer

In vitro studies also revealed that Tregs are involved in the pathogenesis and progression in cancer. An early study, depletion of Tregs using anti-CD25 monoclonal antibodies eradicated syngeneic tumors, suggesting that Tregs were implicated in the growth of these tumors [30]. Similar results were observed in other experiments in which removal of $CD4^+CD25^+$ cells by immunotoxin-conjugated IL-2 lead to augmented antigen-specific T-cell immnue response and inhibit tumor growth [31, 32]. In contrast to this, adoptive transfer of $CD4^+CD25^+$ T cells, but not $CD4^+CD25^-$ T cells derived from animals immunized with Dna J-like 2, a SEREX-defined wild-type antigen, significantly enhanced pulmonary metastasis in recipients, revealing that the promotion of metastasis could be partly due to the immunosuppressive effects of Tregs [33].

Tumors have evolved multiple mechanisms to attract the migration of Tregs. Tumors have the capacity to produce chemokines that promote Tregs recruitment. An early study identified C-C motif chemokine 22 (CCL22) and its receptor C-C chemokine receptor type 4 (CCR4) as important factors that promote the recruitment of Tregs to human ovarian cancers [34]. CCR4 expressed on Tregs, enables Tregs migration toward CCL12 tumor-associated macrophages in the microenvironment, thus promoting the accumulation of Tregs within the tumor microenvironment [34]. Further, the addition of neutralizing antibody against CCL12 greatly attenuated the ability of malignant ascites to recruit Tregs in an in vitro assay [23]. After the initial report, an increasing number of chemokine ligand-receptor pairs, often refereed to as chemokine-receptor axes, have been found to be implicated in the trafficking of Tregs to different types of cancers [35]. The importance of chemokine-driven Tregs trafficking to tumor sites provides a basis for therapies blocking this process.

After migrating to tumor locations, Tregs begin to exert their suppressive functions through diverse pathways. In vitro studies demonstrat-

ed that Tregs derived from tumor tissues blocked the production of INF-γ and IL-2 by effector T cells and attenuated the cytotoxicity of tumor antigen specific T cells [23, 36]. Expression of a dominant-negative TGF-β receptor by $CD8^+$ T cells rendered them resistant to suppression by Tregs, indicating that Tregs suppress tumor-specific $CD8^+$ T cell cytotoxicity through TGF-β signaling [37]. And in a model of ultraviolet-radiation-induced carcinoma, IL-10 produced by Tregs appeared to be important for dampening anti-tumor immunity [38]. Furthermore, in patients with head and neck squamous-cell carcinoma, with the neutralizing antibodies to TGF-β and IL-10, the suppression of Tregs on autologous T cells was completely abrogated [39]. There was an interesting phenomenon in this study in which the combination of neutralizing antibodies to IL-10 and TGF-β completely eliminated suppression of Tregs in the presence or absence of the Transwell insert, while Transwell inserts without the blocking antibodies reduced suppression by 50%, indicating that these cytokines might be indispensable for the suppression by Tregs and surface molecules on Tregs are also implicated in suppression mediated by cell-to-cell contact.

The effect of radiation on Tregs

Different studies have been conducted to decipher the impact of radiation on Tregs. Although the idea that radiation drives Tregs to protect tissues against radiation seems plausible, it should be noted that the effect of radiotherapy on Tregs is context-dependent. Several factors should be taken into consideration including radiation doses, radiation modalities (whole body or located) and models used in the studies (disease or normal).

Irradiation-induced shift in the population of Tregs

The preferential survival of $CD4^+CD25^+$ T cells was noted in mice treated by low dose total body irradiation as the fraction of $CD4^+CD25^+$ T cells increased following radiation [40]. And these $CD4^+CD25^+$ T cells could ameliorate chronic graft-versus-host disease, suggesting that these cells preserved immune regulatory properties. This interesting study raised the possibility that Tregs could survive and even function after irradiation. The radioresistant character of Tregs was further confirmed in the study by Komatsu et al., in this study, peripheral expansion of radioresistant host Tregs restored and maintained normal Tregs homeostasis in lethally irradiated wild-type host mice transplanted with bone marrow cells from scurfy mice genetically lacking Foxp3 expression, thus preventing development of a fatal autoimmune disease [41]. Additonally, a recent study by Qu et al. also confirmed that $CD4^+CD25highFoxp3^+$ Tregs are more resistant to gamma irradiation than other T cells [42].

