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Radiation Exposure–Induced Changes in the Immune Cells and Immune Factors of Mice With or Without Primary Lung Tumor

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Abstract

Recent studies have demonstrated that radiation activates in situ antitumor immunity and consequently induced a synergistic effect of radiotherapy and immunotherapy. However, studies related to radiation-induced changes in immune system of tumor-bearing mice are limited, which are of great significance to improve the efficacy of radioimmunotherapy. In this study, we first established a primary lung tumor mouse model using urethane. Then part of the right lung of the mouse was exposed to X-ray irradiation with a computed tomography–guided small animal irradiator and the changes of immune cells in both peripheral blood and spleen were determined by flow cytometry. Besides, the levels of both cytokines and immunoglobulins in mouse serum were detected by a protein chip. We found that B lymphocytes increased while $CD8^+$ T lymphocytes reduced significantly. Interleukin-3 (IL-3), IL-6, regulated upon activation, normally T-expressed, and presumably secreted factor (RANTES), and vascular endothelial growth factor (VEGF) were found to be decreased after tumor formation, and the similar results have also been observed with kappa, IgG3, IgE, IgM, and IgG2a. After irradiation, lower concentrations of IgD, kappa, and IgM were found in the serum. Our findings indicate that localized tumor irradiation caused some obvious changes like inhibiting the ability of innate immunity, and these changes may be useful in predicting prognosis.

Keywords

ionizing radiation, lung cancer, immune system, side effect

Introduction

Lung cancer is a malignant tumor with high morbidity and mortality[1] and non-small cell lung cancer (NSCLC) is the leading cause of cancer-related death. For patients with early-stage disease, clinical treatment mainly involves surgical resection and mediastinal lymph node dissection. However, many patients are unsuitable for surgery. Numerous studies have reported the efficacy of stereotactic body radiotherapy in achieving local control in NSCLC.[2,3]

Radiotherapy has been used for a long time as a standard treatment for cancer or in combination with surgery and systemic therapies including immunotherapy[4] and in 5% to 50% of patients, as a partial treatment for most cancers.[5] Radiotherapy can induce various types of cell death, including apoptosis, necrosis, necroptosis, and autophagy, all of which have been shown to have immunosuppressive or immunogenic effects.[6] Radiotherapy can also reduce cancer cell viability by inducing

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modifications in the local microenvironment.[7] In addition to these radiological indicators, other factors must be taken into account as well as the cytotoxicity of tumor cells.

Recently, many studies have reported that radiotherapy promotes an inflammatory response in tumors, which supports tumor-specific immunity and in fact, the efficacy in preclinical models seems to depend on concomitant immune stimulation. Irradiation (IR) not only causes the death of tumor cells but also causes tissue atrophy outside the exposure area. This phenomenon is known as a side effect of radiotherapy. Few studies have investigated the ability of radiation to induce an abscopal effect.[8] This side effect is not only a phenomenon in radiological biology but also a way of transmitting signals through the immune system between local and distant cells. Cytokines, chemokines, and other types of biological molecules play a significant role in signal transmission and there is evidence to prove that this side effect can alter immune function.[9] In addition, the immune system is capable of identifying and killing tumor cells; however, during proliferation and differentiation, tumor cells have acquired the ability known as an immune escape thus are not recognized by the immune system. Tumors can cause not only the inhibition of immune function in the solid tumor microenvironment but also inhibition of the immune system.[10] Ionizing radiation can also destroy the immune system.[11] To enhance the effect of cancer treatment, immunotherapy, a new cancer treatment, has been developed with the aim to stimulate the immune system to enhance its killing effect on cancer cells and to inhibit the metastasis of tumor cells.[12] Previous evidence indicated that ionizing radiation can cause inflammation in the tumor microenvironment, and the form of inflammation contributes to specific immunity in killing tumor cells. Other research demonstrated that a single radiation dose of 20 Gy could lead to better local control of tumors and promote the activation of complement in blood serum.[13] Another study proved that ionizing radiation can affect tumor cells by inducing the secretion of a large number of cytokines and chemokines.[4] After IR, a significant increase in the level of interleukin-5 (IL-5) and IL-13 in blood serum was observed in tumor-bearing mice, but a decrease in interferon-γ (IFN-γ) and IL-12.[14] Previous studies also demonstrated that IR not only stimulated immunity in the microenvironment but also stimulated body immunity, and this was mainly due to the infiltration of lymphocytes, especially T lymphocytes.[15] In addition to the effect on immune cells, IR can also lead to changes in serum cytokine levels, and most research has been focused on transforming growth factor-β (TGF-β).[16] Irradiation can also induce a significant decrease in IL-8 in addition to TGF-β[17] and an increase in IL-6 and IL-10.[18] Other cytokines such as IL-1α, IL-1β, IL-2, IL-4, IL-6, and IL-12 also showed a distinct increase after IR, as compared to IFN-γ, which significantly decreased after IR.[19,20] The above studies demonstrate that radiation can induce the death of tumor cells or inhibit their growth. In addition, IR can also influence the microenvironment and systemic immunity. Irradiation, at the appropriate dose, can not only enhance the function of immune cells but also promote the secretion of cytokines to facilitate the killing of cancer cells by the immune system.

In the present study, we used a primary lung tumor mouse model to determine the changes in the immune system after the right lung partially exposed to a single dose of radiation, aiming to evaluate the underlying mechanisms of the side effect phenomenon and to accurately assess the effects of radiotherapy.

Materials and Methods

Mouse Feeding and the Establishment of a Primary Lung Tumor Mouse Model

Male Kunming mice (6-8 weeks old, weighing 15-25 g) purchased from Shanghai Lingchang Biochemistry Corporation were fed in a pathogen-free environment for 1 week before establishing the tumor-bearing model. Humidity in the breeding environment was 55% to 65%, the temperature was 24 °C to 26 °C, and the light-dark cycle was 12/12 hours. The mice were divided into 4 groups, 15 mice each. The detailed information on each group is as follows: (i) Control group (Control): the mice received a saline injection only; (ii) IR group: the mice received the same saline injection as the control group and then underwent irradiation; (iii) tumor group (Tumor): tumor-bearing mice were given an injection of urethane via the enterocoelia but no IR; and (iv) tumor + IR group (Tumor + IR): the mice were given an injection of urethane and then irradiated. Twenty percent urethane (dissolved in saline) solution was administered at a dose of 1 g/kg intraperitoneally. The control group was injected with the same volume of saline. The mice were weighed every week until 1 week before IR. We confirmed tumor formation with cone-beam computed tomography (CBCT) and immunohistochemical staining. Peripheral blood samples were collected and centrifuged at 10 000 g/min for 10 minutes prior to euthanasia. The serum was frozen at −80 °C for subsequent experiments. The remaining blood samples were prepared for flow cytometry analysis. The organs were also collected for subsequent experiments. All animal studies were reviewed and approved by the Soochow University Institutional Animal Care and Use Committee.

