Fractionated but not single dose radiation releases key signals of in situ tumor vaccination

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The balance between pro-inflammatory and immunosuppressive signals in the tumor microenvironment dictates the responsiveness of the immune system. Local radiotherapy (RT) has the potential to switch this balance in favor of anti-tumor immunity by promoting cross-priming of anti-tumor T cells thus generating an individualized in situ vaccine. We have previously shown that the dose and fractionation employed modulate RT ability to synergize with immunotherapy. Indeed, in two tumor models, generation of an in situ vaccine synergistic with anti-CTLA-4 treatment was achieved by irradiation of the tumor with 3 fractions of 8 Gy (8Gy x 3) but not by a single 20 Gy dose (20Gy x 1) (Dewan et al., Clin Cancer Res 2009).

To understand the mechanisms underlying the different outcome obtained with fractionated (3x8Gy) versus single dose (20Gy x 1) RT, TSA tumors growing in syngeneic immunocompetent BALB/c mice were harvested at 4, 24 and 48 hrs post-RT for analysis of purified RNA by microarray for gene expression or infiltrating immune cells by flow cytometry. Expression of key immune genes in TSA cells irradiated in vitro was assessed by qPCR.

Over 100 immune response genes were differentially expressed in irradiated tumors by 8Gy x 3 but not 20Gy x 1, with a dominant type I interferon (IFN) response at 4 and 24 hours, which was confirmed by qRT-PCR. CD8a+ dendritic cells (DC), which are the subset of DC cross-presenting tumor antigens, showed a significant upregulation of activation markers CD86, CD40 and CD70 at 48 hours following 8Gy x 3 but not 20 Gy x 1. Importantly, the in vitro setting (devoid of an immune infiltrate) demonstrated expression of IFNβ and downstream immune genes, including chemokines CXCL9, CXCL10 and CXCL11 by TSA cells irradiated with 8Gy x 3 but not 20Gy x 1.

Data indicate that fractionated RT can mimic, at least in part, a viral infection and activate canonical defense pathways in neoplastic epithelial cells with induction of type-I IFN. In vivo this leads to activation of DC cross-presenting tumor antigens, suggesting that the quality of fractionated-RT generates the key “ingredients” of an in situ tumor vaccine. Further studies to identify the molecular mechanisms of RT-induced tumor vaccination and their modulation by different RT regimens are critical to the rational design of clinical trials testing RT combinations with immunotherapy.

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