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Experimental model for the irradiation-mediated abscopal effect and factors influencing this effect

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Abstract: Radiotherapy (RT) is the primary treatment for cancer. Ionizing radiation from RT induces tumor damage at the irradiated site, and, although clinically infrequent, may cause regression of tumors distant from the irradiated site—a phenomenon known as the abscopal effect. Recently, the abscopal effect has been related to prolongation of overall survival time in cancer patients, though the factors that influence the abscopal effect are not well understood. The aim of this study is to clarify the factors influencing on abscopal effect. Here, we established a mouse model in which we induced the abscopal effect. We injected MC38 (mouse colon adenocarcinoma) cells subcutaneously into C57BL/6 mice at two sites. Only one tumor was irradiated and the sizes of both tumors were measured over time. The non-irradiated-site tumor showed regression, demonstrating the abscopal effect. This effect was enhanced by an increase in the irradiated-tumor volume and by administration of anti-PD1 antibody. When the abscopal effect was induced by a combination of RT and anti-PD1 antibody, it was also influenced by radiation dose and irradiated-tumor volume. These phenomena were also verified in other cell line, B16F10 cells (mouse melanoma cells). These findings provide further evidence of the mechanism for, and factors that influence, the abscopal effect in RT.

Keywords: Abscopal effect, radiotherapy, tumor-specific CD8⁺ T cells, anti-PD1 antibody, radiation dose, irradiated-tumor volume

Introduction

Radiotherapy (RT) is a primary treatment for solid cancers, in both the definitive patient survival time and reduced recurrence [1-3]. The main anti-tumor mechanisms of RT at irradiated sites are direct damage to cellular components, including cell membrane, mitochondria, and irradiatable deoxyribonucleic acid (DNA), as well as indirect damage from free radicals generated by interactions with water within the tissues [4]. Additional immunological effects, ranging from anti-inflammatory to anti-tumor immunity, are caused by ionizing radiation, referred to as systemic anti-tumor effects [5]. The mechanism for radiation-mediated systemic anti-tumor effects is activation of tumor-specific CD8⁺ T cells, which are primed by antigen-presenting cells, which capture tumor-specific antigens derived from the collapsed tumors, and thus play an important role in this process [6]. Primed CD8⁺ T cells induce apoptosis in tumor cells not only at the irradiated sites, but at distant, non-irradiated sites, through Fas/Fas ligand and/or Perforin/Granzyme B pathways [5, 7].

In patients with metastatic cancers, RT occasionally induces tumor regression at sites distant from the irradiated area. This phenomenon, called the abscopal effect, was first defined by Dr. Mole in 1953 [8]. The abscopal effect occurs infrequently in clinical cases, though it has been reported in different tumor types, including melanoma, hepatocellular carcinoma and renal cell carcinoma [9]. Of note, when it does occur, the abscopal effect is associated with prolongation of overall patient survival time [10, 11]. Although the precise mechanism of the abscopal effect is unknown, an immune
response mediated by activated CD8+ T cells has been shown to play a central role [6, 12, 13].

Recently, immune checkpoint inhibitors (e.g., anti-PD1 antibodies) have emerged as powerful therapeutics for the treatment of various metastatic cancer types [14]. Anti-PD1 antibodies are especially effective against tumors with high mutational burden [15], which presumably possess large numbers of neoantigens, leading to activation of an anti-tumor immune response [16].

In patients who have undergone RT, radiation-induced cell deaths increase tumor-associated antigens and consequently promote activation of an immune response [6]. Meanwhile, the activated tumor-specific CD8+ T cells express programmed death 1 (PD1) and release interferon-γ (IFN-γ) [17]. Moreover, tumor cells express the ligand of PD1 (PD-L1) in cytoplasm and/or plasma membrane, facilitating escape from attack by activated tumor-specific CD8+ T cells [18-20]. Thus, although tumor-specific CD8+ T cells were prepared to defend against attacking tumor cells, adaptive immune tolerance is induced in a significant proportion of patients after irradiation, as a function of the PD1/PD-L1 pathway, which inhibits the RT-mediated systemic anti-tumor effects [17, 21-24].

Thus, because the abscopal effect is closely related to the systemic anti-tumor effect, which is due to tumor-specific CD8+ T cells, we hypothesized it could be induced by combination therapy with RT and PD1 blockade, potentially contributing to development of a new strategy for improving metastatic cancer patients’ prognosis. The study was aimed to explain the effects of PD1 blockade, and to identify other factors affecting induction of the abscopal effect in RT. To this end, we sought to establish a feasible experimental model to investigate factors influencing the abscopal effect.