There have been studies elucidating the mechanisms underlying the radioresistance of Tregs. Tregs were less prone to radiation-induced apoptosis than $CD4^+Foxp3^-$ T cells [43]. In the study by Qu et al., Bcl-2, which has the functions to repress cell death, is expressed at higher levels in irradiated $CD4^+CD25high$ T cells than in irradiated $CD4^+CD25^-$ cells [42]. However, the increased expression was not replicated in another study, probably being attributed to different models used in these studies [44]. Expression of GITR was also detected in irradiated Tregs, which makes Tregs relatively resistant to apoptotic signals [45]. However, the preferential survival of $CD4^+CD25^+$ T cells in the irradiated mice might not be simply attributed to decreased apoptosis induced by radiation. Other mechanisms may also have a hand in this process. For example, radiation itself is able to induce and activate TGF-β, which is known to drive Tregs. In another study by Qu et al., the authors found that the percentage of thymic $CD4^+CD8^-CD25^+$ Tregs was also increased, indicating that a low dose of whole body irradiation stimulated the development of $CD4^+CD25^+$ Tregs in the thymus [46]. Intriguingly, the percentage of $CD4^+CD25^+$ Tregs was significantly increased in the periphery of thymectomized mice, reflecting the direct effect of radiation on the peripheral T-cell subset [46]. A more dynamic increase in the proliferation and regeneration of the Tregs population was noted in the mice eleven days after irradiation. These results suggest that both the radioresistance of Tregs and the stimulating effect of radiation on the production of Tregs contribute to a relative high count of Tregs in irradiated mice. Although studies indicated that radiation might spare Tregs or even increase the production of Tregs, there are still several reports with discordant results. Using C57BL/6

mice that were irradiated with 1.25 Gy of gamma irradiation, Liu et al. found that low-dose total body irradiation selectively decreased the percentage and absolute count of Tregs, which was associated with enhanced antitumor immunity [47]. More in vivo and in vitro studies are needed to confirm the radioresistance of Tregs.

Irradiation alters the phenotype and function of Tregs

Radiation modulates Tregs functions by altering the expression of cellular activation markers and cytokine expression. In a study by Cao et al., compared to the untreated group, the irradiated Tregs displayed reduced expression of CD62L, Foxp3, CD45RO and increased expression of GITR in a dose-dependent manner [45]. Interestingly, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), a molecule that downregulates the immune system, was upregulated by low dose of γ-ray (1.8 Gy), whereas high dose of γ-ray (30 Gy) deceased CTLA-4 expression [45]. High dose irradiation (30 Gy) abolished the suppressive capacity of Tregs on autologous T cells while relative low dose had no significant effect on the suppressive function of Tregs, at least partly as a result of reduced CTLA-4 expression [45]. The irradiated Tregs significantly exhibited decreased membrane TGF-β expression, which was previously demonstrated to mediate Tregs suppression on T cells in a cell-to-cell contact way [45]. High dose irradiation caused increased apoptosis as well as enhanced pro-apoptosis protein Bax expression in Tregs [45]. Tregs isolated from mesenteric lymph nodes in mice which received abdominal irradiation failed to suppress $CD4^+$ effector cells, which was correlated with a decreased mRNA level of Foxp3, TGF-β, and CTLA-4 [48]. In another study, although enhanced CTLA-4 and IL-10 expression and unchanged TGF-β were observed in the irradiated Tregs, the irradiated Tregs showed reduced capacity to suppress effector T cell proliferation, suggesting that changes in other molecules might be involved in dampening the functions of Tregs [43].

Radiotherapy modulates Tregs within the tumor microenvironment

Aside from direct effects on tumor cells, radiation has been demonstrated to have pleiotropic effects on the local tumor environment [49]. It is important to recognize the effect of radiation on Tregs within microenvironment, since these Tregs directly affect the anti-tumor immunity. More $Nrp1^+$ Tregs and CCR^+ Tregs were presented in tumor-draining lymph nodes from patients who received higher-dose radiation, compared to those who received lower dose radiation [50]. This change was also accompanied with a significantly inverted CD4/CD8 ratio, which was associated with reduced anti-tumor immunity. Increased Tregs accumulation were also observed in the tumor tissues after radiation in bladder cancer, in this model, radiation-induced Akt activation was demonstrated to promote the radiation-induced tumor-infiltrating Tregs survival [51]. Akt pathway activation also contributed to the irradiation-induced Tregs survival in hepatocellular carcinoma tissues [52]. In a murine mesothelioma model, local radiation of primary tumor caused more Tregs infiltration in primary tumor and secondary tumor [53]. Blockade of TGF-β1 clearly decreased the number of Tregs in the irradiated tumors in a prostate cancer model, suggesting a role of TGF-β1 in the accumulation of Tregs in irradiated tumors [54]. Radiation induced increased TGF-β and adenosine A2A in head and neck squamous cell carcinoma, which can provide both a growth and survival advantage to Tregs [55], to some extent contributing to the accumulation of Tregs within tumor microenvironment. Although several studies confirmed that local radiation promoted the accumulation of Tregs within tumor microenvironment, in a murine D5 melanoma model, after radiation, the relative reduction in the number of Tregs within the tumor was greater than the reduction in conventional T cells [56]. This discrepancy could be due to the fact that a Treg subpopulation that was sensitive to radiation had been induced after tumor implantation.

