OncoImmunology 1:9, 1629–1631; December 2012; © 2012 Landes Bioscience

Enhancing the antitumor effects of radiotherapy with combinations of immunostimulatory antibodies

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Keywords: breast cancer, CD137, CD40, immunotherapy, PD-1, radiotherapy, T cells

Abbreviations: APC, antigen-presenting cell; DC, dendritic cell; IL, interleukin; mAb, monoclonal antibody; MHC, Major histocompatibility complex; PD-(L)1, programmed death (ligand) 1

The development and use of combination immunotherapy-based anticancer regimens is at an early but clearly exciting stage. We now demonstrate that the antibody-based co-targeting of multiple immunostimulatory and/or inhibitory pathways can be used safely and effectively in combination with single dose or fractionated radiotherapy to cure mice bearing established mammary tumors.

Radiotherapy has long been known for its direct cytotoxic effects on tumor cells through the induction of DNA damage. However, over the last decade, significant interest has developed around the idea that first-line anticancer therapy can kill tumor cells in a manner that engages immune effector mechanisms that are capable of contributing to disease control. The immunological effects of radiotherapy on tumor cells includes an increased production of cytokines and peptides, encompassing radiation-specific peptides, as well as an enhanced expression of MHC Class I and adhesion molecules (reviewed in ref. 1). In addition, ionizing radiation has been reported to induce an immunogenic form of cell death, which is associated with exteriorization of calreticulin on the tumor cell surface and the release of the pro-inflammatory protein high mobility group box-1 (HMGB1). Altogether, these promote the recognition and engulfment of dying tumor cells by dendritic cells (DCs) and stimulate antigen processing and cross-presentation (reviewed in ref. 2).

To fully unmask the immunoadjuvant effects of radiotherapy, antibody-based combinatorial immunotherapy may constitute a promising approach. Immunomodulatory antibodies targeting co-stimulatory molecules such as CD137, CD40 or OX40 or immunosuppressive receptors like CTLA-4 have demonstrated that antibody-based immunotherapy can enhance antitumor immune responses elicited by radiotherapy and in some instances mediate promising abscopal effects, i.e., antitumor responses occurring outside the field of radiotherapy (see ref. 3; reviewed in refs. 1 and 4). However, the cure rates achieved with these combinations, particularly in the case of poorly immunogenic tumors, have been generally low. Ultimately, if we are to achieve clinically relevant anticancer immune responses in established disease settings, radio-immunotherapeutic strategies that combine the use of multiple (stimulatory and/or inhibitory) immunomodulatory agents will likely be a necessity to override the diversified mechanisms that are in place to promote tumor escape.

In a recent study,[4] we examined the therapeutic benefit of combining the pro-immunogenic effects of radiotherapy with (1) agonistic antibodies targeting the co-stimulatory molecules CD40 and CD137 to promote DC and T-cell function, respectively or (2) antibodies targeting CD137 and the immunosuppressive receptor program death (PD)-1, the blockade of which may re-engage effector cell activity within irradiated tumors and hence enhance their responsiveness to stimulatory signals. Moreover, the combined targeting of these stimulatory and inhibitory pathways may potentiate anticancer immunity evoked in response to radiation-induced cell death, similar to what was described for the co-administration of anti-CD137 and anti-PD-1 monoclonal antibodies in the context of chronic LCMV infection.[5]

These novel combinations of monoclonal antibodies were tested for therapeutic efficacy in two distinct mouse models of established mammary cancer. We demonstrated that the concomitant targeting of the co-stimulatory molecules CD137 and CD40 enhanced the antitumor effects of radiotherapy and promoted the rejection of established subcutaneous BALB/c-derived 4T1.2 tumors in more than 80%

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Submitted: 07/24/12; Accepted: 07/26/12
http://dx.doi.org/10.4161/onci.21652