Materials and methods

Mice

Five-to-10-week-old male C57BL/6 mice were obtained from CLEA Japan (Tokyo, Japan) and maintained under pathogen-free conditions in the animal facility at Kyoto University. All experiments conformed to the relevant regulatory standards and were approved by the Institutional Animal Care and Use Committee of Kyoto University (Med Kyo 18289).

Cells and reagents

The MC38 cell is a C57BL/6 mouse-derived colon adenocarcinoma cell [25]. The B16F10 cell is a mouse melanoma cell from a C57BL/6 mouse, and it is purchased from RIKEN Cell bank (Tsukuba, Japan). MC38 cells were cultured in RPMI 1640 medium (Life Technoligies, Grand Island, NY, USA) and supplemented with 10% FBS (Life Technologies), 1 U/mL penicillin (Life Technologies), and 1 μg/mL streptomycin (Life Technologies). B16F10 cells were cultured in RPMI 1640 medium and supplemented with 10% FBS.

In vivo mouse models’ RT and/or anti-PD1-monoclonal antibody

MC38 cells or B16F10 cells were subcutaneously injected into two sites, the upper and lower dorsal, of male C57BL/6 mice. Radiation (2 gray [Gy] or 8 Gy) was administered in a single fraction to tumors on the lower, but not the upper, dorsal on days 7, 8, and 9 after tumor cell inoculation. All mice were positioned in a modified 50-mL conical plastic tube to allow irradiation of the tumor area while keeping the rest of the body outside the RT field using a collimator, as shown in other study [12]. The lower dorsal tumors were locally irradiated on days 7, 8, and 9 with 2 Gy or 8 Gy of 137Cs γ-rays using a Gammacell 40 Exactor (MDS Nordion International, Ottawa, ON, Canada). Anti-PD1 mouse monoclonal antibody (mAb) 4H2 (Ono Pharmaceutical, Osaka, Japan), or isotype control IgG antibody (ab6728; Abcam, Cambridge, UK) was administered intraperitoneally at a dose of 10 mg/kg on days 7, 11, and 14. Tumor diameters were measured using callipers, and tumor growth was evaluated every 2 or 3 days until day 17, when all mice were euthanized. Tumors were then resected from mice on day 17 and analysed.

Histological and immunohistochemical staining

Tissue samples were fixed in 10% neutral buffered formalin (Wako Pure Chemical Industries,
Osaka, Japan) overnight, embedded in paraffin, and cut into 4 μm sections for standard hematoxylin and eosin (H&E) staining and immunohistochemistry. Tyramide signal amplification avidin-biotin complex method was used for immunohistochemistry. Incubation and washing procedures were carried out at room temperature. After deparaffinization and antigen retrieval by incubation in 0.1% Trypsin solution at 37°C for 30 min, endogenous peroxidase activity was blocked by 0.3% H₂O₂ in methyl alcohol for 30 min. The glass slides were washed in phosphate-buffered saline (PBS; 6 times, 5 min each) and mounted with 1% horse normal serum in PBS for 30 min. The primary antibody, a mouse monoclonal anti-CD8 alpha antibody (EPR20305; Abcam, Cambridge, UK) at 1:2000 dilution, a mouse monoclonal anti-Perforin antibody (5B10; Abcam) at 1:20 dilution, and a mouse monoclonal anti-single stranded DNA (ssDNA) (F7-26; ELS, New York, NY, USA) at 1:100 dilution were subsequently applied overnight at 4°C. Cells were incubated with biotinylated horse anti-mouse serum (second antibody, Vector Laboratories, Burlingame, CA, USA) diluted to 1:300 in PBS for 40 min, and followed by PBS washes (6 times, 5 min each). Avidin-biotin-peroxidase complex (ABC) (ABC-Elite; Vector Laboratories) diluted 1:100 in bovine serum albumin was applied for 50 min. After washing in PBS (6 times, 5 min each), a colouring reaction was carried out with diaminobenzidine and nuclei were counterstained with hematoxylin.