Irradiation

Irradiation was conducted on only a small volume of tissue (<50% of the lung) at a dose of 22.3 Gy for every model mice 28 weeks after urethane administration. Before imaging and IR, the mice were anesthetized with 90 mg/kg body weight sodium pentobarbital administered intraperitoneally. The irradiated lung volumes, as percentages of the total lung volumes, were determined from computed tomography (CT) data sets for the nominal 5-mm beam path, respectively. The volume percentages of the irradiated lung were generated by counting the volume based on dose-volume histogram data. We constructed a dedicated small animal IR device for focused, high-dose IR, which was easily mounted on an X-RAD 225Cx X-ray source

(Precision X-Ray). Radiation planning using an open-source treatment planning system was developed in MATLAB and was provided by Precision X-ray. The software allows identification of a target point and treatment volume on a CBCT scan of the animal immobilized in the treatment position. The X-RAD 225Cx is a self-contained X-ray system with continuous energy adjustment from 20 to 225 kV. The system has 3 main components: a collimation mechanism for producing small radiation beams that exhibit a sharp lateral falloff; an imaging subsystem consisting of a fluorescent screen coupled to a charge-coupled device camera; and a 3-dimensional (3D) manual positioning stage. The essential characteristics of this device are high-dose rate, small beam size, and precise target localization. In order to reduce extra influences on the experimental mice, we did not use contrast media during the imaging process.

Tissue Collection

Whole blood from the orbit was collected 4 weeks after radiation. The lungs, livers, and hearts were also collected for preservation and fixation. In addition, we conducted immunohistochemistry and hematoxylin–eosin (HE) staining of lung tissues. Spleen tissues were collected for flow cytometry analysis (BD FACSVerse).

Flow Cytometric Analysis

Peripheral blood (100 μL) was mixed with 2-μL antibody. Phosphate-buffered saline (PBS; 500 μL) was added and centrifuged at 12 000 rpm/min for 10 minutes. The samples were then washed twice with PBS. The following antibodies were used, APC-CD-19, PENK-1.1, PE-CD4, Per-CP-CD8e, and FITC-CD3e (BD Pharmingen). Blood samples containing antibodies were incubated at room temperature for 30 minutes in the dark and then subjected to flow cytometry analysis (BD Biosciences). At least 10 000 cells were analyzed for each sample.

Immunohistochemistry and Histopathology

For histopathologic examination, the mice were euthanized with phenobarbital, and lung tissues were fixed by tracheal instillation of 10% neutral-buffered formalin. The lung tissues were rapidly dehydrated and cleared with an automatic tissue processor and embedded in wax. During the embedding procedure, the temperature was maintained below 60 °C. Paraffin sections (4-6 μm thickness) were placed on plus-coated slides, dried, and stored with desiccant. The slices were used either for conventional histology or for immunohistochemistry. For conventional histology, the slides were stained with HE staining to evaluate histopathological changes in animal tissues. Immunohistochemistry was performed on wax embedded lung cancer sections by Ki-67 staining. Sections were incubated with mouse serum (Zhongshan Jinqiao) for unspecific protein blocking and probed with the primary antibody Ki-67 (CST Biotech) at the dilution of 1:200 overnight at 4 °C. Signals were visualized using 3,3′-diaminobenzidine. Ten micrographs were randomly taken from each stained section at 400× magnification and positive cells were then counted.

Cytokines and Immunoglobulins

We used the protein chip (Quantibody Mouse Cytokine Array 1; RayBiotech) to detect the level of cytokines and immunoglobulins in mouse serum. Peripheral blood was collected in an anticoagulant tube for 30 minutes and then centrifuged at 12 000 g/min for 10 minutes. The serum was then isolated and stored at −80 °C. The detected cytokines were granulocyte macrophage colony-stimulating factor (GM-CSF), IFN-γ, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12p70, IL-13, IL-17, keratinocyte chemoattractant (KC), monocyte chemotactic protein-1 (MCP-1), M-CSF, RANTES, TNF-α, and VEGF. The detected immunoglobulins were IgA, IgD, IgE, IgM, IgG1, IgG2a, IgG2b, IgG2b, IgG3, mouse immunoglobin light chains (lambda and kappa).

Radiological Analysis

A CT scan of the chest in anesthetized mice was carried out using a CBCT system (X-RAD SmART, Precision X-Ray). To measure tumor volume and generate 3D images, the final reconstructed data were converted to the Digital Imaging and Communications in Medicine (DICOM) format by 3D-rendering software (IBEX, v1.0β). Voxels thresholds of −700 and −350 Hounsfield Units (HU) were required to obtain reasonable 3D images defining the lung surface. Volumetric analysis was performed from 3D lung images created through isosurface profiling. We used dicompyler (extensible radiation therapy research platform & viewer for DICOM & DICOM RT, Version: 0.4.2) to estimate the tumor volume. To evaluate the radiological responses, CBCT images were obtained from the entire thorax, using a GE eXplore Locus micro-CT scanner (GE Healthcare). To categorize the lung density, we set the HU scale to define normal lung, ground-glass opacity, and consolidation as < -400, > -400 to < -100, and > -100 HU, respectively.

Statistical Analysis

All data were presented as mean ± SE of at least 3 independent experiments. Student *t* test was used to evaluate statistical significance. A *P* value less than .05 was considered as statistically significant.

