REVIEW Libri Oncol. 2017;45(2-3):72–77

IMMUNOMODULATORY EFFECTS OF RADIOTHERAPY

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Summary

It is well known that radiotherapy, as mainly locoregional treatment, has systemic effects as well. The term abscopal effect (latin: *ab* – away from; *scopus* – target) has been used to describe the regression of distant tumor metastases after completion of primary tumor radiation therapy. It has been shown that radiotherapy can initiate systemic antitumor immunologic response which includes the activation of cytotoxic T-lymphocytes via numerous complex mechanisms. Latest findings indicate that there is an extremely complex interaction between the effects of radiotherapy, the activation of different signal pathways, tumor microenvironment and immunologic response. Depending on different tumor and host immune system characteristics, radiation therapy may have either immunostimulatory or immunosuppressive effects. Several case reports and smaller studies have described tumor regression after concurrent application of immunotherapy and radiation therapy, mainly in metastatic melanoma, but also in other cancers, such as castration-resistant prostate cancer, breast cancer, non-small cell lung cancer, etc. Many studies researching possible immunomodulatory properties of radiotherapy, as well as the combination of radiotherapy and immunotherapy are currently in progress. Better understanding of molecular mechanisms of tumor immunologic regulation and the immunomodulatory effects of radiotherapy will open possibilities of creating new more effective therapeutic options in treatment of malignant diseases.

KEY WORDS: *radiotherapy, immunotherapy, abscopal effect*

IMUNOMODULATORNI UČINCI RADIOTERAPIJE

Sažetak

Poznato je da radioterapija, kao primarno lokoregionalno liječenje ima i svoje sistemske učinke. Radioterapijski apskopalni učinak (latinski: *ab* – od; *scopus* – cilj, meta), odnosi se na povlačenje udaljenih presadnica nakon provedene radioterapije primarnog tumora. Pokazano je da radioterapija može potaknuti sustavni protutumorski imunosni odgovor koji uključuje aktivaciju citotoksičnih T-limfocita putem složenih mehanizama. Najnovija istraživanja ukazuju da postoji izuzetno složeno međudjelovanje zračenja, aktivacije različitih signalnih putova, tumorskog mikro okoliša i imunosnog odgovora. Radioterapija može djelovati imunostimulativno i imunosupresivno, ovisno o obilježjima tumora i imunološkog sustava. Opisana su moguća povlačenja tumora nakon istovremene primjene imunoterapije i radioterapije u metastatskom melanomu, kastracijski rezistentnom raku prostate, raku dojke, raku pluća nemalih stanica, itd. U tijeku su ispitivanja mogućih imunomodulatornih učinaka radioterapije, kao i djelovanje kombinacije radioterapije i imunoterapije. Bolje razumijevanje molekularnih mehanizama i radioterapijskog imunomodulatornog djelovanja otvara mogućnosti učinkovitijeg pristupa liječenju zloćudnih bolesti.

KLJUČNE RIJEČI: *radioterapija, imunoterapija, apskopalni učinak*

INTRODUCTION

For many years radiotherapy has been established as one of the most important modalities of cancer treatment. Whether it is performed in definitive, neoadjuvant, adjuvant or palliative setting, it is primarily a locoregional treatment, but systemic effects of radiotherapy are observed as well. Over the past six decades, number of clinical cases describing regression and even eradication of distant metastases following radiation therapy, were published. The patients presented in case reports differed by age, sex and type of tumor. However, it was observed that this effect more frequently occurred in patients with immunogenic tumors, such as leukemias and lymphomas, while it seemed to be less common in solid tumors (1). This phenomenon of possible regression of distant tumor metastases after completion of radiotherapy is known as the abscopal effect. The name derives from the Latin word *ab*, meaning *away from* and the Latin word *scopus*, which means *targe*t, and was first introduced in 1953 by Mole (2).

Immunomodulatory effects of radiotherapy and the abscopal effect

Abscopal effect of radiotherapy has been known for many years. The first theory proposed radiation scattering as a possible cause of abscopal effect. However, although the quantity of scattered radiation is dependent on the radiation volume and intensity, it does not normally exceed 3% of the total dose, and cannot possibly result in any systemic antitumor activity (1,3,4). It is known today that radiotherapy has numerous immunomodulatory effects and these complex mechanisms are still under investigation (5).

It has been shown that immune system can be affected and modified by radiation therapy on many levels. Radiation therapy leads to a specific type of cell death, known as the immunogenic cell death. The immunogenic cell death is characterized by the unique processes that occur on the molecular level within the cell. This includes the release of ATP, the upregulation of costimulatory molecules, the translocation of calreticulin (a protein that plays an important role in cancer cell adhesion) on cell surface, as well as the increase in the release of DAMP (damage-associated molecular patterns) molecules. DAMP activate dendritic cells (DCs) of the immune system in a way similar to the antigens of pathogenic origin, which ultimately leads to the activation of cytotoxic T-lymphocytes (6–8). The best known DAMP molecule is HMBG-1 (high-mobility group box 1), an agonist of toll-like receptor 4 (9,10). Toll-like receptors are a group of transmembrane proteins which are expressed in many cells, including antigen-presenting cells, where they play a key role in initiation of innate immune response via recognition of PAMP (pathogen-associated molecular patterns) and DAMP molecules (11,12), and are currently the focus of numerous studies in the field of immunotherapy.

