RESEARCH HIGHLIGHT

Cancer-host battles: measures and countermeasures in radiation-induced caspase activation and tumor immunogenicity

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Radiotherapy exerts its effects on tumor cells directly by damaging their DNA and consequently activating a programmed cell death process (apoptosis) and indirectly by stimulating an immune response towards tumor antigens. Tumor cells are known to activate several mechanisms to preserve their survival and limit the host response, including resistance to apoptosis and immune evasion. Recently, Han et al.[1] published that to limit the induction of the host immune response, tumor cells can exploit the intrinsic apoptosis program rather than block it, an apparent paradox. When exposed to the caspase inhibitor emricasan, which blocks this immune-evasion mechanism, tumor cells "adopt" countermeasures by upregulating the expression of PD-L1, which negatively controls immune responses. To eliminate this additional tumor adaptive reaction and protect the animals from tumor growth, the authors used a combination of radiation, caspase inhibition, and immune checkpoint inhibitor (anti-PD-L1 antibody) treatment.

The form of tumor cell death (apoptosis vs. necrosis and their variants) induced by radiation depends on several factors, including the quality of radiation (type, dose, fractions, dose rate) and tumor features (type, metabolic status, microenvironment). How cell death is perceived by (innate) immune cells is conditioned by the presence (or absence) of "danger signals" associated with the release of DAMPs (damage-associated molecular patterns), including fragmented DNA, by exposed tumor cells, which can result in immunogenic cell death. Damaged DNA is also expected to be detected within tumor cells and induce the production of type I interferon (IFN-I). IFN-I plays a preeminent role in radiotherapy-induced as well as in chemotherapy-induced systemic antitumor responses and the generation of immunological memory.[2] Notably, the action of apoptotic caspases can accelerate DNA fragmentation, prevent the production of IFN-I, and favor the "silent" removal of dying cells.[3] At variance, the release of tumor antigens in an immunogenic context allows innate immune cells to react, and thus to promote tumor-antigen-specific responses by adaptive immunity. The radiation-induced immune response is based on the synergistic effects of radiotherapy and immunotherapy through immune checkpoint inhibitors (anti-CTLA-4 and anti-PD-1/PD-L1 antibodies).[2]

Han et al. found that emricasan, a pancaspase inhibitor used to reduce fibrosis and cell death in chronic liver diseases, increased IFN-I production in mouse colon cancer cells (MC38 cell line) in vitro. Emricasan further increased IFN-I expression when tumor cells were irradiated, a finding also observed using another caspase inhibitor (Q-VD-oph). To understand how inhibition of caspases could license IFN-I production in tumor cells, Han et al. explored the extrinsic and intrinsic apoptosis pathways by exposing caspase 8 ($Casp8^{-/-}$)- and caspase 9 ($Casp9^{-/-}$)-de®-cient tumor cells to ionizing radiation. Casp8 represents a key factor for the induction of apoptosis through the extrinsic pathway, as, for instance, it is activated upon stimulation of death receptors such as CD95. Casp9, together with APAF1, is a key component of the apoptosome complex of the mitochondrion-triggered intrinsic pathway. Both pathways result in downstream activation of the effector caspase-3, which is known to restrict IFN-I production, among other effects.[4]

Exposure to ionizing radiation did not induce IFN-I production in $Casp8^{-/-}$ tumor cells or in wild-type (WT) controls. In contrast, exposure of tumor cells deficient in either Casp9 or APAF1 resulted in a lack of Casp3 activation and high IFN-I expression, as was also observed with emricasan. The results were confirmed in another tumor cell line using different radiation doses. The role of the intrinsic apoptosis pathway was further verified at the molecular level by showing that mitochondrial DNA depletion abolished IFN-I production after irradiation of WT emricasan-treated or $Casp9^{-/-}$ tumor cells.

Han et al. verified whether impairment of the Casp9-dependent intrinsic apoptosis pathway could result in a better therapeutic effect in vivo. By inoculating $Casp9^{-/-}$ or WT tumor cells in recipient mice and treating them with radiation, he found that most $Casp9^{-/-}$ tumors regressed completely after exposure, whereas WT tumors did not. Notably, in the absence of radiation, all $Casp9^{-/-}$ tumor-bearing mice died in one month, showing that synergy between impairment of intrinsic apoptosis and radiotherapy is required to improve survival. Remarkably, cured mice did not relapse and became resistant to subsequent tumor challenges, suggesting the involvement of protective immune responses. By using immune-deficient recipient mice ($Rag1^{-/-}$) or depleting specific cell populations, Han et al. showed that radiation-induced tumor regression was dependent on the presence of $CD8^{+}$ cells. Secretion of IFN-I in the tumor microenvironment is required for the induction of antigen-specific T cell-mediated effects of radiotherapy and chemotherapy. IFN-I "licenses" dendritic cells (DCs) to cross-prime T cells, that is, DCs are able to present exogenous tumor-derived antigens to CD8 T cells in association with MHC class I molecules.[5] Using an in vitro culture system, Han et al. showed that IFN-I released by irradiated $Casp9^{-/-}$ tumor cells induced IFN-γ production in CD8