Results

The Response of Primary Lung Cancer Mice to X-Ray IR

The mouse model established using urethane allowed us to study the effects of IR on the immune system. We divided the mice into 4 groups: (1) Control group, (2) IR group, (3) Tumor group, and (4) Tumor + IR group. Twenty-eight weeks after

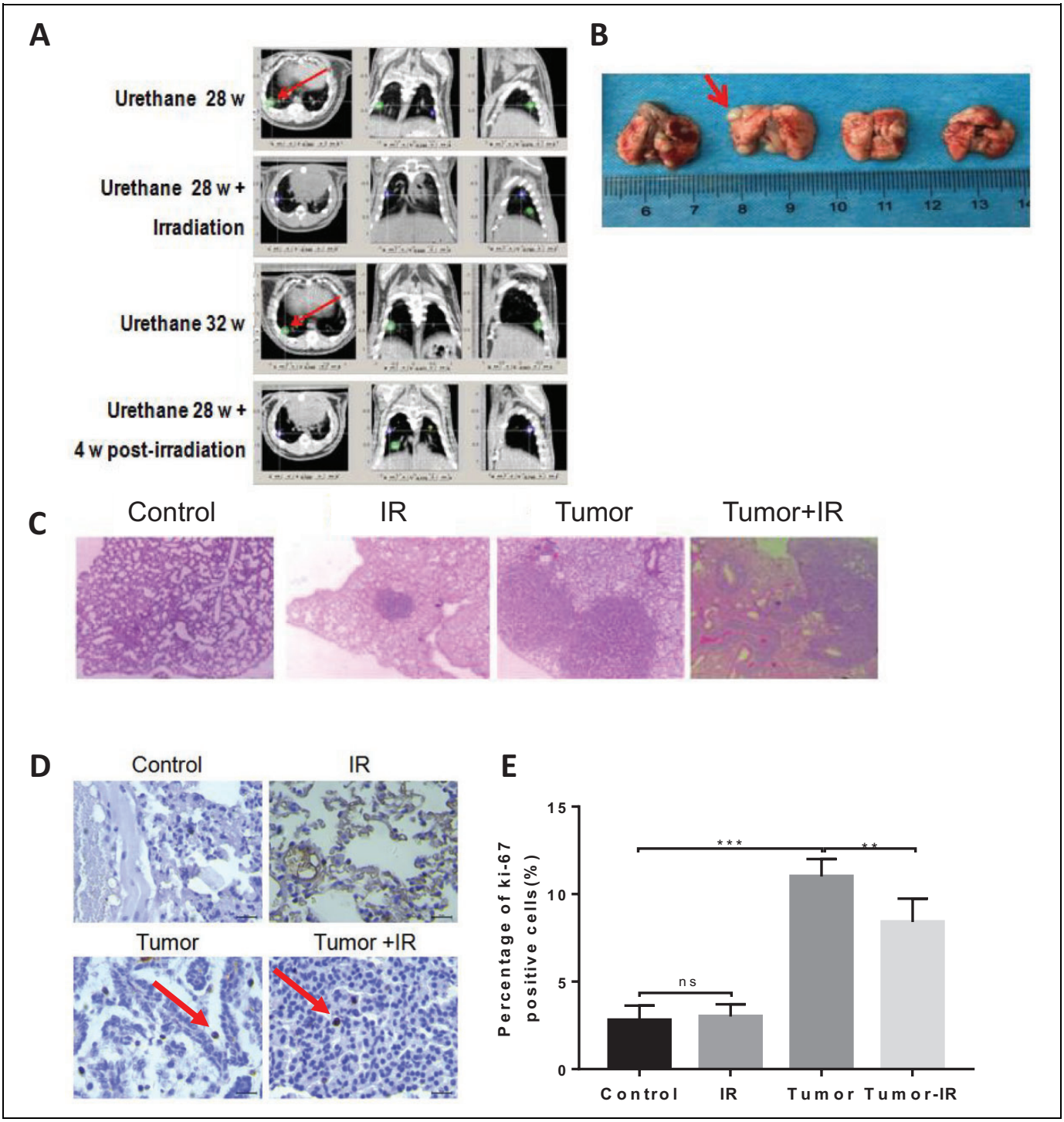

Figure 1. Response of primary lung tumor to X-ray IR. A, Response of primary lung tumor to IR as revealed by computed tomography scanning at the 28th and the 32th week. B, Lung tumors induced by urethane. C, Hematoxylin and eosin staining of tumor samples from the mouse model. D, Representative immunohistochemical staining of Ki-67 in tumor samples. E, Quantitative analysis of the percentage of Ki-67-positive cells in tumor samples. *$P < .05$; **$P < .01$; ***$P < .001$; ****$P < .0001$. CT indicates computed tomography; IR, irradiation; ns: no statistical significance.

urethane administration, we performed CT scanning to confirm whether changes had occurred. According to the CT results, nodules at the 32nd week were larger than those at the 28th week, and the tumor volume at the 28th week following radiation was smaller than that without radiation. Similar results were observed in the Tumor + IR group at the 32nd week (Figure 1A). When the mice were killed, the lungs were isolated and nodules were noted on the lung surface (Figure 1B). The number of nodules was counted, the volumes were measured, and it was found that the number of nodules in the tumor

Table 1. Tumor Incidence in the 4 Groups.

Groups	Tumor number	Tumor incidence	Tumor number/mouse ($\bar{X} \pm s$)	Tumor size ($\bar{X} \pm s$)
Control	0	0/15	0	0
IR	0	0/15	0	0
Tumor	93	15/15	6.2 ± 0.687	1.839 ± 1.012[a]
Tumor + IR	114	15/15	7.6 ± 0.744	1.274 ± 0.6656[a,b]

Abbreviation: IR, irradiation.
[a] Compared with control group.
[b] Compared with tumor group.

+ IR group was greater than that in the tumor group. However, the volume of nodules post-IR (tumor + IR) was smaller compared to the tumor group ($P < .05$; Table 1). To ensure that these nodules are tumor nodules induced by urethane, HE staining was conducted and increased cell atypia was found in the nodules, indicating that the nodules in both tumor and Tumor + IR groups were all tumor nodules (Figure 1C). To further confirm the results of HE staining, we used the Ki-67 antibody to detect cell proliferation. Ki-67 is a proliferation-related nucleolus-associated constituent used as a marker of cell cycling in tumor diagnosis and is used to identify cells in different phases of the cell cycle and in lung cancer pathology.[21,22] The immunohistochemistry figures showed that there was no obvious change in the percentage of Ki-67 positive cells after IR compared with the control group. However, the percentage of Ki-67 positive cells in the tumor group showed a significant increase ($P < .001$) and was markedly decreased in the Tumor + IR group ($P < .01$; Figure 1D, 1E).

Changes in Mouse Immunocytes After X-Ray IR

Immune cells were detected by flow cytometry and it was found that the number of natural killer (NK) cells in the tumor group did not change not only in peripheral blood but also in the spleen compared with the control group (Figure 2A and B). However, NK cells in peripheral blood in the IR group increased significantly ($P < .05$; Figure 2A). The number of B lymphocytes either in the blood or in the spleen did not change. Similar results were seen in the tumor group and Tumor + IR group (Figure 2E and F). However, the number of B lymphocyte in the blood increased significantly in IR group compared with the control group ($P < .05$; Figure 2E). In addition, we also found that the number of CD4-positive lymphocytes did not change markedly in the blood or spleen (Figure 2C and D). The number of CD8-positive lymphocytes was also unchanged in blood. However, the level of CD8-positive lymphocytes decreased significantly in the spleen ($P < .01$). But this change did not occur in the tumor group (Figure 2G and H).