**Figure 1.** Establishment of experimental model using MC38 cells (mouse colon adenocarcinoma cells) in which the abscopal effect can be induced with radiotherapy (RT). A. Experimental protocol. MC38 cells were subcutaneously injected into C57BL/6 mice at two sites (lower dorsal: 2.5 × 10⁶ or 5.0 × 10⁶ cells [red-filled circle] and upper dorsal: 2.5 × 10⁶ cells [green-filled circle]) (day 0). Lower dorsal tumor irradiated (8 Gy × 3 fr), upper dorsal tumor unirradiated. [#1]: control group, [#2]: small tumor-irradiated group, [#3]: large tumor-irradiated group. Tumor size measured every 2 or 3 days until day 17. B. Time course of tumor volume at irradiated sites (lower dorsal: red-filled circle): Direct RT effect. Tumors irradiated with 2.5 × 10⁶ and 5.0 × 10⁶ cells showed significant tumor reduction compared to control tumors. ***P < 0.001, between-groups (n = 8). C. Time course of tumor volume in unirradiated sites (upper dorsal: green-filled circle): Abscopal effect. Unirradiated tumors also showed tumor reduction after RT. The abscopal effect was significantly enhanced as irradiated-tumor volume increased. **P < 0.01. ***P < 0.001, between-groups (n = 8). Immunostained tissues were assessed using a BIOREVO BZ-9000 microscope (Keyence,
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Figure 2. Establishment of experimental model using B16F10 cells (mouse melanoma cells) in which the abscopal effect can be induced with radiotherapy (RT). A. Experimental protocol. B16F10 cells were subcutaneously injected into C57BL/6 mice at two sites (lower dorsal: red-filled circle) with 0.1 × 10^6 or 0.25 × 10^6 cells (day 0). Lower dorsal tumor irradiated (8 Gy × 3 fr), upper dorsal tumor unirradiated. [#1]: control group, [#2]: small tumor-irradiated group, [#3]: large tumor-irradiated group. Tumor size measured every 2 or 3 days until day 17. B. Time course of tumor volume at irradiated sites (lower dorsal: red-filled circle): Direct RT effect. Tumors irradiated with 0.1 × 10^6 and 0.25 × 10^6 cells showed significant tumor reduction compared to control tumors. ***P < 0.001, between-groups (n = 5). C. Time course of tumor volume in unirradiated sites (upper dorsal: green-filled circle): Abscopal effect. Unirradiated tumors also showed tumor reduction after RT. The abscopal effect was significantly enhanced as irradiated-tumor volume increased. **P < 0.01, ***P < 0.001, between-groups (n = 5). D. Time course of body weight changes in mice of each group (n=5 in each group). Animal weights on day 17 were not significantly different between the groups.

Osaka, Japan), for staining of anti-CD8 alpha antibody, anti-Perforin antibody, and anti-ssDNA antibody. Staining was assessed by a pathologist (T.H). Positive cells were scored by counting at least 300 cells per high-power field (n = 5) under light microscopy.

Statistical analysis

Data are presented as mean ± standard deviation. Data were first tested for normality of distribution. Between-groups differences were analysed using two-tailed, Student’s t-tests for paired-samples with equal variance.

The interaction of RT and anti-PD1 antibody treatment was assessed using two-way ANOVA. When significant interactions were found, analysis for more than two groups were conducted with Tukey’s Honest Significant Difference test.
A P < 0.05 was considered significant. Statistical analyses were performed using SPSS software (v. 21; IBM SPSS Inc., Armonk, NY, USA).

Results

RT-induced abscopal effect in a mouse model and association with irradiated tumor volume

To establish an experimental model to investigate the radiation-mediated abscopal effect, we injected MC38 cells (2.5 × 10^6 cells in the upper dorsal [green circle] and 2.5 × 10^6 [small tumor-irradiated group] or 5.0 × 10^6 [large tumor-irradiated group] cells in the lower dorsal [red circle]) or B16F10 cells (0.1 × 10^6 cells in the upper dorsal [green circle] and 0.1 × 10^6 [small tumor-irradiated group] or 0.25 × 10^6 [large tumor-irradiated group] cells in the lower dorsal [red circle]) subcutaneously into C57BL/6 mice (Figures 1A and 2A, respectively). Radiation (8 Gy in a single fraction) was administered to the lower (red circle) but not upper (green circle) dorsal tumors on days 7, 8, and 9 after tumor cell inoculation. Tumor volume was monitored up to day 17.