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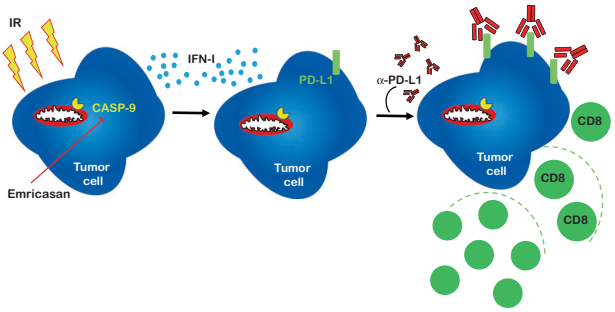

Fig. 1 Ionizing radiation (IR) induces genomic and mitochondrial DNA damage and activates the apoptosis program in tumor cells. In the presence of a caspase inhibitor (emricasan), the activity of caspase 9 (Casp9), a key component of the mitochondrion-triggered intrinsic pathway, is suppressed, and tumor cells can sense fragmented DNA. Tumor cells are induced to produce type I interferon (IFN-I), which activates innate and adaptive immune cells. Tumor cells also react to IFN-I by upregulating PD-L1, a ligand for the immune checkpoint PD-1 that negatively controls immune responses and limits the action of tumor-antigen-specific cytotoxic CD8 T cells. Systemic administration of an anti-PD-L1 antibody, intratumor administration of emricasan and local radiotherapy synergize to induce an effective systemic antitumor response able to control primary and secondary tumor growth through a CD8 T-cell-dependent abscopal effect.

cells stimulated by DCs. Altogether, these results demonstrated that when the intrinsic caspase pathway is compromised, radiotherapy can induce a robust antitumor memory immune response by sustaining T-cell priming through the induction of IFN-I.

However, even if irradiated Casp9$^{-/-}$ primary tumors were regressing, animals were not able to control the growth of secondary distal tumors, suggesting the possibility that tumor adaptive resistance could restrain systemic immunity. Indeed, tumors often react to radiotherapy with "countermeasures" that favor immune evasion, such as upregulation of immune checkpoint ligands.[6] Han et al. found that impairment of Casp9 sustained the expression of PD-L1, a ligand for the PD-1 inhibitory receptor (immune checkpoint), and that radiation further increased its expression. PD-L1 upregulation was sustained by radiation/Casp9 deficiency-dependent IFN-I production. Thus, freeing IFN-I production by Casp9 to promote the immune response also resulted in the activation of a negative regulatory pathway that can limit antitumor immunity. This is part of the (required) physiological mechanisms that regulate the amplitude of immune responses, but under therapeutic settings, it represents a bottleneck. To overcome the inhibitory effects of the PD-L1/PD-1 axis on the systemic response, Han et al. added an anti-PD-L1 antibody to the therapeutic scheme. Applying different treatment combinations, the authors found that Casp9 deficiency in primary tumors, which unleashes IFN-I production and sustains priming of an immune response, facilitated an effective response to the combination of radiation and anti-PD-L1 antibody also in distal tumors. In a subsequent set of experiments, they showed that intratumor administration of emricasan associated with local radiotherapy and systemic anti-PD-L1 antibody generated the best therapeutic effects on primary and secondary tumors (Fig. 1). These findings were confirmed using another mouse tumor cell line (TS/A) and a humanized mouse model inoculated with a human lung cancer cell line (A549).

Overall, Han et al. demonstrated that inhibition of intrinsic apoptosis, radiotherapy, and immune checkpoint blockade synergize to induce an effective systemic antitumor response. The use of caspase inhibitors in cancer radiotherapy might appear to be a paradox. Indeed, cell death evasion is considered a major mechanism in resistance to radiotherapy and/or to the action of cytotoxic T cells, while caspase activation can radiosensitize tumor cells. However, radiation is also known to activate signals that can contribute to cell survival, a delicate equilibrium to be considered in combination therapies. The dose and timing of therapeutic treatments and tumor microenvironment factors, including oxygen availability, contribute to the fate of exposed/treated tumor cells. Delayed apoptosis and/or a redirected type of cell death can contribute to the immunogenicity of cancer cells and therefore create the basis for more effective combinations with immunotherapies. Further research is desirable to better address the mechanisms involved in these complex interactions.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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