Changes in Cytokines After X-ray IR

Other factors that may promote the immune stimulatory activity of IR are cytokines, chemokines, and other immune molecules.[23] Thus, to further study the effect of ionizing radiation on immune system, we determined cytokines using a protein chip. The data showed that some molecules were markedly changed in the tumor group and IR group, as shown in Figure 3. According to the protein chip results, we found that IL-17 decreased significantly in the IR group ($P < .05$; Figure 3C). Serum VEGF also markedly decreased in the IR group ($P < .05$; Figure 3E). Compared with the control group, after tumorigenesis, the level of IL-3 decreased ($P < .05$; Figure 3A) and the same results were found for IL-6 ($P < .01$; Figure 3B), VEGF ($P < .05$; Figure 3E), and RANTES ($P < .05$; Figure 3F). While no significant change was found for other cytokines, including IL-2, 4, 5, 9, 13, MCP-1, M-CSF, and KC, and so on. (Supplemental Figure 1).

Changes in Immunoglobulins in Peripheral Blood Induced by IR

According to the cytokine data, we hypothesized that ionizing radiation can induce changes in the levels of immunoglobulins; thus, we used the protein chip to determine the levels of serum immunoglobulins. The data showed that after tumorigenesis, the level of IgE decreased markedly ($P < .01$; Figure 4C). Similar results were observed for IgG2a ($P < .005$; Figure 4E), IgM ($P < .05$; Figure 4F), kappa ($P < .01$; Figure 4B), and IgG3 ($P < .05$; Figure 4D). When the tumor group was irradiated, a significant decrease in the level of kappa ($P < .005$; Figure 4B) was observed. A similar result was noted for IgD ($P < .05$; Figure 4A) and IgM ($P < 005$; Figure 4F). However, others like IgG1 and IgG2 did not change markedly (Supplemental Figure 2).

Discussion

Irradiation has a significant effect on the immune system by stimulating the release of cytokines, chemokines, and growth factors. These factors, in turn, can affect tumor metastatic ability, tissue remodeling, and the balance between cell survival and death. Therefore, the analysis of cancer cell cytokine characteristics will facilitate a better understanding of the function of IR in regulation of the immune system of the tumor patients. In this study, we used urethane to construct a Kunming mouse lung cancer model to study the effect of IR on the immune system of tumor-bearing mice. In the constructed tumor-bearing mouse model, the lung cancer incidence rate was 100% when observed 28 weeks after

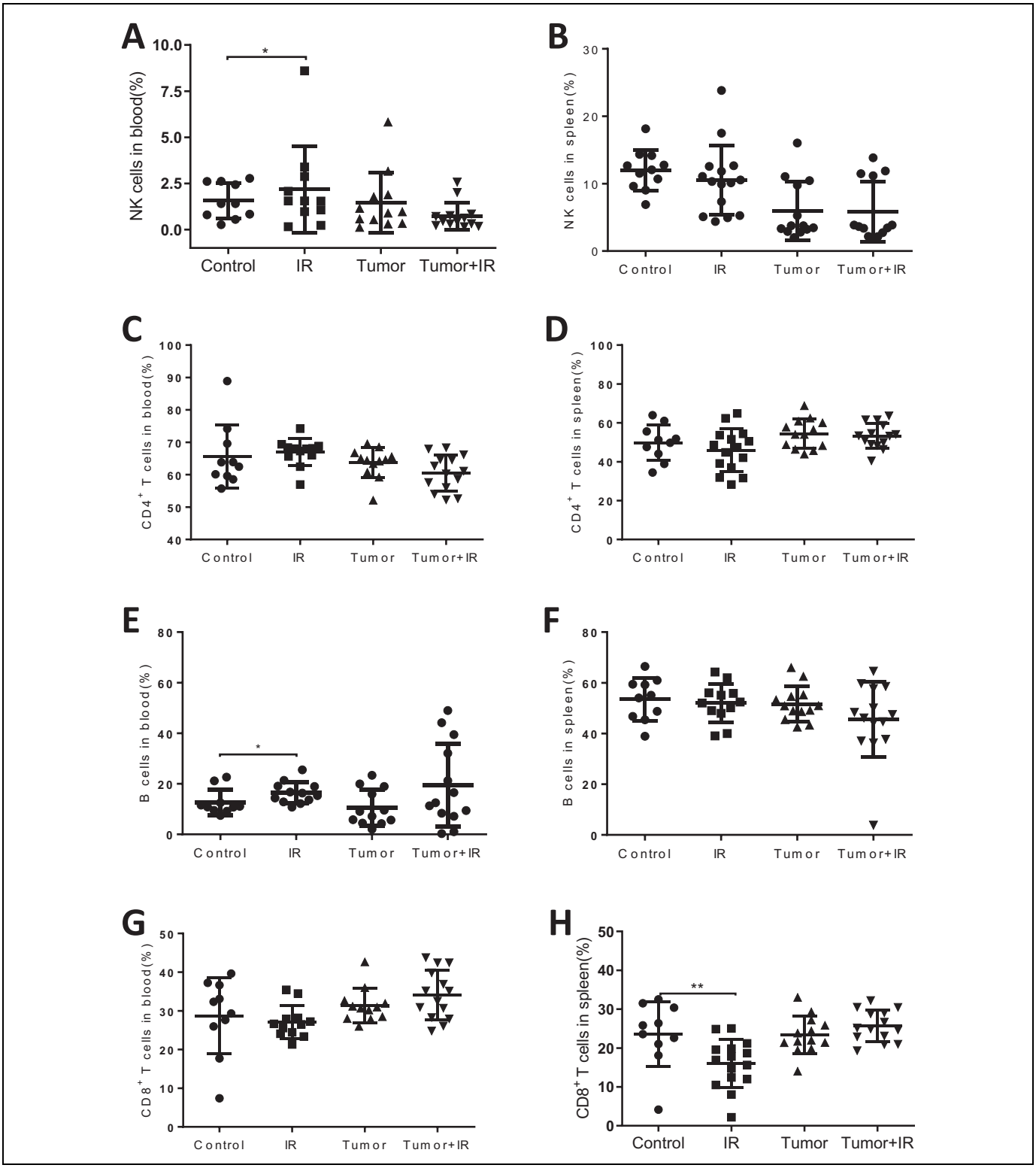

Figure 2. Changes in mouse immunocytes after X-ray IR analyzed by flow cytometry. A and B, NK cells were detected by NK1.1 antibody. E and F, B lymphocytes were detected by CD19 antibody. C, D and G, H, CD4+, CD8+ T lymphocytes were detected by CD3, CD4, and CD8 antibodies. IR, irradiation; NK, natural killer.

urethane injection, which is consistent with previous report. The pulmonary nodules were further confirmed as tumor nodules by immunohistochemistry.