As shown in Figure 1B, MC38 cell-derived tumors in the irradiated sites (lower dorsal; red circle) showed significant growth reduction in both small and large tumor-irradiated groups, compared with tumors in the control group (inhibitory rate: [#2] vs. [#1]: 96.9%, P < 0.001; [#3] vs. [#1]: 95.3%, P < 0.001). Similarly, direct RT effect was observed in B16F10 cell-derived tumors (inhibitory rate: [#2] vs. [#1]: 95.5%, P < 0.001; [#3] vs. [#1]: 93.8%, P < 0.001) (Figure 2B).

MC38 cell-derived or B16F10 cell-derived tumors in the unirradiated sites (upper dorsal; green circle) also showed growth reduction compared with tumors in the control group (MC38 cells: inhibitory rate: [#2] vs. [#1]: 34.4%, P = 0.0524; [#3] vs. [#1]: 67.9%, P = 0.0021, Figure 1C), (B16F10 cells: inhibitory rate: [#2] vs. [#1]: 20%, P = 0.0594; [#3] vs. [#1]: 38.6%, P = 0.00168, Figure 2C), demonstrating the abscopal effect in these models.

Of note, tumor volume in unirradiated sites in the large tumor-irradiated group ([#3]) was sig-
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Figure 4. Effect of anti-PD1 antibody on abscopal effect (B16F10 cell-derived experimental model). A. Experimental protocol. B16F10 cells were subcutaneously injected into C57BL/6 mice at two sites (lower dorsal: \(0.1 \times 10^6\) cells [red-filled circle] and upper dorsal: \(0.1 \times 10^6\) cells [green-filled circle]) (day 0). Radiation administered on days 7, 8, and 9 (8 Gy \(\times 3\) fr) to lower dorsal tumors. Anti-PD1 or isotype control IgG antibody was administered intraperitoneally at 10 mg/kg on days 7, 11, and 14. [#1]: control group, [#2]: RT alone group, [#3]: anti-PD1 antibody group, [#4]: RT+anti-PD1 antibody group. Upper dorsal tumor (green-filled circle) diameters measured every 2 or 3 days until day 17. B. Time course of tumor volume in unirradiated sites (Abscopal effect). C. Time course of body weight changes in mice of each group (n=5 in each group). Animal weights on day 17 were not significantly different between the groups.
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11.4%, 66.4 ± 23.6% and 21.4 ± 3.8%, respectively. Interaction between RT and anti-PD1 antibody was not significant (P = 0.558696). Therefore, we evaluated the abscopal effect by RT or the antitumor effect by anti PD-1 antibody administration. Non-irradiated tumor volume in the irradiated group ([#2] and [#4]) was significantly (P < 0.01) smaller than that in the non-irradiated group ([#1] and [#3]) (tumor growth rate: mean tumor volume on day 17 in [#1] and [#3] = 52.3%). In addition, the tumor volume in groups receiving anti-PD1 antibody treatment ([#3] and [#4]) was significantly (P < 0.01) smaller than that in the untreated groups ([#1] and [#2]) (tumor growth rate: mean tumor volume on day 17 in [#3] and [#4]/mean tumor volume on day 17 in [#1] and [#2] = 52.8%).

In B16F10-derived tumors, tumor growth rates (on day 17) in the group ([green circle]) receiving RT ([#2]), anti-PD1 antibody ([#3]), and/or combination therapy with RT and anti-PD1 antibody ([#4]) compared with the control group ([#1]) were 80.9 ± 9.9%, 72.6 ± 16.2% and 20.4 ± 7.1%, respectively. Because interaction between RT and anti-PD1 antibody was significant (P = 0.0125), we conducted a multiple comparison of all groups. As shown in Figure 4B, the tumor growth in treatment group receiving RT and anti-PD1 antibody ([#4]) was significantly suppressed compared with the control group ([#1]) (P < 0.0001). Moreover, the tumor volume of the unirradiated sites in the group treated with RT and anti-PD1 antibody ([#4]) was significantly smaller than that in the group treated with RT alone ([#2]) or anti-PD1 antibody treatment alone ([#3]) ([#4] vs. [#2], P < 0.0001, [#4] vs. [#3], P < 0.0001). No mice died and apparent ill effects were not observed in these experiments. No significant weight loss was observed in groups on day 17 of the experiment (Figure 4C).

Thus, the abscopal effect was more effectively induced when anti-PD1 antibody was administered.