Tregs contribute to the resistance of tumor to radiation

Tregs can also play an important role in the resistance of tumor cells to radiotherapy. When k1106 cells (A human B lymphoma cell line) were cocultured with regulatory T cells (Tregs) and irradiated, the apoptosis of k1106 cells was significantly reduced, indicating an acquired resistance to irradiation [57]. When mice with inoculated prostate cancer cell lines

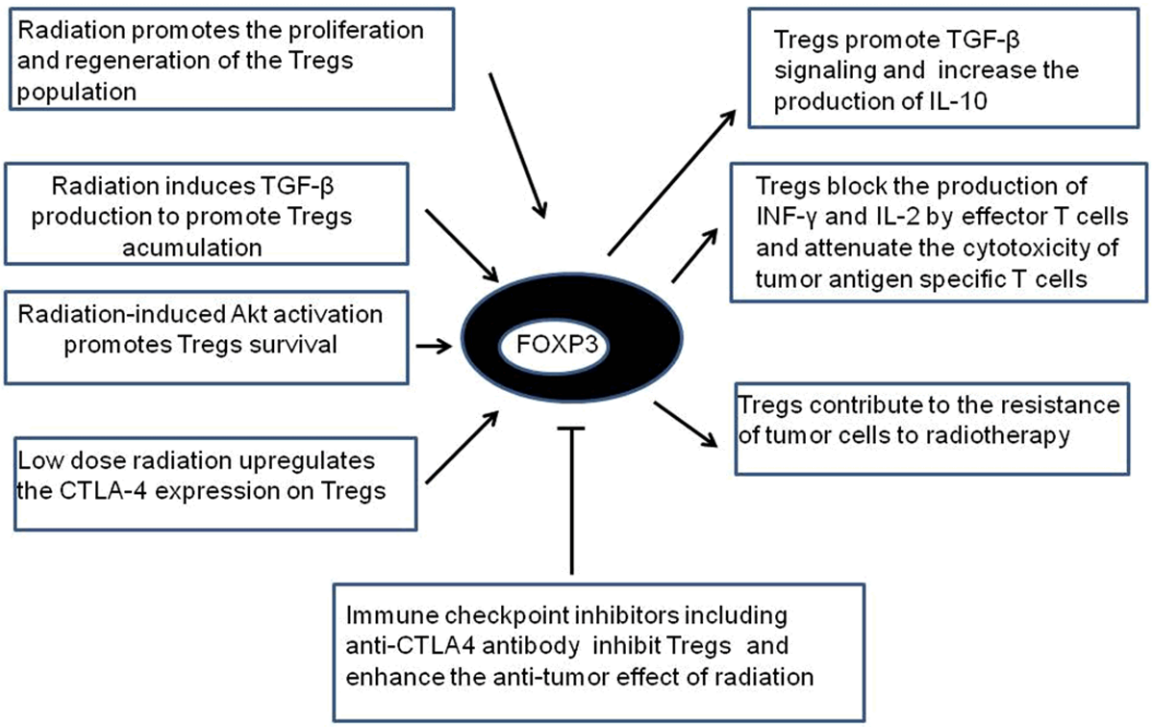

Figure 1. Several mechanisms are involved in the effects of radiation on Tregs. Tregs impair the anti-tumor effect of tumor antigen specific T cells and contribute to the resistance of tumor to radiation. Immune checkpoint inhibitors can enhance anti-tumor effect by inhibiting Tregs.

were irradiated with a sub-lethal dose, the regrowth of irradiated tumors was correlated with TGF-β1 levels and Tregs infiltration in vivo [54], whereas blocking TGF-β1 attenuated Tregs accumulation and improved radiation response. These findings, although indirectly, suggest that Tregs contribute to the resistance of tumors to radiotherapy [54].

