The Future of Combining Carbon-Ion Radiotherapy with Immunotherapy: Evidence and Progress in Mouse Models

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Abstract

After >60 years since the first treatment, particle radiation therapy (RT) is now used to treat various types of tumors worldwide. Particle RT results in favorable outcomes, especially in local control, because of its biological properties and excellent dose distribution. However, similar to other types of cancer treatment, metastasis control is a crucial issue. Notably, immunotherapy is used for cancer treatment with high risk for recurrence and/or metastasis. These 2 cancer therapies could be ideal, complementary partners for noninvasive cancer treatment. In this review, we will focus on preclinical studies combining particle RT, especially carbon ion RT, and immunotherapy.

Keywords: immunotherapy; mouse; radiotherapy; dendritic cell; carbon-ion

Introduction

When compared with conventional photon radiation therapy (RT), carbon ion (C-ion) RT has a higher antitumor effect and is less damaging to normal tissues surrounding the tumor [1–3]. For some difficult cancers, such skull base, sarcoma, and pancreatic cancer, C-ion RT alone or combined with chemotherapy significantly prevented or delayed the development of distant metastasis, with improved survival and local control [1]. However, metastasis and local recurrence are crucial issues for the improvement of the C-ion RT outcomes.

In this review, we discuss the progress of animal models treated with C-ion RT combined with immunotherapy (IT) and future developments of this combination therapy.

Immunotherapy as a Partner of Radiation Therapy

The human immune system has potential to eliminate cancer, as shown by spontaneous regression in some cases. However, the immune system is not able to block tumor growth for several reasons, such as immunosuppression caused by myeloid-derived suppressor cells, regulatory T cells, and cytokines [4–6] during the escape phase of cancer immunoediting [7, 8]. Therefore, IT was designed to boost the natural immune system and eliminate cancer by administering cancer-specific antibodies, cytokines, cancer vaccines, and immune-checkpoint inhibitors, as well as other therapies (**Figure 1**). In addition, IT is able to attack small tumors that cannot be detected by conventional methods and can retain the ability to kill cancer cells long after treatment [9].

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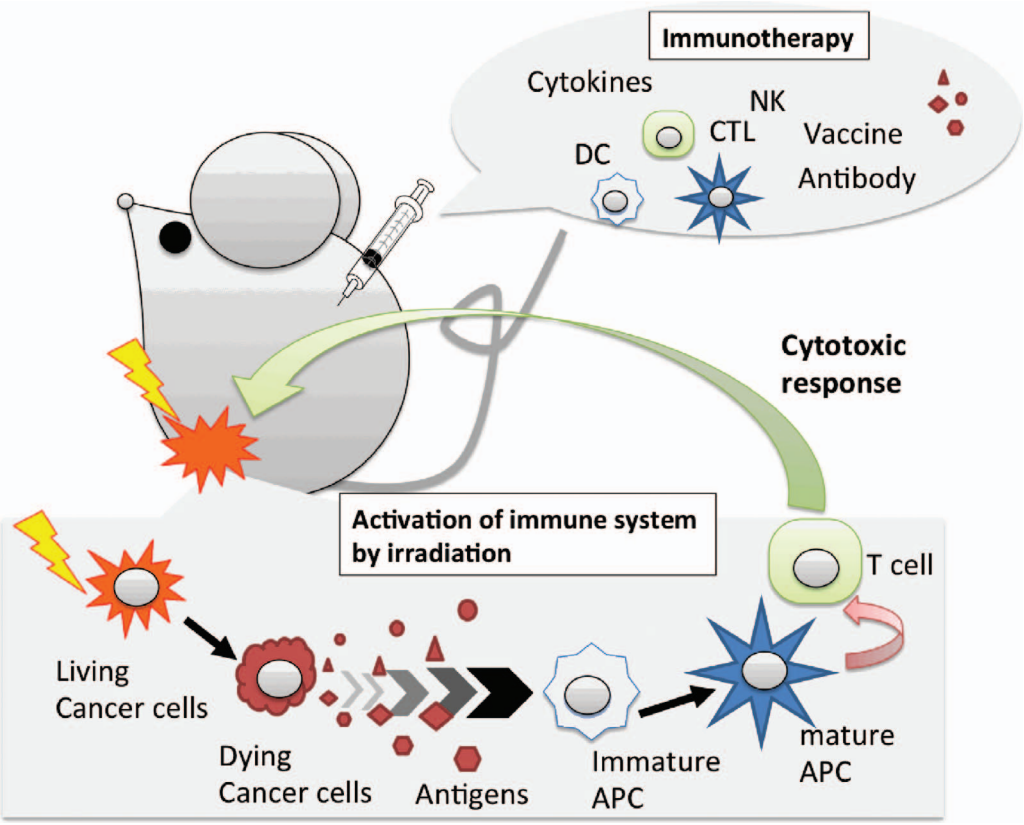

