

Radiotherapy supports protective tumor-specific immunity

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Radiotherapy is an important therapeutic option for the treatment of cancer. Growing evidence indicates that, besides inducing an irreversible DNA damage, radiotherapy promotes tumor-specific immune response, which significantly contribute to therapeutic efficacy. We postulate that radiotherapy activates tumor-associated dendritic cells, thus changing the tolerogenic tumor environment into an immunogenic one.

The vertebrate immune system must be capable of protecting the host from pathogens while remaining unresponsive to self. The dependence of adaptive immune responses on innate or pro-inflammatory signals may be a consequence of evolution, as such signals usually accompany infections and thus indicate a perturbation of immune homeostasis. Innate signals result in the activation and maturation of dendritic cells (DCs), which is a prerequisite for the induction of protective immunity.¹ In contrast, immature DCs promote peripheral T-cell tolerance through a variety of mechanisms, including signals that are transduced via PD-1 and CTLA-4.¹

While malignant cells can be efficiently recognized by the immune system, protective immunity is most often insufficient in cancer patients. Besides local immunosuppression and immunoediting,² we think that an inadequate DC maturation contributes to impaired immunity in the context of cancer. The observation that the frequency of tumor-infiltrating regulatory T cells (Tregs) correlates with poor prognosis in many different cancer types² may be partially explained by the capacity of Tregs to keep DCs in an immature state.³ Accordingly, DC activation without further manipulations has been shown to result in tumor control in preclinical models.^{4,5} The remarkable clinical efficacy

of Coley's toxin—a mixture of heat-killed *Streptococcus pyogenes* and *Serratia marcescens* that was used by William Coley in the 19th century to treat cancer patients⁶—may also result from the activation of DCs, which obviously was not appreciated at that time.

Radiotherapy and chemotherapy are standard treatments for cancer and both have been considered as immunosuppressive measures for a long time. However, a growing body of evidence suggests that chemotherapy, at least in some instances, actually supports tumor-specific immunity and that DC activation is a crucial aspect herein.^{5,7} Also radiotherapy, especially when administered in limited fractions of high dose, positively influences tumor-specific immunity. For example, radiotherapy results in local production of Type I interferon,⁸ which enhances the cross-presentation of tumor-derived antigens by DCs and improves qualitative as well as quantitative aspects of tumor-specific immunity.⁹ We are currently investigating whether tumor-associated plasmacytoid dendritic cells (pDCs) are a source of radiation-induced Type I interferon. We have found that the therapeutic efficacy of a single dose of X-rays (10 Gy) crucially depends on the presence of CD8⁺ T cells and of DCs during and/or immediately after radiotherapy.⁵ Importantly, this was

the case for multiple transplantable syngeneic tumors in at least two different genetic backgrounds, suggesting a rather general mechanism. In contrast, macrophages or CD4⁺ T cells were dispensable for the therapeutic efficacy of radiotherapy. Moreover, we found that local high-dose radiotherapy activates tumor-associated DCs, in a fashion that is independent of MYD88, NALP3 or DNGR1 (CLEC9A) (unpublished observations). In contrast to what has been described for particular types of chemotherapy,⁷ radiotherapy apparently does not induce the upregulation of the non-histone chromatin binding protein HMGB1 (unpublished observations). This suggests that the activation of DCs induced by radiotherapy and chemotherapy involves different signaling pathways. Maturation rather than the mere presence of tumor-associated DCs is a crucial aspect of radiotherapy, because blockade of CD70—an important co-stimulatory molecule for the priming of protective CD8⁺ T cell-dependent immunity—completely abolished the therapeutic efficacy of radiotherapy.⁵ Based on our data, we think that radiotherapy does not necessarily promote the infiltration of tumor-specific T cells, but supports their proliferation and/or survival within the tumor. In addition, tumor-specific CD8⁺ T cells seem to better maintain their effector function

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within irradiated, as compared with non-irradiated, tumors. Whether other soluble factors or cell populations other than activated DCs contribute to the increased number and function of tumor-infiltrating T cells after radiotherapy is the subject of ongoing investigation.