Combining radiation with therapies targeting Tregs

The role of Tregs in the development and progression of tumor provides a basis for targeting Tregs in cancer treatment. Further, the possibility that Tregs attenuate the therapeutic effect of irradiation makes it rational to combine radiation and therapies that target Tregs. Several in vitro studies have been conducted to examine the combining effect of Tregs depletion and irradiation therapy. In a murine prostate tumor model, the administration of anti-CD25 antibody significantly improved the efficacy of radiation, resulting in delayed tumor growth and transient tumor regression [58]. This result suggested targeting Tregs allowed enhancement of radiotherapeutic benefit through immune modulation. In the advanced intracerebral B16 mouse melanoma model, tumors in mice receiving radiation therapy (RT) plus immunotherapy with Treg-depleting mAb were significantly smaller than tumors in mice treated only with radiation [59]. Aside from anti-CD25 antibodies, cyclophosphamide has emerged as a clinically feasible agent that can suppress Tregs [60]. In mouse models of lung and colon cancer, combined treatment of low-dose cyclophosphamide with radiation significantly depleted Tregs in the spleen and tumor compared with radiation, which was also associated with increased effector T cell, improved survival, and suppressed irradiated and nonirradiated tumor growth.

CTLA-4, which is upregulated on the cell surface of Tregs, is a main therapeutic target for suppressing Tregs function. The study by Wing et al. revealed that CTLA-4 expressed on Tregs is critically important for their in vivo and in vitro suppression [61]. The study by Twyman-Saint et al. showed that anti-CTLA4 antibody predominantly inhibits T-regulatory cells, thereby increasing the $CD8^+$ T-cell to Tregs ratio, suggesting that anti-CTLA-4 antibody exerts its

immune-enhancing function by modulating Tregs [62]. In the murine mesothelioma model, CTLA4 blockade using anti-CTLA-4 antibody reversed the proportion of Tregs over effector T cells that was elicited by local radiation, further activating $CD8^+$ T cells [53]. Combination of local RT with CTLA-4 blockade inhibited metastases in a mouse model of breast cancer [63]. In this model, combination of local RT with CTLA-4 blockade led to the development of an efficient $CD8^+$ T cell-dependent antitumor immunity, which could not only inhibit primary tumor growth but also suppress the formation of lung metastasis [63]. In clinic, immune checkpoint inhibitors including anti-CTLA-4 antibody has shown promise in some types of cancers, which can be combined with local radiotherapy, especially in the setting of oligometastatic disease [64, 65]. Increasing preclinical data and several case reports which show the presence of abscopal effects when radiotherapy is co-administered with immune checkpoint inhibitors, suggesting that this combination may enhance out-of-field tumor response outside of the primary treatment site [66, 67]. There are ongoing clinical trials investigating therapeutic efficacy of radiotherapy and anti-CTLA4 antibody ipilimumab in metastatic, castration-resistant prostate cancer [68, 69]. And the preliminary results of these studies are encouraging.

Conclusion

In this review, we discussed the effect of radiation on Tregs in physiological conditions and in cancer. Main points of this review were summarized in **Figure 1**. Tregs are implicated in initiation and progression of cancer and also contribute to the resistance of neoplasm in radiotherapy. Therefore, it is important to target Tregs in cancer therapeutics, further, combination of radiation and novel drugs that eliminate Tregs proved to be efficient in clinical trials. More efforts should be devoted to the discovery of new medications targeting Tregs in cancer therapy.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Xinchen Sun, Department of Radiation Oncology, The First Affiliated Hospital of Nanjing Medical University, 300 Guanzhou Road, Nanjing 210029, China. Tel: (086) 25-68135700; E-mail: sunxinchen2012@163.com

References

[1] Demaria S, Ng B, Devitt ML, Babb JS, Kawashima N, Liebes L and Formenti SC. Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. Int J Radiat Oncol Biol Phys 2004; 58: 862-870.

[2] Postow MA, Callahan MK, Barker CA, Yamada Y, Yuan J, Kitano S, Mu Z, Rasalan T, Adamow M, Ritter E, Sedrak C, Jungbluth AA, Chua R, Yang AS, Roman RA, Rosner S, Benson B, Allison JP, Lesokhin AM, Gnjatic S and Wolchok JD. Immunologic correlates of the abscopal effect in a patient with melanoma. N Engl J Med 2012; 366: 925-931.