Figure 1. Radiation-induced immune activation and combination with immunotherapy. Radiation therapy is able to induce cancer cell death, including immunogenic cell death. The dying cells act as a source of tumor antigen to antigen-presenting cells, such as dendritic cells and macrophages. Mature antigen-presenting cells display tumor antigens combined with major histocompatibility complexes and stimulate T cells to become cytotoxic T cells. The activated cytotoxic T cells are expected to attack cancer, including micrometastases and circulating tumor cells. If we find ways to control the mechanisms in any patients, radiation therapy is able to use an inducer of in situ vaccine. For immunotherapy, these key factors, such as immune cells and cytokines, are used for the treatment. Therefore, combination immunotherapy–radiation therapy is expected to enhance the antitumor effects.

It is thought that most cancer treatments, including RT, involve immune-mediated systems. In particular, RT is believed to possess strong potential in combination with IT. Unlike surgical therapy, irradiated cancer cells die in the body after irradiation. These dead cancer cells may act as tumor antigens and be taken up by phagocytes (**Figure 1**). In previous reports [10–13], RT- and chemoRT-induced immunogenic cell death is preceded or accompanied by the emission of a series of immunostimulatory, damage-associated molecular patterns in a precise spatiotemporal configuration [14, 15]. The radiation-induced immunogenic cell death might act as an in situ tumor vaccine and is a crucial process to initiate anticancer immune responses [16]. In addition, clinical case reports demonstrated that RT sometimes induced tumor regression of nonirradiated tumors [17–21]. These phenomena, known as the *abscopal effect*, may be involved in the immune response, secondary to irradiation-induced cancer cell death. If it becomes possible to regulate and/or enhance the RT-induced abscopal effect, we may move a step closer to cancer control. Therefore, an increasing body of basic research combining RT and IT has been carried out in immune-competent animal models [13, 22–40]. These studies can be categorized based on the index of evaluation, as shown **Figure 2**. There may be a tendency showing that the abscopal effect was enhanced by, or was only functional by, combination treatment with IT. However, even though it does not show a clear abscopal effect, RT combined with IT treatment prevented tumor growth or distant metastasis [41]. In addition, several clinical trials have already been started for combined photon RT (or chemoRT) and IT [42–46].

Basic Studies on the Combination of Particle Beam and Immunotherapy

Even though particle beams might have several biological advantages for use in combination with IT, basic science studies on the combination are still limited (**Table 1**).

Mouse studies on the combination of IT with C-ion were published in 2010 by 2 independent groups [47, 48]. Both articles used bone marrow (BM)-derived dendritic cells (DCs) as IT. The DCs were administered by intratumoral injection after