Figure 5. Effect of anti-PD1 antibody and irradiated-tumor volume on abscopal effect (MC38 cell-derived experimental model). A. Experimental protocol. MC38 cells were subcutaneously injected into C57BL/6 mice at two sites (lower dorsal: 2.5 × 10^6 (small tumor-irradiated group) or 5.0 × 10^6 (large tumor-irradiated group) cells (red-filled circle) and upper dorsal: 2.5 × 10^6 cells (green-filled circle) (day 0). Lower dorsal tumor (red-filled circle) was irradiated (8 Gy × 3 fr) on days 7, 8, and 9, and upper dorsal tumor (green-filled circle) unirradiated. Anti-PD1 antibody was administered intraperitoneally at 10 mg/kg on days 7, 11, and 14. Upper dorsal tumor (green-filled circle) diameters were measured every 2 or 3 days to day 17. B. Time course of tumor volume in unirradiated sites (green-filled circle). Abscopal effect in large tumor-irradiated group was significantly higher than that in small-tumor-irradiated group. ***P < 0.001, n = 8.
Effect of anti-PD1 antibody and irradiated-tumor volume on the abscopal effect

Next, we examined whether irradiated-tumor volume influenced induction of the abscopal effect with RT and anti-PD1 antibody combination treatment. MC38 cells or B16F10 cells were subcutaneously injected: (MC38 cells: $2.5 \times 10^6$ cells) or B16F10 cells ($0.1 \times 10^6$ cells) in the upper dorsal [green circle] and $0.25 \times 10^6$ cells [small tumor-irradiated group] or $0.5 \times 10^6$ cells [large tumor-irradiated group] in the lower dorsal [red circle]; we administered anti-PD1 antibody (10 mg/kg) to the mice with RT (8 Gy × 3-irradiation) (Figures 5A and 6A, respectively). Tumor growth of the unirradiated tumors in the large tumor-irradiated group was significantly suppressed compared with those in the small tumor-irradiated group (P < 0.001, Figure 5B, P < 0.01, Figure 6B).

Effect of anti-PD1 antibody and irradiation dose on the abscopal effect

Next, we examined whether irradiation dose affects induction of the abscopal effect with RT and anti-PD1 antibody combination treatment. MC38 cells ($2.5 \times 10^6$ cells) or B16F10 cells ($0.1 \times 10^6$ cells) were subcutaneously injected in the upper dorsal (green circle) and lower dorsal (red circle), and we administered anti-PD1 antibody (10 mg/kg) to the mice with RT (2 Gy or 8 Gy × 3-irradiation) (Figures 7A and 8A, respectively). As shown, growth of unirradiated tumors in mice with high-dose irradiation (8 Gy × 3-fr) was significantly suppressed compared with mice with low-dose irradiation (2 Gy × 3-fr) (P < 0.001, Figure 7B, P < 0.001, Figure 8B).

Histological and immunohistochemical examinations

To investigate the pathology of the abscopal effect in this experimental model, histological and immunohistochemical examinations were conducted. In the unirradiated-site tumors of
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The group treated with RT and anti-PD1-antibody ([#4]) was significantly higher compared with those in control ([#1]), monotherapy with anti-PD-1 antibody ([#2]) and RT alone group ([#3]) groups. (CD8: [#4] vs. [#1], P < 0.001; [#4] vs. [#2], P < 0.001; [#4] vs. [#3], P < 0.001; respectively, Perforin: [#4] vs. [#1], P < 0.001; [#4] vs. [#2], P < 0.001; [#4] vs. [#3], P < 0.001; respectively, ssDNA: [#4] vs. [#1], P < 0.001; [#4] vs. [#2], P < 0.001; [#4] vs. [#3], P = 0.0012; respectively).

Discussion

We successfully established an experimental mouse model in which to induce the fractionated radiation-mediated abscopal effect. There were three major findings in this study. First, induction of the abscopal effect is influenced by irradiated-tumor volume. Second, treatment with anti-PD1 antibody enhances the abscopal effect. Third, the abscopal effect from RT and anti-PD1 antibody combination therapy is enhanced based on irradiated-tumor volume and radiation dose.