[3] Nishioka A, Ogawa Y, Kubonishi I, Kataoka S, Hamada N, Terashima M, Inomata T and Yoshida S. An augmentation of Fas (CD95/APO-1) antigen induced by radiation: flow cytometry analysis of lymphoma and leukemia cell lines. Int J Mol Med 1999; 3: 275-278.

[4] Gasser S, Orsulic S, Brown EJ and Raulet DH. The DNA damage pathway regulates innate immune system ligands of the NKG2D receptor. Nature 2005; 436: 1186-1190.

[5] Gonzalez S, Lopez-Soto A, Suarez-Alvarez B, Lopez-Vazquez A and Lopez-Larrea C. NKG2D ligands: key targets of the immune response. Trends Immunol 2008; 29: 397-403.

[6] Champsaur M and Lanier LL. Effect of NKG2D ligand expression on host immune responses. Immunol Rev 2010; 235: 267-285.

[7] Kershaw MH, Devaud C, John LB, Westwood JA and Darcy PK. Enhancing immunotherapy using chemotherapy and radiation to modify the tumor microenvironment. Oncoimmunology 2013; 2: e25962.

[8] Dieckmann D, Plottner H, Berchtold S, Berger T and Schuler G. Ex vivo isolation and characterization of CD4(+)CD25(+) T cells with regulatory properties from human blood. J Exp Med 2001; 193: 1303-1310.

[9] Persa E, Balogh A, Safrany G and Lumniczky K. The effect of ionizing radiation on regulatory T

cells in health and disease. Cancer Lett 2015; S0304-3835: 00170-6.
[10] Vignali DA, Collison LW and Workman CJ. How regulatory T cells work. Nat Rev Immunol 2008; 8: 523-532.
[11] Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, Kelly TE, Saulsbury FT, Chance PF and Ochs HD. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet 2001; 27: 20-21.
[12] Dejaco C, Duftner C, Grubeck-Loebenstein B and Schirmer M. Imbalance of regulatory T cells in human autoimmune diseases. Immunology 2006; 117: 289-300.
[13] Williams LM and Rudensky AY. Maintenance of the Foxp3-dependent developmental program in mature regulatory T cells requires continued expression of Foxp3. Nat Immunol 2007; 8: 277-284.
[14] Bettini ML and Vignali DA. Development of thymically derived natural regulatory T cells. Ann N Y Acad Sci 2010; 1183: 1-12.
[15] Ohkura N, Kitagawa Y and Sakaguchi S. Development and maintenance of regulatory T cells. Immunity 2013; 38: 414-423.
[16] Raimondi G, Turner MS, Thomson AW and Morel PA. Naturally occurring regulatory T cells: recent insights in health and disease. Crit Rev Immunol 2007; 27: 61-95.
[17] Jonuleit H, Schmitt E, Stassen M, Tuettenberg A, Knop J and Enk AH. Identification and functional characterization of human CD4(+) CD25(+) T cells with regulatory properties isolated from peripheral blood. J Exp Med 2001; 193: 1285-1294.
[18] Bilate AM and Lafaille JJ. Induced CD4+Foxp3+ regulatory T cells in immune tolerance. Annu Rev Immunol 2012; 30: 733-758.
[19] Curotto de Lafaille MA and Lafaille JJ. Natural and adaptive foxp3+ regulatory T cells: more of the same or a division of labor? Immunity 2009; 30: 626-635.