Reits et al. described in 2006 that the γ irradiation of cancer cells leads to an increased expression of MHC Class I molecules in an mTOR-dependent fashion. This observation prompted us to investigate whether also cancer-testis antigens (CTAs), a particular class of tumor-associated antigens that are often targeted spontaneously by the immune system in cancer patients, are upregulated upon γ irradiation. Indeed, we found that irradiation upregulates the expression of MHC Class I molecules and various CTAs (including some cases of de novo expression) in cancer cell lines and fresh human tumor material *ex vivo*, in xenografted human cell lines *in vivo* and in biopsies from sarcoma patients.¹⁰ Obviously, the upregulation of both MHC Class I molecules and CTAs makes cancer cells more visible to cytotoxic T cells, which presumably contributes to the therapeutic efficacy of radiotherapy. Retrospective analyses of a cohort of sarcoma patients, from whom paired biopsies were collected before and after radiotherapy, showed a striking impact of radiotherapy on multiple immune parameters within the tumor. For example, we found a consistent increase of immune effector cells and molecules including CD8⁺ cells, perforin and interferon γ . Along similar lines, we

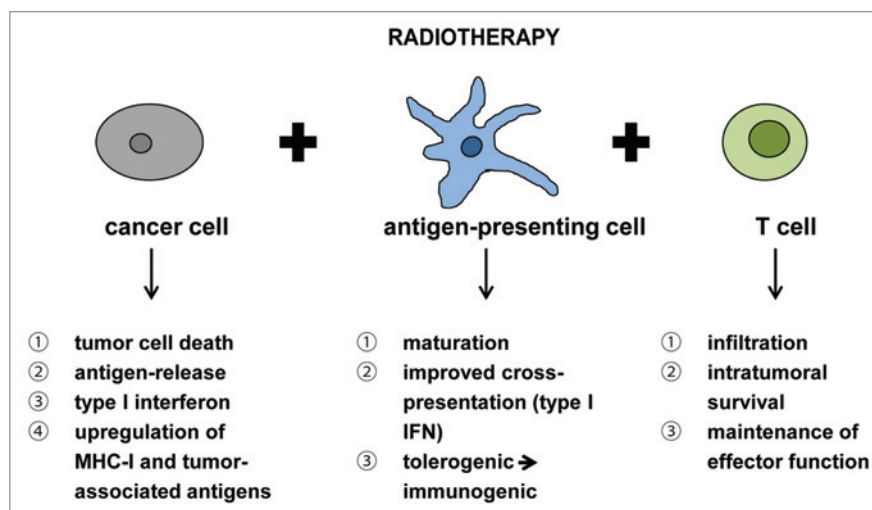


Figure 1. Radiotherapy supports tumor-specific immunity at multiple tumor-intrinsic and tumor-extrinsic levels. Radiotherapy (1) induces the death of tumor cells, resulting in the release of tumor-associated antigens and presumably in their cross-presentation by DCs, which is further promoted by radiation-induced Type I interferon; (2) induces the upregulation of MHC Class I molecules and CTAs, which makes cancer cells better targets for the immune system; (3) disturbs immune homeostasis in a way that results in DC maturation; and (4) supports the survival, proliferation and maintenance of effector functions of tumor-infiltrating T cells, resulting in a qualitatively and quantitatively improved T-cell infiltrate.

found a significant decrease of molecules involved in immunosuppression such as interleukin-10, transforming growth factor β and PD-L1 (Sharma A et al., manuscript in preparation).

In summary, we think that radiotherapy supports tumor-specific immunity at both tumor-intrinsic and tumor-extrinsic levels (Fig. 1). First, radiotherapy induces the death of tumor cells, resulting in the release of tumor-associated antigens and presumably in their cross-presentation by DCs, which is further promoted by radiation-induced Type I interferon. Second,

radiotherapy induces the upregulation of MHC Class I molecules and CTAs, which makes cancer cells better targets for the immune system. Third, radiotherapy disturbs immune homeostasis in a way that results in DC maturation. Fourth, radiotherapy supports the survival, proliferation and maintenance of effector functions of tumor-infiltrating T cells, resulting in a qualitatively and quantitatively improved T-cell infiltrate. We propose that combining radiotherapy with immunostimulation may result in synergistic anticancer effects.

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