It has been shown that radiation therapy results in increased expression of Fas molecule (a trigger of programmed cell death) and the major histocompatibility complex - MHC I (8,13,14), which is responsible for antigen presentation to cytotoxic $CD8^+$ lymphocytes. It appears that radiation therapy can also activate $CD8^+$ lymphocytes through the induction of type I interferons (15,16). Several studies on animal models have confirmed that the activation of $CD8^+$ lymphocytes is a key marker of radiosensitivity of the primary tumor (17–20), and is crucial for the development of abscopal effect (21).

The mechanisms of combination of radiotherapy and immunotherapy have been studied for several years. Golden et al. showed that the addition of immunotherapy to standard radiotherapy treatment consistently leads to abscopal effect in patients with metastatic solid tumors (22). The study included 41 patients with stable or progressive malignant disease and at least three measurable tumor lesions. Patients were diagnosed with different types of cancer, although a slight majority had non-small cell lung and breast cancer. In addition to standard treatment regimen, patients received subcutaneous injections of granulocyte-macrophage colonies stimulating factor (GM-CSF), and radiation to one of the measurable lesions at a dose of 35 Gy in 10 fractions (3.5 Gy per fraction). Evaluation of therapeutic response was performed using clinical exam and CT scans 7-8 weeks after completion of radiation therapy. The abscopal effect was defined by 30% reduction in size of any of non-irradiated lesions. The results showed that abscopal effect occurred in 27% of subjects. Also, patients who developed abscopal effect had longer overall survival compared to the others (21 months vs. 8 months).

Several case reports and smaller studies have described tumor regression after concurrent application of immunotherapy and radiation therapy, mainly in metastatic melanoma, but also in other cancers, such as castration-resistant prostate cancer, breast cancer, non-small cell lung cancer, etc. (19-24).

Latest findings indicate that there is an extremely complex interaction between the effects of radiotherapy, the activation of different signal pathways, tumor microenvironment and immunologic response. Depending on different tumor and host immune system characteristics, radiation may have either immunostimulatory or immunosuppressive effects. It seems that radiation therapy under certain conditions can aid the tumor to evade the immune response. Radiation therapy can upregulate TGF-β (transforming growth factor beta), the known mediator of tumor invasiveness and the epithelial-mesenchymal transition (23), the recruitment of T-regulatory lymphocytes (Tregs) and the polarization of M2 macrophages (24). It has been shown that radiation therapy can lead to the upregulation of some of the receptors expressed on the surface of tumor cells that have inhibitory effect on T-lymphocytes. These are CTLA-4 (cytotoxic T lymphocyte antigen-4), PD-1 (programmed cell death protein 1) and PD-L1 (programmed death-ligand 1) /PD-1 (1).

Radiotherapy and immune checkpoint inhibitors

The knowledge of the mechanisms of tumor immune tolerance led to the development of specific immunomodulators, checkpoint inhibitors, such as ipilimumab inhibitor of CTLA-4 (cytotoxic T lymphocyte antigen-4)), pembrolizumab, nivolumab, PD-1 (programmed cell death protein 1) and atezolizumab, inhibitor of PD-L1 (programmed cell death protein 1 ligand). While radiation therapy evidently can facilitate antitumor immune responses, the immunosuppressive effects of radiotherapy are also known, mainly by upregulating of the expression of PD-L1 (19).

Many studies researching possible immunomodulatory properties of radiotherapy, as well as the combination of radiotherapy and immunotherapy are currently in progress (for example radiotherapy with addition of checkpoint inhibitors such as anti CTLA-4, anti PD-1 (programmed cell death protein 1), anti PDL1/L2, or with addition of TGFβ (transforming growth factor beta) antagonists or TLR (toll-like receptor) agonists (22-24).