[20] Roncarolo MG, Gregori S, Battaglia M, Bacchetta R, Fleischhauer K and Levings MK. Interleukin-10-secreting type 1 regulatory T cells in rodents and humans. Immunol Rev 2006; 212: 28-50.
[21] Nishikawa H and Sakaguchi S. Regulatory T cells in cancer immunotherapy. Curr Opin Immunol 2014; 27: 1-7.
[22] Strauss L, Bergmann C, Gooding W, Johnson JT and Whiteside TL. The frequency and suppressor function of CD4+CD25highFoxp3+ T cells in the circulation of patients with squamous cell carcinoma of the head and neck. Clin Cancer Res 2007; 13: 6301-6311.
[23] Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, Evdemon-Hogan M, Conejo-Garcia JR, Zhang L, Burow M, Zhu Y, Wei S, Kryczek I, Daniel B, Gordon A, Myers L, Lackner A, Disis ML, Knutson KL, Chen L and Zou W. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. Nat Med 2004; 10: 942-949.
[24] Sato E, Olson SH, Ahn J, Bundy B, Nishikawa H, Qian F, Jungbluth AA, Frosina D, Gnjatic S, Ambrosone C, Kepner J, Odunsi T, Ritter G, Lele S, Chen YT, Ohtani H, Old LJ and Odunsi K. Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. Proc Natl Acad Sci U S A 2005; 102: 18538-18543.
[25] Leffers N, Gooden MJ, de Jong RA, Hoogeboom BN, ten Hoor KA, Hollema H, Boezen HM, van der Zee AG, Daemen T and Nijman HW. Prognostic significance of tumor-infiltrating T-lymphocytes in primary and metastatic lesions of advanced stage ovarian cancer. Cancer Immunol Immunother 2009; 58: 449-459.
[26] Correale P, Rotundo MS, Del Vecchio MT, Remondo C, Migali C, Ginanneschi C, Tsang KY, Licchetta A, Mannucci S, Loiacono L, Tassone P, Francini G and Tagliaferri P. Regulatory (FoxP3+) T-cell tumor infiltration is a favorable prognostic factor in advanced colon cancer patients undergoing chemo or chemoimmunotherapy. J Immunother 2010; 33: 435-441.
[27] Salama P, Phillips M, Grieu F, Morris M, Zeps N, Joseph D, Platell C and Iacopetta B. Tumor-infiltrating FOXP3+ T regulatory cells show strong prognostic significance in colorectal cancer. J Clin Oncol 2009; 27: 186-192.
[28] Tosolini M, Kirilovsky A, Mlecnik B, Fredriksen T, Mauger S, Bindea G, Berger A, Bruneval P, Fridman WH, Pages F and Galon J. Clinical impact of different classes of infiltrating T cytotoxic and helper cells (Th1, th2, treg, th17) in patients with colorectal cancer. Cancer Res 2011; 71: 1263-1271.
[29] Haas M, Dimmler A, Hohenberger W, Grabenbauer GG, Niedobitek G and Distel LV. Stromal regulatory T-cells are associated with a favourable prognosis in gastric cancer of the cardia. BMC Gastroenterol 2009; 9: 65.
[30] Onizuka S, Tawara I, Shimizu J, Sakaguchi S, Fujita T and Nakayama E. Tumor rejection by in vivo administration of anti-CD25 (interleukin-2 receptor alpha) monoclonal antibody. Cancer Res 1999; 59: 3128-3133.
[31] Attia P, Maker AV, Haworth LR, Rogers-Freezer L and Rosenberg SA. Inability of a fusion pro-