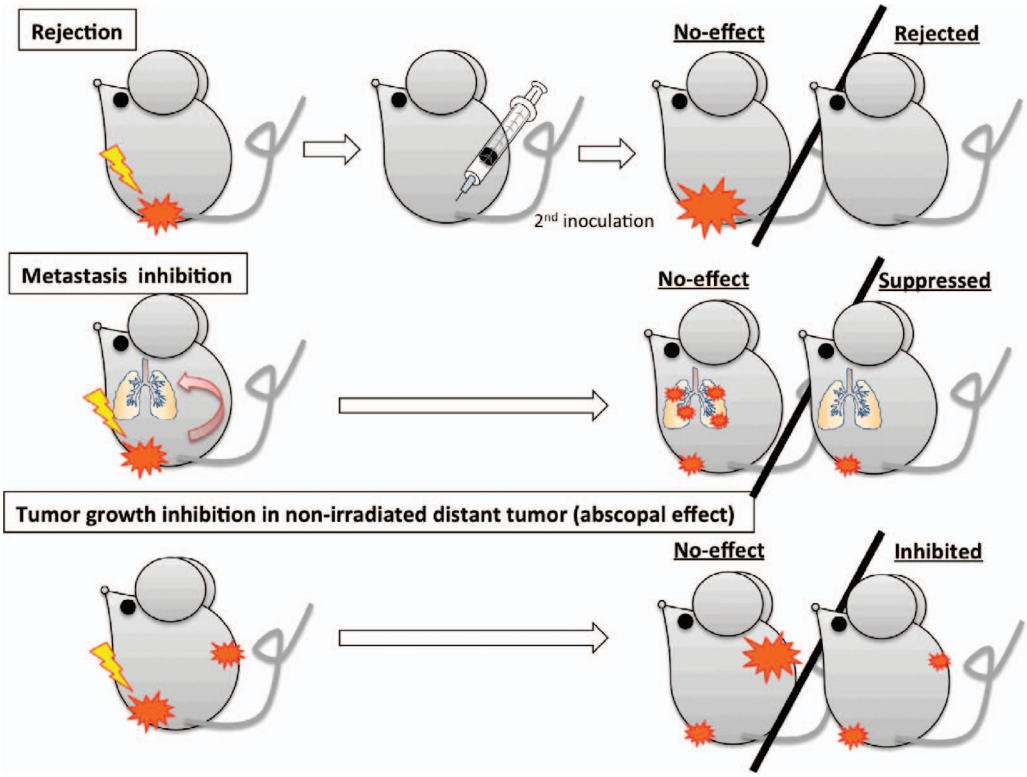

Figure 2. Types of evaluation for effectiveness of radiation therapy–induced immune response. If irradiation induces antitumor immune response in the tumor-bearing mouse, evaluation of activation is possible by the following methods: rejection—evaluate rejection rate of secondary tumor inoculation after treatment of the first tumor; metastasis suppression—evaluate number of metastases after treatment; and abscopal effect—evaluate nonirradiated tumor growth after irradiation of another tumor.

irradiation. They are the most potent population of the antigen-presenting cells and the key mediator in generating therapeutic immunity against cancer [49, 50]. Matsunaga et al [47] used 2 experimental models: (1) syngeneic C3H/He mice inoculated with poorly immunogenic squamous cell carcinoma (SCC VII), and (2) mammary carcinoma FM3A cells. Before administration, DCs were activated by infection with recombinant Sendai virus after a coculture with SCC VII lysate as the tumor antigen. These were administered multiple times following irradiation. Researchers showed that C-ion irradiation resulted in tumor elimination, rejection of secondary tumor inoculation, and T-cell activation. These antitumor effects were enhanced by the combination of DC IT.

On the other hand, we reported a study [48] that used a syngeneic model of highly metastatic SCC NR-S1-implanted C3H/He mouse. In the article [48], we evaluated the effect of the combination treatment based on metastasis suppression. For IT, we administrated α-galactosylceramide (α-GalCer)–treated, BM-derived DCs on day 1.5 after irradiation by intratumoral injection. We chose lower radiation doses, which could not significantly repress the growth of the irradiated tumor. Because high-dose irradiation significantly inhibits tumor growth, it is difficult to evaluate the nature of C-ion–induced metastasis inhibition. Specifically, it is unclear whether such irradiation has a direct effect on the metastatic

Table 1. Immune-competent mouse model experiments combining particle radiation therapy with immunotherapy.

Source, y	Cell line	Mouse strain	Radiation therapy	Immunotherapy	Immunotherapy administration	Effect
Matsunaga et al, 2010 [47]	SCC VII; FM3A, mammary carcinoma	C3H/He; BALB/c–nude	290 MeV/n C-ion; 77 keV/μm; <10 Gy/min	BM-derived; DCs (SCC VII lysate treated, then rSeV/dF infected)	Immunotherapy, d 2, 9, 17 after IR	Second tumor rejection
Ohkubo et al, 2010 [48]	NR-S1, SCC	C3H/He	290 MeV/n C-ion; 6 cm SOBP; 6 Gy	BM-derived; α-GalCer–pulsed DCs	Immunotherapy, d 1.5 after IR	Lung metastasis

Abbreviations: BM, bone marrow; C-ion, carbon ion; DC, dendritic cell; SCC, squamous cell carcinoma; SOBP, spread-out Bragg peak; α-GalCer, α-galactosylceramide.