In this study, the abscopal effect was induced by radiation monotherapy in xenografted tumors derived from MC38 mouse colon adenocarcinoma cells as well as B16F10 mouse melanoma cells. Although it has been reported that abscopal effect induction is infrequent with radiation monotherapy, even in experimental mouse models [12, 25], we suggest that tumor size at the time of irradiation in previous studies was relatively small, probably due to the small number of transplanted tumor cells [12, 25]. In contrast, we injected a large number of MC38 cells (i.e., 2.5 × 10^6 or 5.0 × 10^6 cells) into C57BL/6 mice, and the average tumor size (from 2.5 × 10^6 or 5.0 × 10^6 cells) at irradiation was 45.42 ± 42.79 mm^3 and 68.58...
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As for B16F10 cells, the average tumor size was 123.0 mm$^3$ and 145.5 mm$^3$, respectively, although the number of cells transplanted was small ($0.1-0.25 \times 10^6$ cells). We expect that sufficient tumor destruction following irradiation of these large tumors contributed to activating the tumor-specific CD8$^+$ T cells necessary to induce the abscopal effect. Our data thus support the notion that induction of the abscopal effect is influenced by irradiated-tumor volume.

Further, we suggest that MC38 and B16F10 cell-derived xenograft tumors might be suitable as an experimental tumor model to induce the RT-mediated abscopal effect. It has been reported that PD-L1 in MC38 and B16F10 cells is strongly induced by stimulation of IFN-γ [26], which is released by activated CD8$^+$ T cells [24], and that PD1/PD-L1 blockade exerts an effective anti-tumor effect on MC38 and B16F10 cells [26]. Because CD8$^+$ T cells were massively infiltrated on unirradiated tumors in our experimental model, as shown in the immunohistochemical studies, maintenance of CD8$^+$ T cells due to administration of anti-PD1 antibody may contribute to inducing the abscopal effect. Moreover, infiltration of CD8$^+$ T cells may be involved in the expression of Perforin, and subsequent induction of apoptosis, represented by our finding of ssDNA-positive cells.

In this study, we demonstrated that the abscopal effect is effectively induced by fractionated RT in combination with anti-PD1 antibody, and that their effects are radiation dose- and tumor volume-dependent. Our results are consistent with a previous report showing that fractionated RT, but not single-dose RT, in combination with anti-PD1/PD-L1 blockade, is the important factor for inducing the abscopal effect [12, 25, 27]. Thus, repetitive tumor-antigen collapse due to fractionated RT, as well as blockade of immune tolerance due to anti-PD1 antibody, may cooperate to effectively induce the abscopal effect.

Here we summarize the mechanism of our experimental model regarding abscopal effect due to RT and anti-PD1 antibody (Figure 10). Our findings indicate that irradiation of large tumors might be expected to induce a systemic anti-tumor effect, and that RT and anti-PD1 antibody combination therapy may be a
reasonable treatment strategy in such cancers. Moreover, our data support the idea that irradiation of as much tumor tissues as possible within the irradiatable region, along with additional administration of anti-PD1 antibody, may be effective for inducing a systemic anti-tumor effect, even in patients with stage IVB in whom cancer has spread throughout the body.

Figure 9. Images of representative histological and immunohistochemical. A. Unirradiated tumors (MC38 cells) in the group treated with or without radiotherapy (8 Gy × 3 fr) and/or anti-PD1 antibody (10 mg/kg) ([#1]: control group, [#2]: anti-PD1 antibody group, [#3]: RT alone group, [#4]: RT+anti-PD1 antibody group) were stained with hematoxylin and eosin, anti-CD8 antibody, anti-Perforin antibody, and anti-ssDNA antibody. Scale bar = 150 µm. B. The index of CD8-, Perforin-, and ssDNA-positive stained cells. [#1]: control group, [#2]: anti-PD1 antibody group [#3]: RT alone group, [#4]: RT+anti-PD1 antibody group. CD8-, Perforin-, and ssDNA-positive cells quantified by counting under high-power field with light microscopy (n = 5). ***P < 0.001, between-groups.
This study was not without some limitations. First, we were unable to demonstrate PD-L1 expression on MC38 and/or B16F10-derived xenograft tumors because we were unable to find appropriate positive control samples and PD-L1 antibody for immunostaining mouse tissues. Second, it is unclear whether the T cells we found in tumor cells are tumor-specific. Additional studies will be required to further explain the relationship between irradiation and immune stimulation.

In conclusion, the abscopal effect is significantly enhanced from RT and anti-PD1 antibody combination therapy, and is influenced by radiation dose and irradiated-tumor volume. Our findings may provide a basis for better understanding induction of the RT-associated abscopal effect.

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Disclosure of conflict of interest

None.
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