Previous reports have demonstrated the cytotoxic effect of ionizing radiation on tumor cells, which was primarily due to the production of DNA double-strand breaks followed by cell

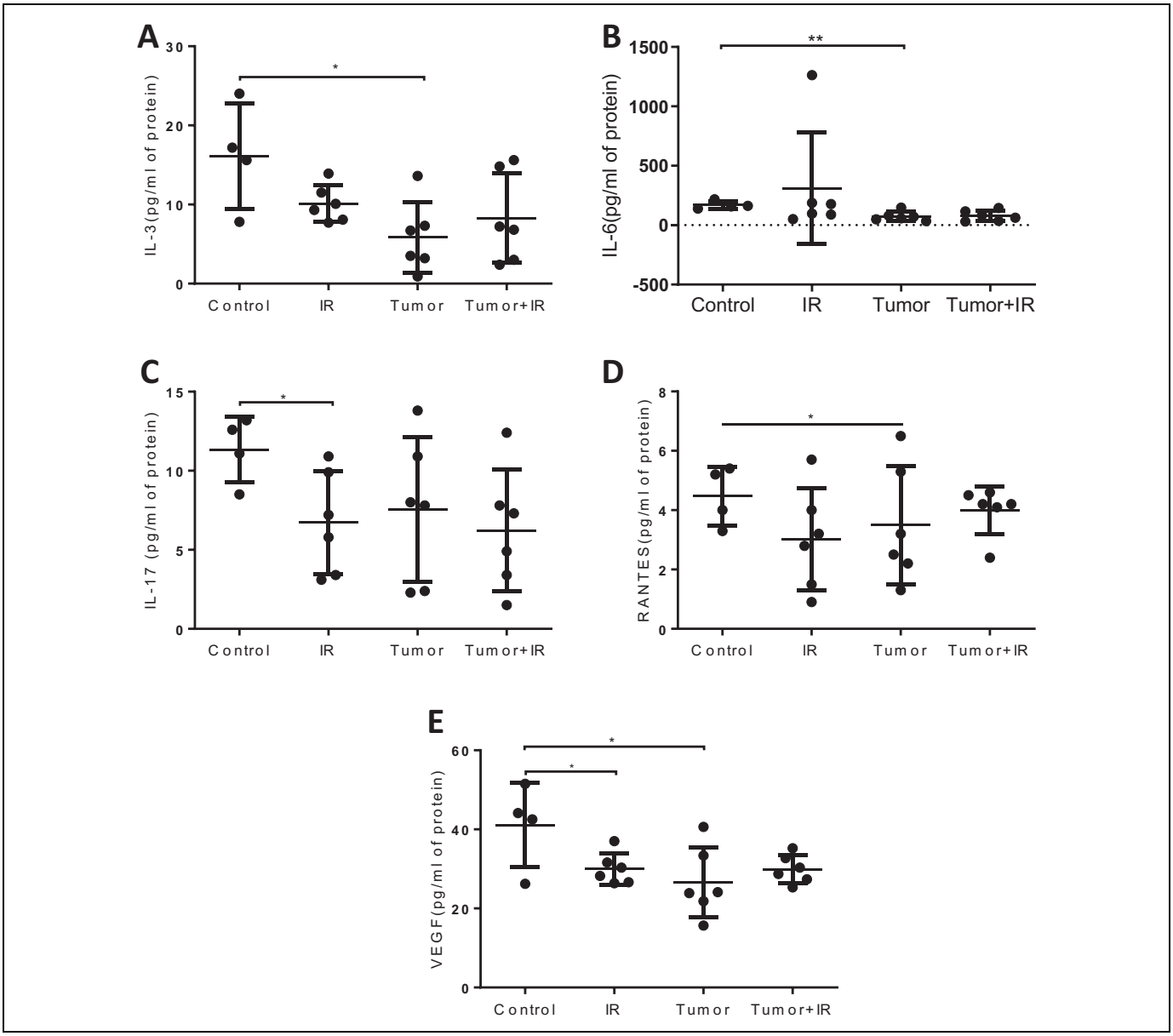

Figure 3. Changes in cytokines after X-ray IR analyzed by protein microarray. Changes of (A) IL-3, (B) IL-6, (C) IL-17, (D) RANTES, and (E) VEGF in serum samples of mice subjected to IR. IL indicates interleukin; IR, irradiation.

death via apoptosis, necrosis, or autophagy.[24] Due to the effects of radiation, radiotherapy such as hypofractionated and stereotactic radiotherapy has been widely used in the clinic. Stereotactic ablative body radiotherapy (SABR) is a noninvasive method of precision radiation that offers a radical departure from conventional therapy, and it is typically delivered in a daily dose of 2 Gy, 5 days a week for up to 7 weeks. Stereotactic ablative body radiotherapy is currently used in the treatment of many cancers, such as NSCLC, prostate cancer, renal cancer, and liver cancer.[25] The success of SABR in the clinic is largely attributed to tumors treated with much larger biologically effective doses of radiation.[26] Recent studies have shown that radiotherapy does not only make a contribution to the tumor microenvironment and the host's antitumor immunity but promotes an inflammatory response.[27] Ionizing radiation can also affect the release of immune molecules and co-stimulatory molecules.[28] Other studies have shown that following radiotherapy, the toxicity of Treg cells is enhanced, and the number of NK cells significantly increase after IR.[29,30]

Due to the inherent sensitivity of naive immune cells to radiation, radiotherapy has long been viewed as an immunosuppressive form of cancer therapy.[31] However, some data suggest that ionizing radiation has the capacity to engage host immune effector mechanisms that may contribute to the control or eradication of cancer.[32] For this reason, the field of combination-based therapies is receiving more attention as it is now abundantly clear that no single cancer therapy will be successful in more than a very small fraction of patients.[33]