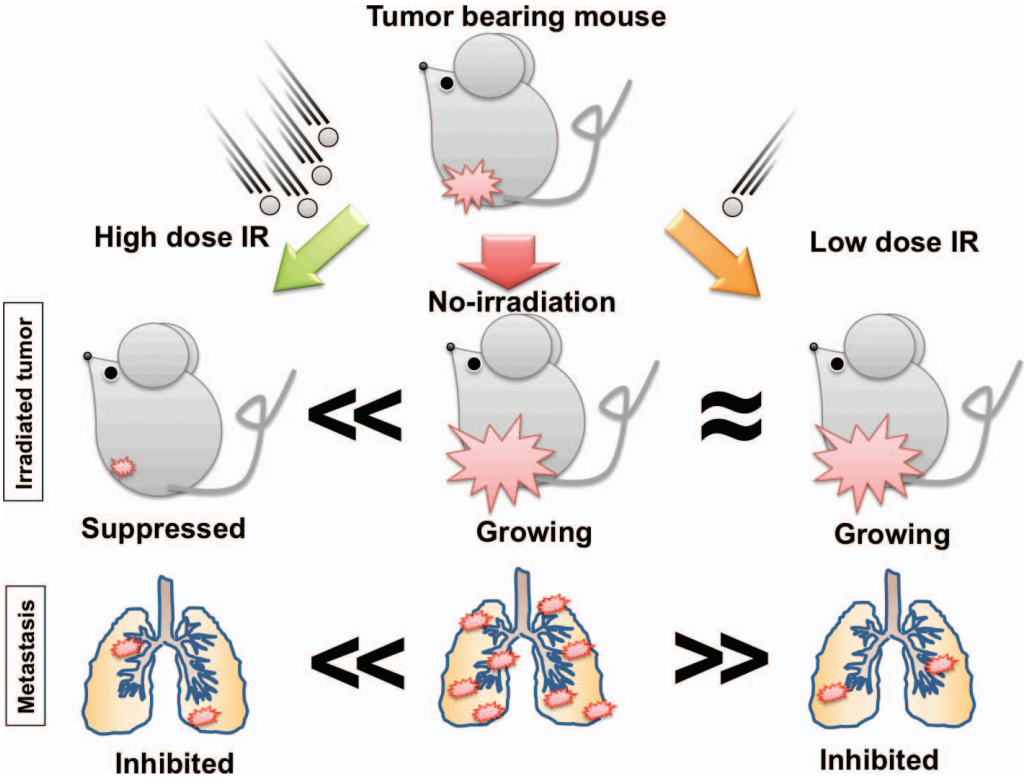

Figure 3. The possible mechanisms of metastasis reduction. High-dose irradiation is able to suppress the growth of the irradiated tumor, and distant lung metastasis may also be inhibited. However, it is difficult to evaluate whether the inhibition of metastasis results from immune response or is a consequence of primary tumor regression. In contrast, low-dose irradiation has less effect on tumor growth. The irradiated tumor is able to sustain growth. If lung metastasis is inhibited significantly in this condition, it might indicate a direct effect of carbon-ion radiation therapy in metastases. Moreover, the radiation-induced immune response may be evaluated.

process, such as the invasive potential or if it is just a consequence of growth inhibition (**Figure 3**). In this setting, treatments with either α-GalCer DCs or C-ion irradiation decreased the numbers of metastatic nodules. When the combination of immature DCs IT and C-ion irradiation was used, the number of lung metastatic nodules was drastically reduced. Interestingly, DC treatment had no effect on tumor growth whether with C-ion treatment or without. We further showed that the lung tissue of the NR-S1–implanted mice exhibited increased expression of S100A8, which is a marker of premetastatic change. The S100A8 protein and messenger RNA expression were not affected by either C-ion irradiation or DC treatment. However, the lung tissues of the combination therapy group showed repressed S100A8 expression at 7 days after C-ion irradiation.

In many reports, BM-derived DCs were used as IT in tandem with RT. However, the DCs were treated with different activators or modifiers, such as α-GalCer [48] or tumor lysate [47], even though the combination treatment resulted in significant effects in all cases. In addition, radiation treatment combined with the administration of Flt3 ligand, which stimulates the proliferation and differentiation of DCs, also expanded radiation-induced antitumor effects [34, 38]. This suggests that BM-derived DCs themselves or the various treatments for immune activation were the essential factors. For example, DCs with α-GalCer treatment are thought to activate natural killer T cells. Therefore, we evaluated whether these modifications are essential or not for the combination treatment. When we used BM-derived immature DCs or α-GalCer-treated DCs in combination with C-ion RT, lung metastasis was suppressed in both cases [51]. This result highlights that C-ion irradiation has enough potential to activate immature DCs without pre-treatment. Furthermore, we also compared different methods for the administration of DCs. Because particle therapy has an advantage in treating tumors that are located deep in the body, intratumoral injection is not a suitable way for combination treatment. Among the methods compared, intravenous injection was shown to be highly effective in preventing lung metastasis. Further investigation is necessary to elucidate the precise mechanisms involved, such as tracking the injected DCs. In addition, we confirmed the efficacy with other mouse models using different cancer cell lines and mouse strains to expand the application of combination C-ion RT and DC IT. As a result, C-ion RT combined with DC IT significantly suppressed lung metastasis. Furthermore, we have shown that, even when exposed to the equivalent, relative biological effectiveness dose of C-ion and photon, the combination with photon could not be induced to the same level [51]. This result highlights that C-ions may be more effective in activating the immune system.

