TREX1 dictates the immune fate of irradiated cancer cells

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Abstrekt

The optimal radiation dose and fractionation to induce anti-tumor immunity remain elusive. We recently found that the exonuclease TREX1 abrogates the immunogenicity of irradiated cancer cells by degrading interferon-stimulatory cytosolic dsDNA. TREX1 upregulation by radiation dose per fraction beyond a threshold of 10–12 Gy results in poor synergy with immune checkpoint blockers.

Radiation therapy (RT) has been recognized to convey immunogenicity to an irradiated neoplastic lesion by inducing pro-inflammatory signals amenable to the immune system recognition. The ability of RT to jumpstart systemic immune responses provides a putative explanation for the rare, but well established clinical observation that occasional patients with metastatic disease experienced responses of metastasis outside the irradiated field (abscopal effect). How radiation should be administered in terms of dose and fractionation to facilitate such abscopal responses, particularly when combined with modern immunotherapy, is currently being debated.

In our recent publication in Nature Communications, we performed side-by-side comparisons of different radiation doses and fractionation in mice bearing bilateral TSA murine breast carcinoma to evaluate the mechanisms by which tumor-targeted RT synergizes with anti-CTLA-4 or anti-PD-1 response. We found that in terms of in field control of the irradiated tumor a single high dose of 30 Gy was comparable to radiation administered in 3 fractions of 8 Gy given in consecutive days (8 Gy X 3); however, only the fractionated regimen (8Gy X 3) but not a single 30 Gy dose induced abscopal responses when combined with anti-CTLA-4. Gene expression analysis of the irradiated tumors revealed that 8Gy X 3 induced the interferon type I (IFN-I) genes signature while single high dose RT failed to do so. To investigate the mechanisms underlying these differences, we then performed a series of in vitro and in vivo experiments, which revealed that RT activated the production of IFNb and expression of IFN-stimulated genes (ISGs) in the cancer cells, which was mediated by cGMP-AMP synthase (cGAS aka Mab-21 domain containing 1 – MB21D1) and its adaptor protein named stimulator of interferon genes (STING aka transmembrane protein 173 – TMEM173). Findings were confirmed in murine TSA, 4T1 and MCA-38 and human MDA-MB-231 carcinoma cells. Conditional silencing of cGAS or STING in the cancer cells at the irradiated tumor site demonstrated that cancer-cell cGAS/STING pathway activation was essential for the RT-driven initiation of systemic anti-tumor immunity via the recruitment of BATF3-dependent dendritic cells (DCs) to the tumor, a DC subset well-known to be critical for cross-priming of antitumor CD8+ T cells.

Because cGAS is a nucleotidyltransferase that selectively binds cytosolic double stranded (ds) DNA to initiate IFN-I responses upon STING stimulation, we investigated dsDNA content in the cytosol of cells subjected to radiation doses ranging from 0 Gy to 30 Gy. Strikingly, as the radiation dose increased, more cytosolic dsDNA accumulated to a critical threshold when it abruptly decreased, generally with doses that varied between 12 to 18 Gy in different carcinoma murine and human cells.

In a series of distinct experiments we then demonstrated that the main reason as to why doses above this threshold did not confer immunogenicity was the dose-dependent upregulation of the 3 prime repair exonuclease 1 (TREX1). TREX1 is a DNA nuclease whose main role is to degrade cytoplasmic ds- and single stranded (ss) DNA. We found in TSA cells that at RT single doses above 12 Gy cytosolic dsDNA was cleared by TREX1, precluding the activation of cGAS pathway to induce IFN-I, therefore abolishing RT-induced abscopal effect. During immune checkpoint blockade with either anti-PD1 or anti CTLA-4 enforced TREX1 expression in TSA cells completely abrogated the immunogenicity of a regimen of 8 Gy X 3 whereas knockdown of TREX1 restored the immunogenicity of a single dose of 20 Gy, hence confirming the critical role of TREX1 as a regulator of RT immunogenicity. Interestingly, to achieve robust abscopal effects repeated doses were
important, as demonstrated in vitro by the marked upregulation of IFNβ production by carcinoma cells treated with 8 Gy X 3 versus 8 Gy single dose, and in vivo by the requirement for repeating the dose 2 to 3 times, even when 20 Gy was used in a setting of TSA cells with TREX1 knockdown.

While the mechanisms leading to TREX1 upregulation upon RT treatment are currently under investigation, our results clearly identify TREX1 as an upstream regulator of radiation-induced anti-tumor immunity, and provide a candidate biomarker to guide the selection of the radiation dose and fractionation in the clinic (Fig. 1). Importantly, in the mouse and human carcinoma cells tested, the radiation dose threshold for TREX1 upregulation sufficient to clear cytoplasmic dsDNA was consistently above 10 Gy, while exhibiting some variation, with some cell lines preserving their immunogenicity up to doses of 15 Gy in a single fraction. The relationship between intrinsic radiosensitivity of the cancer cells and TREX1 induction remains to be determined. Likewise, it is unknown if in vivo, the presence of tumor hypoxia may shift the threshold for TREX1 induction.

Interestingly, DNA derived from cancer cells has been shown to stimulate IFNβ production via the cGAS/STING pathway not only in cancer cells but also in DC infiltrating the tumor, by gaining access to their cytoplasm via undefined mechanisms. It is also possible that TREX1 upregulation may also reduce the amount of DNA taken up by DCs, further decreasing the activation of anti-tumor CD8+ T cells.

Despite the many open questions remaining, including the mechanisms that control dsDNA accumulation in the cytoplasm of irradiated cells, the new mechanisms identified advance the current understanding of dose-dependency of radiation immunogenicity, and provides information critical to the design of clinical trials testing the combination of radiation with immunotherapy.

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