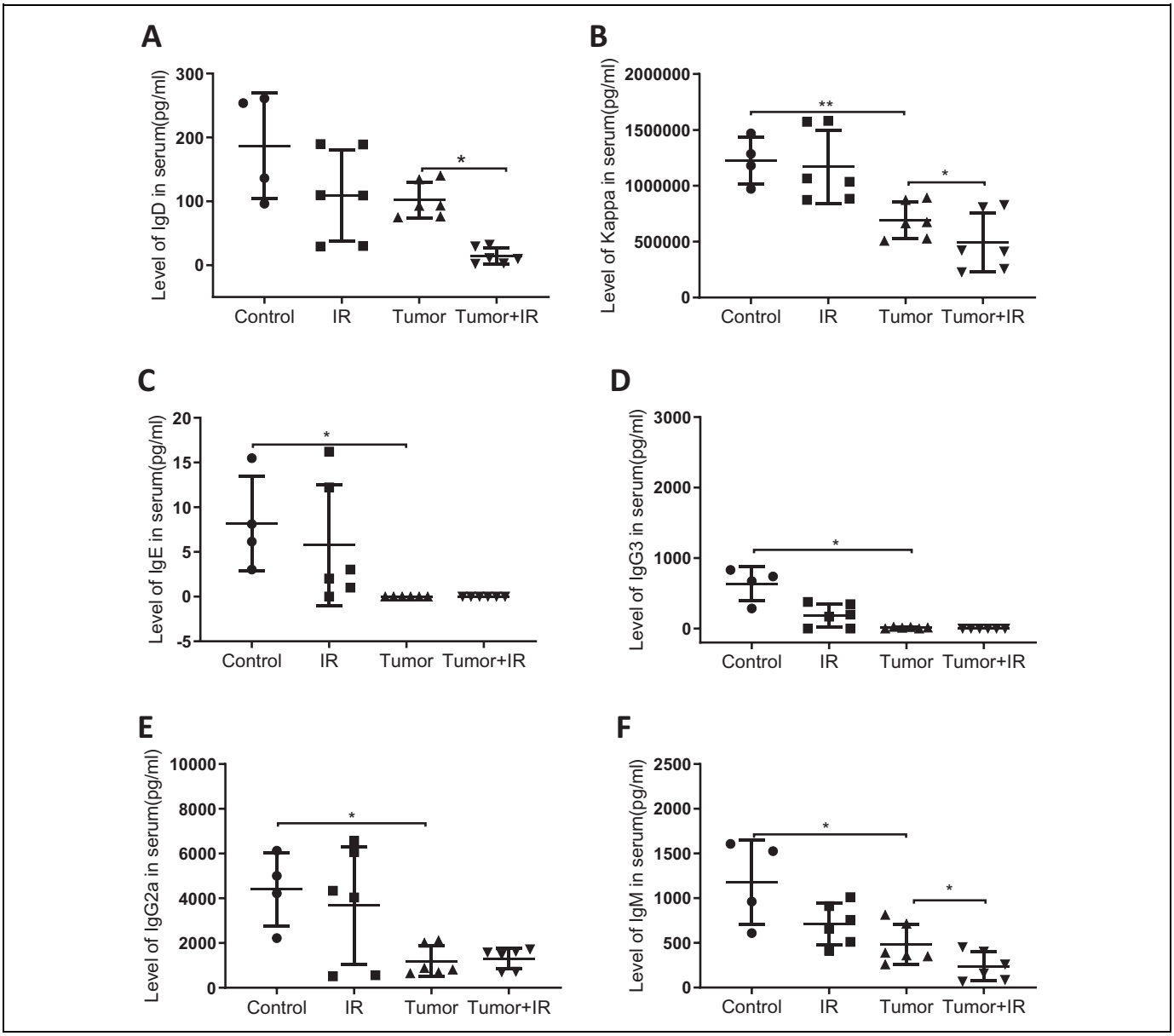

Figure 4. Changes in immunoglobulins in peripheral blood after X-ray IR analyzed by protein microarray. Changes of (A) IgD, (B) Kappa, (C) IgE, (D) IgG3, (E) IgG2a, and (F) IgM in peripheral blood samples of mice subjected to IR. IL indicates interleukin; IR, irradiation.

Many reports have shown that radiation has the ability to stimulate the immune system. A previous study showed that IR can both induce multiple cell death such as tumor cells and damage immune cells.[7] Radiotherapy can not only kill tumor cells but can induce reactions in the immune system and cause modulation of major histocompatibility complex (MHC) class I expression, which indicates that it can increase T cell recognition of irradiated tumor cells, making them vulnerable to cytotoxic T lymphocyte-mediated clearance.[34] Clinical evidence shows that the impact of radiotherapy on the immune system is modulated by CD8-positive T lymphocytes.[6] Notably, by increasing the release of antigens from cancer, radiation can also result in the transient expression of tumor-specific MHC/peptide complexes on stromal cells.[35] Recent studies have shown that radiotherapy is effective in stimulating not only cellular immunity but also innate immunity. Jones et al showed that radiation therapy can stimulate innate immunity to enhance tumor control by targeting Toll-like receptors and other innate recognition pathways.[36] In the present study, it was shown that B lymphocytes increased significantly, and $CD8^+$ T lymphocytes decreased markedly after IR. Following blood analysis, the level of NK cells was increased by IR, and a significant increase in the level of B lymphocytes was noted. The level of B lymphocytes in the spleen did not change markedly. In addition, there was no obvious change in CD4-positive lymphocytes both in the blood and spleen. A significant decrease in spleen CD8-positive lymphocytes was observed. All these data show IR can inhibit the ability of adaptive immunity.