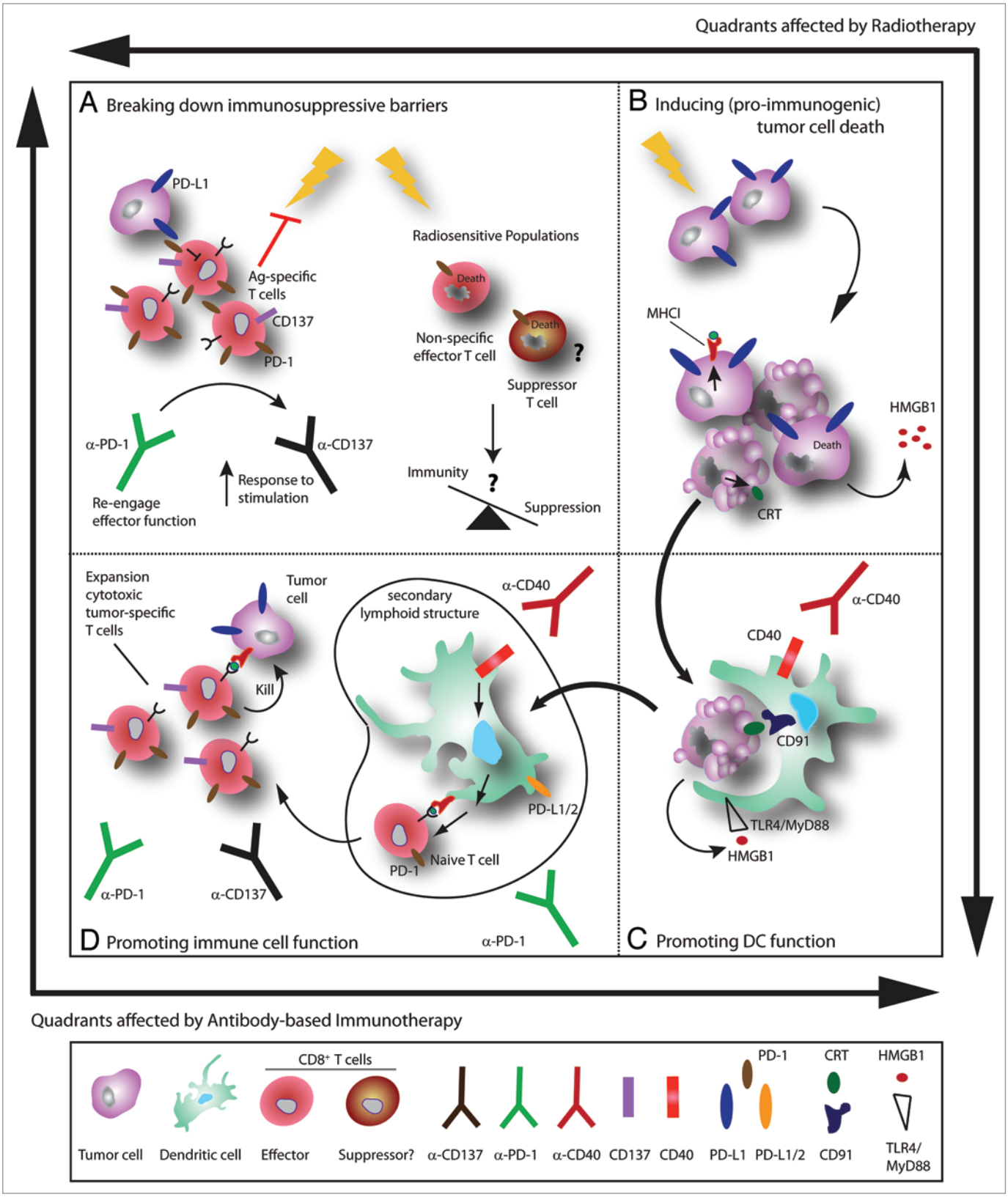

Figure 1. For figure legend, see page 1631.