tein of IL-2 and diphtheria toxin (Denileukin Diftitox, DAB389IL-2, ONTAK) to eliminate regulatory T lymphocytes in patients with melanoma. J Immunother 2005; 28: 582-592.
[32] Litzinger MT, Fernando R, Curiel TJ, Grosenbach DW, Schlom J and Palena C. IL-2 immunotoxin denileukin diftitox reduces regulatory T cells and enhances vaccine-mediated T-cell immunity. Blood 2007; 110: 3192-3201.
[33] Nishikawa H, Kato T, Tanida K, Hiasa A, Tawara I, Ikeda H, Ikarashi Y, Wakasugi H, Kronenberg M, Nakayama T, Taniguchi M, Kuribayashi K, Old LJ and Shiku H. CD4+ CD25+ T cells responding to serologically defined autoantigens suppress antitumor immune responses. Proc Natl Acad Sci U S A 2003; 100: 10902-10906.
[34] Wei S, Kryczek I, Edwards RP, Zou L, Szeliga W, Banerjee M, Cost M, Cheng P, Chang A, Redman B, Herberman RB and Zou W. Interleukin-2 administration alters the CD4+FOXP3+ T-cell pool and tumor trafficking in patients with ovarian carcinoma. Cancer Res 2007; 67: 7487-7494.
[35] Mailloux AW and Young MR. Regulatory T-cell trafficking: from thymic development to tumor-induced immune suppression. Crit Rev Immunol 2010; 30: 435-447.
[36] Yu P, Lee Y, Liu W, Krausz T, Chong A, Schreiber H and Fu YX. Intratumor depletion of CD4+ cells unmasks tumor immunogenicity leading to the rejection of late-stage tumors. J Exp Med 2005; 201: 779-791.
[37] Chen ML, Pittet MJ, Gorelik L, Flavell RA, Weissleder R, von Boehmer H and Khazaie K. Regulatory T cells suppress tumor-specific CD8 T cell cytotoxicity through TGF-beta signals in vivo. Proc Natl Acad Sci U S A 2005; 102: 419-424.
[38] Loser K, Apelt J, Voskort M, Mohaupt M, Balkow S, Schwarz T, Grabbe S and Beissert S. IL-10 controls ultraviolet-induced carcinogenesis in mice. J Immunol 2007; 179: 365-371.
[39] Strauss L, Bergmann C, Szczepanski M, Gooding W, Johnson JT and Whiteside TL. A unique subset of CD4+CD25highFoxp3+ T cells secreting interleukin-10 and transforming growth factor-beta1 mediates suppression in the tumor microenvironment. Clin Cancer Res 2007; 13: 4345-4354.
[40] Anderson BE, McNiff JM, Matte C, Athanasiadis I, Shlomchik WD and Shlomchik MJ. Recipient CD4+ T cells that survive irradiation regulate chronic graft-versus-host disease. Blood 2004; 104: 1565-1573.
[41] Komatsu N and Hori S. Full restoration of peripheral Foxp3+ regulatory T cell pool by radioresistant host cells in scurfy bone marrow chimeras. Proc Natl Acad Sci U S A 2007; 104: 8959-8964.
[42] Qu Y, Jin S, Zhang A, Zhang B, Shi X, Wang J and Zhao Y. Gamma-ray resistance of regulatory CD4+CD25+Foxp3+ T cells in mice. Radiat Res 2010; 173: 148-157.
[43] Balogh A, Persa E, Bogdandi EN, Benedek A, Hegyesi H, Safrany G and Lumniczky K. The effect of ionizing radiation on the homeostasis and functional integrity of murine splenic regulatory T cells. Inflamm Res 2013; 62: 201-212.
[44] McFarland HI, Puig M, Grajkowska LT, Tsuji K, Lee JP, Mason KP, Verthelyi D and Rosenberg AS. Regulatory T cells in gamma irradiation-induced immune suppression. PLoS One 2012; 7: e39092.
[45] Cao M, Cabrera R, Xu Y, Liu C and Nelson D. Gamma irradiation alters the phenotype and function of CD4+CD25+ regulatory T cells. Cell Biol Int 2009; 33: 565-571.
[46] Qu Y, Zhang B, Liu S, Zhang A, Wu T and Zhao Y. 2-Gy whole-body irradiation significantly alters the balance of CD4+ CD25- T effector cells and CD4+ CD25+ Foxp3+ T regulatory cells in mice. Cell Mol Immunol 2010; 7: 419-427.
[47] Liu R, Xiong S, Zhang L and Chu Y. Enhancement of antitumor immunity by low-dose total body irradiationis associated with selectively decreasing the proportion and number of T regulatory cells. Cell Mol Immunol 2010; 7: 157-162.
[48] Billiard F, Buard V, Benderitter M and Linard C. Abdominal gamma-radiation induces an accumulation of function-impaired regulatory T cells in the small intestine. Int J Radiat Oncol Biol Phys 2011; 80: 869-876.
[49] Burnette B and Weichselbaum RR. Radiation as an immune modulator. Semin Radiat Oncol 2013; 23: 273-280.
[50] Battaglia A, Buzzonetti A, Martinelli E, Fanelli M, Petrillo M, Ferrandina G, Scambia G and Fattorossi A. Selective changes in the immune profile of tumor-draining lymph nodes after different neoadjuvant chemoradiation regimens for locally advanced cervical cancer. Int J Radiat Oncol Biol Phys 2010; 76: 1546-1553.
[51] Wang M, Gou X and Wang L. Protein kinase B promotes radiation-induced regulatory T cell survival in bladder carcinoma. Scand J Immunol 2012; 76: 70-74.
[52] Li CG, He MR, Wu FL, Li YJ and Sun AM. Akt promotes irradiation-induced regulatory T-cell survival in hepatocellular carcinoma. Am J Med Sci 2013; 346: 123-127.
[53] Wu L, Wu MO, De la Maza L, Yun Z, Yu J, Zhao Y, Cho J and de Perrot M. Targeting the inhibitory receptor CTLA-4 on T cells increased apscopal effects in murine mesothelioma model. Oncotarget 2015; 6: 12468-12480.
[54] Wu CT, Hsieh CC, Yen TC, Chen WC and Chen MF. TGF-beta1 mediates the radiation re-