The previous study showed that radiation not only had an influence on immune cells but also cytokines, chemokines, and other immune molecules like promoting the secretion of cytokines.[37] Another research demonstrated that the production of IFN-γ and TNF-α is correlated with radiation, and the level of intercellular adhesion molecule-1 (ICAM-1) increased after exposure.[38] On the other hand, the toxicity induced by radiotherapy resulted in changes in 30 types of cytokines.[39] In addition, radiotherapy can also induce the secretion of immune activators to enhance the sensitivity of the tumor to immunity.[40] According to the reports, IR can not only play a tumor-killing role but also regulate the immune environment of the tumor, and this regulation is closely related to the release of various cytokines. During the process of tumorigenesis, the release of ILs is involved in the complex tumor microenvironment, and IR can cause changes in various immune factors such as IFN-γ, IL-6, IL-10, IL-11, IL-12, IL-3, IL-33, IP-10, MCP-1, MCP-3, MDC, MIP-1α, MIP-1β, MIP-3α, MIP-3β, TGF-β1, TGF-β2, TGF-β3, TIMP-1, TNF-α, and VEGF.[32] However, the reaction of cytokines to radiotherapy is complicated and involves many factors. In addition, local radiation can stimulate tumor cells or epithelial cells to secrete TNF-α, IL-1, and IL-6, and these cytokines were found to promote dendritic cell maturation.[41] Radiation can also induce the secretion of IFN-γ in the microenvironment by stimulating T lymphocytes and NK cells.[42] Interleukin-1α plays an important role in immune activation after tumor cell death. It can also affect the secretion of IL-1β.[43] The same reaction was found for TNF-α and also found increased secretion of IL-1α, IL-6, and GM-CSF.[44] Interleukin-6 and IL-8 were also increased in glioma specimens.[45] In our study, IL-17 and VEGF significantly decreased in the IR group, and IL-3, IL-6, RANTES, and VEGF decreased in the tumor group. According to our results, the tumor can promote immune evasion by suppression of proliferation of hematopoietic cells, B cell maturation as well as pro-inflammatory factors, while IR suppressed both innate immunity and angiogenesis to some extent.

The abscopal effect was first described by Mole in 1953.[46] The phenomenon that enhanced eradication of local and distant tumors by genetically produced IL-12 and radiation has been increasingly reported in both preclinical and clinical studies.[47] Demaria et al reported that localized radiotherapy or hypofractionated IR plus cytotoxic T-lymphocyte-associated protein-4 blockage resulted in an abscopal effect in a mouse tumor model.[48] Some studies have demonstrated that X-ray radiation can induce the release of some cytokines such as IL-6 and can promote the production of MCP-1, whose main function is to move immune cells to inflammatory regions via chemotaxis. Another report showed that the release of MCP-1 was maintained at a high level for 21 days after IR, but IL-17 levels were lower[23] which are consistent with our results. Furthermore, we also found changes in immunoglobulins. The relevant data showed a marked decrease in IgD, IgM, and kappa in tumor + IR group, and significant decrease in IgE, IgM, IgG3, IgG2a, and kappa in tumor group, indicating that IR inhibited the biosynthesis and secretion function of innate immune cells.

In conclusion, there is a close correlation between the immune system of the tumor-bearing organism and the IR. Our work established a practical model for investigating the effects of radiotherapy on immune cells and immune factors and further found that some cytokines are involved in the response of the tumor to radiation. Besides we found IR decreased the secretion of IgM, which is a crucial functional component of innate immunity, resulting in the inhibition of the innate immunity. These findings paved a way for further revealing that underlying mechanisms of radiotherapy combined with immunotherapy.

Authors' Note

Shuxian Pan and Jingjie Wang authors contributed equally to this work. Dr Wentao Hu and Dr Bingyan Li conceived and designed the study. Anqing Wu, Jingjie Wang, and Shuxian Pan helped to irradiate the mice. Immunohistochemistry and flow cytometry were carried out by Shuxian Pan.

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Declaration of Conflicting Interests

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Supplemental Material

Supplemental material for this article is available online.

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Tieulent JL, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin*. 2015;65(2): 87-108.
2. Fakiris AJ, McGarry RC, Yiannoutsos CT, et al. Stereotactic body radiation therapy for early-stage non-small-cell lung carcinoma: four-year results of a prospective phase II study. *Int J Radiat Oncol Biol Phys*. 2009;75(3):677-682.
3. Timmerman R, Paulus R, Galvin J, et al. Stereotactic body radiation therapy for inoperable early stage lung cancer. *JAMA*. 2010; 303(11):1070-1076.
4. Surace L, Guckenberger M, van den Broek M. Radiation holidays stimulate tumor immunity. *Oncotarget*. 2015;6(18):15716-15717.
5. de Castria TB, da Silva EM, Gois AF, Riera R. Cisplatin versus carboplatin in combination with third-generation drugs for advanced non-small cell lung cancer. *Cochrane Database Syst Rev*. 2013;16(8):CD009256.