Advantage of Particle Radiotherapy for Use in Combination with Immunotherapy

As a partner to immune combination therapy, are particle beams as effective as, or more advantageous than, photon beams? Even when exposed to the same relative biological effectiveness dose, particle and photon beams are known to induce different bioresponses.

There are several reports demonstrating the effect of particle beams on metastatic potential. In some cases, x-ray treatment–enhanced metastatic potential [52], but the C-ion beam effectively suppressed it [53], with examples like migration [54–58] and invasion [55–57, 59] of cancer cell lines with in vitro assays. In addition, C-ion beam treatment inhibited in vitro angiogenesis at sublethal doses [60] as well as the expression of angiogenesis mediators [59, 61]. In immune-competent mouse models, C-ion significantly suppressed lung metastasis [55, 62].

In recent years, increasing amounts of research assert that substantial heterogeneity derived from clonal evolution exists within tumors, leading to varying radioresistance within a target [63–66]. Coupled with cancer stem cell (CSC)-like cells and also quiescent cells are known for their photon irradiation resistance and are correlated with repopulation of local recurrence after treatments, and the treatment of any individual tumor grows increasingly complex. In vitro experiments showed that CSC-like cells and quiescent cells have more resistance to irradiation with x-rays than C-ion compared with non-CSC-like cells or total cells [67–69]. In addition, Zhang et al [58] reported that CSC-like cells were more sensitive to proton irradiation than photons were. These findings indicated that particle RT is suitable for suppressing the dependency on the heterogeneity within tumors.

Some articles showed that particle beams induce the opposite results or that they induce similar bioresponses as photon irradiation [70–73]. The presence of contradicting reports may indicate the need to clarify the differences in outcome between the RT types used and between different cancers. Basic biological research focused on particle beams is still limited. In particular, to compare the effects on immunological response between different types of RT and/or methods of therapy (hypofractionation, hyperfractionation, among others) are essential issues for further development of particle RT combined with IT.

Future Developments

Despite the superior local control achieved with particle therapy, improvement in overall survival is limited because of distant metastasis [74]. Therefore, it is essential to find the optimal "combination partner" for particle RT to improve clinical outcomes.

Basic research on particle beams combined with IT is still limited to experimental models and the types of IT. However, these results show that, even with a lower dose, C-ion RT has substantial potential to activate the immune system. Such lower irradiated dose might be able to induce cell death in some tumor cells. However, it is enough to activate the immune system in both in vitro assays and mouse models [41]. Excellent dose distribution of particle therapy reduced damaged volume of normal tissues, including the BM and skin. In addition, C-ion RT showed good local control. These features allow us to select different types of IT as a partner for C-ion RT, such as immune system modulators, immune checkpoint inhibitors, myeloid-derived suppressor cells inhibitors, and cytokines, which act via the immune system of the patient.

Preclinical experiments for combining photon RT with various types of IT were previously reported. Combination with an immune-checkpoint inhibitor was first reported in 2005 [37], and many reports were published in the past few years [25, 28, 31, 35, 36, 40, 75, 76]. In addition, clinical trials for photon RT combined with immune-checkpoint inhibitors have already been started. Because particle RT could have an advantage for cancer treatment, it is expected that immune-checkpoint inhibitors may also be efficient in combination with particle RT. Moreover, because of the difference in mechanisms involved among conventional ITs and immune-checkpoint inhibitors, it may be of value for evaluating triple-combination antitumor effects, as already described in some reports [28, 35]. However, there are many variations in immune response, including the differing effects of immune cell lines. Combination therapy approaches considering the tumor microenvironment could serve as starting points [77].

The reports regarding RT combined with IT demonstrate drastic antitumor effects, but there have not been any reports, to our knowledge, on mouse models in which the combination effect could not be observed (except for immunodeficient or CD8-depleted mice). Because the clinical results showed that spontaneous regression (or abscopal effects) was not observed in all cases [78], it is important to investigate inefficient models for combination RT as well as the efficient cases. In addition, very aggressive or treatment-resistance models, such as the 4T1 breast cancer model [4, 39], are required to develop C-ion combined IT because these difficult-to-cure cancers are the targets for C-ion RT. It is expected that comparison among these

mouse models might lead us to further understand the underlying mechanisms of combination RT, which influence the outcomes of individual patients.

ADDITIONAL INFORMATION AND DECLARATIONS

Conflicts of interest: The authors have no conflicts of interest to disclose.

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