sponse of prostate cancer. J Mol Med (Berl) 2015; 93: 73-82.
[55] Park B, Yee C and Lee KM. The effect of radiation on the immune response to cancers. Int J Mol Sci 2014; 15: 927-943.
[56] Wei S, Egenti MU, Teitz-Tennenbaum S, Zou W and Chang AE. Effects of tumor irradiation on host T-regulatory cells and systemic immunity in the context of adoptive T-cell therapy in mice. J Immunother 2013; 36: 124-132.
[57] Li Q, Xu X, Zhong W, Du Q, Yu B and Xiong H. IL-17 induces radiation resistance of B lymphoma cells by suppressing p53 expression and thereby inhibiting irradiation-triggered apoptosis. Cell Mol Immunol 2015; 12: 366-72.
[58] Kachikwu EL, Iwamoto KS, Liao YP, DeMarco JJ, Agazaryan N, Economou JS, McBride WH and Schaue D. Radiation enhances regulatory T cell representation. Int J Radiat Oncol Biol Phys 2011; 81: 1128-1135.
[59] Smilowitz HM, Sasso D, Lee EW, Goh G, Micca PL and Dilmanian FA. Therapy model for advanced intracerebral B16 mouse melanoma using radiation therapy combined with immunotherapy. Cancer Immunol Immunother 2013; 62: 1187-1197.
[60] Le DT and Jaffee EM. Regulatory T-cell modulation using cyclophosphamide in vaccine approaches: a current perspective. Cancer Res 2012; 72: 3439-3444.
[61] Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, Nomura T and Sakaguchi S. CTLA-4 control over Foxp3+ regulatory T cell function. Science 2008; 322: 271-275.
[62] Twyman-Saint Victor C, Rech AJ, Maity A, Rengan R, Pauken KE, Stelekati E, Benci JL, Xu B, Dada H, Odorizzi PM, Herati RS, Mansfield KD, Patsch D, Amaravadi RK, Schuchter LM, Ishwaran H, Mick R, Pryma DA, Xu X, Feldman MD, Gangadhar TC, Hahn SM, Wherry EJ, Vonderheide RH and Minn AJ. Radiation and dual checkpoint blockade activate non-redundant immune mechanisms in cancer. Nature 2015; 520: 373-377.
[63] Demaria S, Kawashima N, Yang AM, Devitt ML, Babb JS, Allison JP and Formenti SC. Immune-mediated inhibition of metastases after treatment with local radiation and CTLA-4 blockade in a mouse model of breast cancer. Clin Cancer Res 2005; 11: 728-734.
[64] Silk AW, Bassetti MF, West BT, Tsien CI and Lao CD. Ipilimumab and radiation therapy for melanoma brain metastases. Cancer Med 2013; 2: 899-906.
[65] Barker CA and Postow MA. Combinations of radiation therapy and immunotherapy for melanoma: a review of clinical outcomes. Int J Radiat Oncol Biol Phys 2014; 88: 986-997.
[66] Grimaldi AM, Simeone E, Giannarelli D, Muto P, Falivene S, Borzillo V, Giugliano FM, Sandomenico F, Petrillo A, Curvietto M, Esposito A, Paone M, Palla M, Palmieri G, Caraco C, Ciliberto G, Mozzillo N and Ascierto PA. Abscopal effects of radiotherapy on advanced melanoma patients who progressed after ipilimumab immunotherapy. Oncoimmunology 2014; 3: e28780.
[67] Golden EB, Demaria S, Schiff PB, Chachoua A and Formenti SC. An abscopal response to radiation and ipilimumab in a patient with metastatic non-small cell lung cancer. Cancer Immunol Res 2013; 1: 365-372.
[68] Slovin SF, Higano CS, Hamid O, Tejwani S, Harzstark A, Alumkal JJ, Scher HI, Chin K, Gagnier P, McHenry MB and Beer TM. Ipilimumab alone or in combination with radiotherapy in metastatic castration-resistant prostate cancer: results from an open-label, multicenter phase I/II study. Ann Oncol 2013; 24: 1813-1821.
[69] Kwon ED, Drake CG, Scher HI, Fizazi K, Bossi A, van den Eertwegh AJ, Krainer M, Houede N, Santos R, Mahammedi H, Ng S, Maio M, Franke FA, Sundar S, Agarwal N, Bergman AM, Ciuleanu TE, Korbenfeld E, Sengelov L, Hansen S, Logothetis C, Beer TM, McHenry MB, Gagnier P, Liu D and Gerritsen WR. Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184-043): a multicentre, randomised, double-blind, phase 3 trial. Lancet Oncol 2014; 15: 700-712.