6. Honscheid P, Datta K, Muders MH. Autophagy: detection, regulation and its role in cancer and therapy response. *Int J Radiat Biol*. 2014;90(8):628-635.
7. Formenti SC, Demaria S. Systemic effects of local radiotherapy. *Lancet Oncol*. 2009;10(7):718-726.
8. Reynders K, Illidge T, Siva S, Chang JY, De Ruysscher D. The abscopal effect of local radiotherapy: using immunotherapy to make a rare event clinically relevant. *Cancer Treat Rev*. 2015; 41(6):503-510.
9. Prise KM, O'Sullivan JM. Radiation-induced bystander signalling in cancer therapy. *Nat Rev Cancer*. 2009;9(5):351-360.
10. Schaue D, Kachikwu EL, McBride WH. Cytokines in radiobiological responses: a review. *Radiat Res*. 2012;178(6):505-523.
11. Lumniczky K, Safrany G. The impact of radiation therapy on the antitumor immunity: local effects and systemic consequences. *Cancer Lett*. 2015;356(1):114-125.
12. Cosimi AB, Brunstetter FH, Kemmerer WT, Miller BN. Cellular immune competence of breast cancer patients receiving radiotherapy. *Arch Surg*. 1973;107(4):531-535.
13. Koski GK, Czerniecki BJ. Combining innate immunity with radiation therapy for cancer treatment. *Clin Cancer Res*. 2005; 11(1):7-11.
14. Bravata V, Minafra L, Forte GI, et al. Cytokine profile of breast cell lines after different radiation doses. *Int J Radiat Biol*. 2017; 93(11):1217-1226.
15. Chen J, Wang Y, Mei Z, et al. Radiation-induced lung fibrosis in a tumor-bearing mouse model is associated with enhanced Type-2 immunity. *J Radiat Res*. 2016;57(2):133-141.
16. Liang H, Deng L, Chmura S, et al. Radiation-induced equilibrium is a balance between tumor cell proliferation and T cell-mediated killing. *J Immunol*. 2013;190(11):5874-5881.
17. Anscher MS, Kong FM, Andrews K, et al. Plasma transforming growth factor beta1 as a predictor of radiation pneumonitis. *Int J Radiat Oncol Biol Phys*. 1998;41(5):1029-1035.
18. Stenmark MH, Cai XW, Shedden K, et al. Combining physical and biologic parameters to predict radiation-induced lung toxicity in patients with non-small-cell lung cancer treated with definitive radiation therapy. *Int J Radiat Oncol Biol Phys*. 2012;84(2): e217-e222.
19. Arpin D, Perol D, Blay JY, et al. Early variations of circulating interleukin-6 and interleukin-10 levels during thoracic radiotherapy are predictive for radiation pneumonitis. *J Clin Oncol*. 2005; 23(34):8748-8756.
20. Hawkins PG, Boonstra PS, Hobson ST, et al. Radiation-induced lung toxicity in non-small-cell lung cancer: understanding the interactions of clinical factors and cytokines with the dose-toxicity relationship. *Radiother oncol*. 2017;125(1): 66-72.
21. Schonk DM, Kuijpers HJ, van Drunen E, et al. Assignment of the gene(s) involved in the expression of the proliferation-related Ki-67 antigen to human chromosome 10. *Hum Genet*. 1989;83(3): 297-299.
22. Wong FW. Immunohistochemical detection of proliferating tumor cells in cervical cancer using monoclonal antibody Ki-67. *Gynecol Obstet Invest*. 1994;37(2):123-126.
23. Desai S, Kumar A, Laskar S, Pandey BN. Cytokine profile of conditioned medium from human tumor cell lines after acute and fractionated doses of gamma radiation and its effect on survival of bystander tumor cells. *Cytokine*. 2013;61(1): 54-62.
24. Golden EB, Pellicciotta I, Demaria S, Barcellos-Hoff MH, Formenti SC. The convergence of radiation and immunogenic cell death signaling pathways. *Front Oncol*. 2012;2:88.
25. Dhakal S, Corbin KS, Milano MT, et al. Stereotactic body radiotherapy for pulmonary metastases from soft-tissue sarcomas: excellent local lesion control and improved patient survival. *Int J Radiat Oncol Biol Phys*. 2012;82(2): 940-945.
26. Brown JM, Carlson DJ, Brenner DJ. The tumor radiobiology of SRS and SBRT: are more than the 5 Rs involved? *Int J Radiat Oncol Biol Phys*. 2014;88(2):254-262.
27. Formenti SC, Demaria S. Combining radiotherapy and cancer immunotherapy: a paradigm shift. *J Natl Cancer Inst*. 2013; 105(4):256-265.
28. Golden EB, Frances D, Pellicciotta I, Demaria S, Helen Barcellos-Hoff M, Formenti SC. Radiation fosters dose-dependent and chemotherapy-induced immunogenic cell death. *Oncoimmunology*. 2014;3:e28518.
29. Friedman EJ. Immune modulation by ionizing radiation and its implications for cancer immunotherapy. *Curr Pharm Des*. 2002; 8(19):1765-1780.
30. Nakagawa K, Yoshida F, Omori N, Tsunoda T, Nose T. The effect of radiation therapy combined with natural killer cells against spontaneous murine fibrosarcoma. *Biotherapy*. 1990; 2(1):69-75.
31. Wasserman J, Blomgren H, Rotstein S, Petrini B, Hammarstrom S. Immunosuppression in irradiated breast cancer patients: in vitro effect of cyclooxygenase inhibitors. *Bull N Y Acad Med*. 1989;65(1):36-44.
32. McBride WH, Chiang CS, Olson JL, et al. A sense of danger from radiation. *Radiat Res*. 2004;162(1):1-19.
33. Haikerwal SJ, Hagekyriakou J, MacManus M, Martin OA, Haynes NM. Building immunity to cancer with radiation therapy. *Cancer Lett*. 2015;368(2):198-208.
34. Reits EA, Hodge JW, Herberts CA, et al. Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. *J Exp Med*. 2006;203(5): 1259-1271.
35. Zhang B, Bowerman NA, Salama JK, et al. Induced sensitization of tumor stroma leads to eradication of established cancer by T cells. *J Exp Med*. 2007;204:49-55.
36. Jones RM, Sloane VM, Wu H, et al. Flagellin administration protects gut mucosal tissue from irradiation-induced apoptosis via MKP-7 activity. *Gut*. 2011;60(5):648-657.
37. Lugade AA, Moran JP, Gerber SA, Rose RC, Frelinger JG, Lord EM. Local radiation therapy of B16 melanoma tumors increases the generation of tumor antigen-specific effector cells that traffic to the tumor. *J Immunol*. 2005;174(12): 7516-7523.

38. Kim JY, Son YO, Park SW, et al. Increase of NKG2D ligands and sensitivity to NK cell-mediated cytotoxicity of tumor cells by heat shock and ionizing radiation. *Exp Mol Med*. 2006;38(5): 474-484.
39. Hartsell WF, Scott CB, Dundas GS, et al. Can serum markers be used to predict acute and late toxicity in patients with lung cancer? Analysis of RTOG 91-03. *Am J Clin Oncol*. 2007;30(4): 368-376.
40. Demaria S, Ng B, Devitt ML, et al. Ionizing radiation inhibition of distant untreated tumors (abscopal effect) is immune mediated. *Int J Radiat Oncol Biol Phys*. 2004;58(3): 862-870.
41. Siva S, MacManus M, Kron T, et al. A pattern of early radiation-induced inflammatory cytokine expression is associated with lung toxicity in patients with non-small cell lung cancer. *PLoS One*. 2014;9(10):e109560.
42. Chiang CS, McBride WH. Radiation enhances tumor necrosis factor alpha production by murine brain cells. *Brain Res*. 1991; 566(1-2):265-269.
43. Lugade AA, Sorensen EW, Gerber SA, Moran JP, Frelinger JG, Lord EM. Radiation-induced IFN-gamma production within the tumor microenvironment influences antitumor immunity. *J Immunol*. 2008;180(5):3132-3139.
44. Ishihara H, Tsuneoka K, Dimchev AB, Shikita M. Induction of the expression of the interleukin-1 beta gene in mouse spleen by ionizing radiation. *Radiat Res*. 1993;133(3):321-326.
45. Zhang JS, Nakatsugawa S, Niwa O, Ju GZ, Liu SZ. Ionizing radiation-induced IL-1 alpha, IL-6 and GM-CSF production by human lung cancer cells. *Chin Med J (Engl)*. 1994;107(9): 653-657.
46. Mole RH. Whole body irradiation - radiobiology or medicine. *Br J of radiol*. 1953;26:234-241.
47. Seetharam S, Staba MJ, Schumm LP, et al. Enhanced eradication of local and distant tumors by genetically produced interleukin-12 and radiation. *Int J Oncol*. 1999;15(4):769-773.
48. Deng L, Liang H, Burnette B, et al. Irradiation and anti-PD-L1 treatment synergistically promote antitumor immunity in mice. *J Clin Invest*. 2014;124(2):687-695.