Figure 1 (See opposite page). Schematic representation of the immunological elements that may contribute to the antitumor effects of radio-immunotherapy. (**A**) Removal of immunosuppressive barriers that can limit endogenous anticancer immune responses: (1) antibody-mediated blockade of PD-1 interaction with the inhibitory ligand PD-L1 on tumor cells re-engages PD-1^{high}CD137^{+} effector-cell activity and increases their responsiveness to anti-CD137 therapy, (2) radiation-induced elimination of tumor-associated suppressor T cells. (**B**) Radiotherapy-induced cell death has been linked to tumor cell expression of MHC Class I molecules and calreticulin (CRT) as well as to the release of HMGB1, two hallmarks of an immunogenic form of cytotoxicity. (**C**) Stimulation of dendritic cell (DC) function: (1) CRT expression can promote DC recognition and phagocytosis of dying tumor cells, (2) agonistic anti-CD40 monoclonal antibodies and (3) HMGB1 signaling through TLR4/MyD88 may promote DC maturation and tumor antigen cross-presentation. (**D**) Stimulation of immune-cell function: (1) agonistic anti-CD137 monoclonal antibodies promote effector-cell function and re-engage the activity of memory T cells, (2) antibody-mediated blockade of the inhibitory receptor PD-1 may help to prolong effector cell function.

of mice. Interestingly, in mice bearing established subcutaneous C57BL/6-derived AT-3 tumors, which, unlike the 4T1.2 tumors, do not contain a necrotic core, this combination was non-curative. Rejection of irradiated AT-3 tumors was only achieved when the anti-CD137 therapy was combined with an anti-PD-1 approach. Strikingly, up to 100% of mice bearing orthotopically implanted mammary tumors were cleared of tumor burden when antibodies targeting both PD-1 and CD137 were administered with single or low-dose fractionated radiotherapy.

Mechanistically, we identified that tumor-associated CD8^{+} T-cells were essential for curative radio-immunotherapy. In both the 4T1.2 and AT-3 experimental systems, tumor-resident CD8^{+} T cells expressed the immunotherapeutic targets CD137 and/or PD-1, a phenomenon that has recently been linked to hypoxia mediated by HIF-1α.[6] Interestingly in the AT-3 model, CD137 expression was restricted to a subset of PD-1^{high}CD8^{+} T cells, which included terminally differentiated, tumor-specific CD8^{+} T cells. Enrichment of this population was observed in response to radiotherapy, resulting from the temporary loss of PD-1^{low}CD137^{-}CD8^{+} T cells from the tumor microenvironment. We have postulated that this differential responsiveness of the PD-1^{high} and PD-1^{low} CD8^{+} T cell subsets to radiotherapy may be linked to the maturation status of the T cells of each population. Indeed, the acquisition of radio-resistance by T cells upon the encounter of tumor antigens in vivo has been reported.[7] Alternatively, PD-1^{low}CD8^{+} T cells may include a population of suppressor T cells, which are characteristically more radiosensitive than other T cells.[8] If the latter were true, this would suggest that radiotherapy might also have the capacity to shift the suppressive nature of the tumor microenvironment in favor of immunity, which may have contributed to the profound therapeutic efficacy of anti-CD137/ anti-PD-1 therapy in irradiated AT-3 tumors.

In light of our findings, as an extension to the three-pronged approach to cancer therapy,[9,10] we now propose a four-pronged regimen in the context of radio-immunotherapy (**Fig. 1**), which supports the idea that (1) the blockade of immunosuppressive barriers within tumors through the combined actions of radiotherapy and immunotherapy is an important first step in increasing the permissiveness of established tumors to the effects of radio-immunotherapy, (2) radiation-induced cell death may possess vaccine-like properties, which along with anti-CD40 therapy can promote DC function and (3) drive the expansion of tumor-specific immune responses with the support of immunotherapy. Notably, we are yet to validate to what extent radiation-induced changes in tumor cell immunogenicity contribute to the therapeutic outcome of each of the radio-immunotherapy combinations tested in our preclinical models. Ultimately, if we are to fully harness the therapeutic power of radiotherapy in synergistic treatment regimens, it will be important to learn more about the immunological consequences of radiation-induced cell death, particularly in the setting of neo-oncogenesis.

It is our hope that establishing robust anticancer immune responses within irradiated tumors through the concomitant targeting of multiple immune regulatory mechanisms will increase the incidence of curative therapeutic responses and in turn decrease the morbidity associated with the metastatic spread of cancer.

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