It has been shown that the efficacy of stereotactic radiotherapy was higher in PD-L1 knockout mice, as well as in mice treated with anti-PD-L1 (25). Also, the combination of anti-PD-L1 and stereotactic radiotherapy showed efficacy in mice with intracranial glioma (26). The investigators who studied the efficacy of combination of anti-CTLA-4 and radiation therapy in mouse model of metastatic breast cancer found tumor shrinkage and the inhibition of lung metastases in mice that were treated with combined regimen, with significantly prolonged overall survival (20). On the basis of the promising results from preclinical studies and some case reports, several small clinical trials (mainly phase I and II) were conducted. Prompted by previously published case report of a patient with metastatic melanoma who, after treatment with combination of ipilimumab and palliative radiotherapy (total dose of 28.5 Gy in 3 fractions) had achieved regression of melanoma lesions and stabilization of disease (27), Hiniker et al. treated a patient with asymptomatic metastatic melanoma with combination of ipilimumab and radiotherapy (total dose 54 Gy in 3 fractions). The treatment resulted in complete regression of primary and metastatic lesions (28). This pointed to the connection between the total dose of radiation therapy and the observed abscopal effect. The combination of ipilimumab and radiotherapy was also studied in patients with castration-resistant prostate cancer. Patients received ipilimumab concurrently with palliative bone radiotherapy. Clinical response was observed in 25% of patients (29). In the phase I study that examined the effect of the combination of stereotactic radiotherapy and interleukin-2 in patients with metastatic melanoma and renal cell carcinoma, 8 of 12 patients had clinical response. Also, patients who responded to the therapy had a higher number of $CD4^+$ lymphocytes (30).

Victor et al. conducted a clinical study of combination regimen of ipilimumab plus radiotherapy in 22 patients with advanced melanoma, and later, retested the results on mouse model (31). In this clinical trial, a single lesion was treated with stereotactic radiotherapy (6-8Gy/2-3 fractions). Three to five days after the completion of

radiotherapy, the patients were given 4 cycles of ipilimumab. The evaluation of results was performed using RECIST criteria, and only non-irradiated lesions were assessed. Partial response was observed in 18% of the patients, another 18% had stable disease, while the rest of the patients had disease progression. The investigators repeated the test using mouse model, and, while getting better results in a form of tumor regression, they still found that some mice were resistant to treatment (31). They conducted further studies on the selected mice and found that after the completion of combined radiotherapy plus immunotherapy, the resistant tumors had lower $CD8^+$ $CD4^+$ to Treg ratio in comparison to sensitive tumors. Further investigation showed that combination of anti-CTLA-4, anti-PD-L1 and radiation therapy increased $CD8^+$ to Treg ratio, working in favor of antitumor immunity over the evasion of immune response. Also, it was shown that the tumors that were resistant to combination therapy had upregulated PD-L1. Testing this theory, authors conducted the same therapy in mice with PD-L1 knockout melanoma cells and observed the restored response. By treating mice with resistant melanomas with combination of anti-PD-L1, anti-CTLA-4 and radiation, the authors achieved the improved response. Similar results were also observed in mouse models of breast and pancreatic cancer. However, it is important to mention that the complete tumor response was not observed in all treated mice, meaning that the upregulation of PD-L1 is not only mechanism of resistance to combined RT and anti-CTLA-4 therapy (31,32). It seems that a number of lymphocytes could be a predictor of abscopal effect in patients treated with the combination of ipilimumab and radiotherapy (33).

However, the total dose of radiation therapy and the fractionation of the dose for individual tumors has not yet been defined. Based on the literature, Sharabi et al. recommended hypofractionated regimen (5-20 Gy per fraction) (10), but definitive guidelines require further studies. Furthermore, Dovedi et al. found that the concurrent administration of immunotherapy with radiation therapy seemed to be more effective than sequential application of the same therapy (19).

Further research is needed to determine the best modality treatment of radiotherapy and immunotherapy combination, as well as to define the subgroup of patients who would benefit the most of this combined therapy.

Toxicity of immune checkpoint inhibitors and combination regimens

The studies have been shown that the combination of radiotherapy and immunotherapy may lead to increased treatment toxicities. The immune checkpoint inhibitors are known to increase the secretion of cytokines, which results in toxicity. The adverse effects of immunotherapy range from mild (which are most common) to severe, and even potentially lethal (34,35). For example, skin rash, hypophysitis, colitis and diarrhea are known adverse effects of CTLA-4 blockade (ipilimumab), while pneumonitis may occur as a side effect of PD-L1 blockade (1,36). The combination of radiotherapy and immunotherapy may lead to increased toxicities. For example, the risks of severe adverse effects in form of pneumonitis, dyspnea or cough, in patients who receive anti-PD-L1 and anti-CTLA-4 ranges between 2 and 4% (14). The risk of the radiation induced pneumonitis as an adverse effect of irradiation of the lung is reasonable to consider when combining these two treatment modalities. However, the improvement of radiation techniques that enable to minimize the treatment field (which leads to the reduction of the radiation induced toxicity) brings confidence in the possibility of using the combination treatment without the fear of severe adverse effects. Based on his, there are several ongoing clinical trials which are addressing this problem by investigating the safety and tolerability of combination of immunotherapy and radiation therapy.

CONCLUSION

Many studies researching possible immunomodulatory properties of radiotherapy, as well as the combination of radiotherapy and immunotherapy are currently in progress. Although number of preclinical studies, case reports and small clinical trials suggest therapeutic benefits, further research is needed to determine the safety and efficacy of this combined treatment. Better understanding of molecular mechanisms of tumor immunologic regulation and the immunomodulatory effects of radiation therapy will open the possibilities of creating new therapeutic options in the treatment of malignant diseases.

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