Commentary

STING-cytosolic DNA sensing: the backbone for an effective tumor radiation therapy

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Local irradiation has been broadly used in the treatment of primary and metastatic tumors. It is commonly believed that treatment effect of radiation is based on a direct killing of cancer cells. However, recent studies have gradually found that tumor regression following ablative irradiation mainly depends on type I interferon signaling and $CD8^+$ T cell response. In this issue of Immunity, Deng and colleagues (1) found that anti-tumor effects of radiation are contributed by both innate and adaptive immune responses. They present compelling evidence that cytosolic DNA sensing pathways bridge the known irradiation-mediated DNA damage to anti-tumor immune response.

Anti-tumor effect of radiotherapy (RT) relies on host immunity

RT has been widely used for various tumor therapy alone or in combination with surgery or chemotherapy. RT such as ionizing radiation can either damage DNA directly or indirectly through creating charged particles (free radicals) within the cells. Besides direct tumoricidal effect, radiation could also lead to transient depletion and rebound effects of resident leukocytes, which impact the ultimate therapy results.

Understanding about the mechanism of radiation-mediated tumor regression has a breakthrough recently. Lee *et al*. revealed that ablative RT increases T-cell priming in draining lymphoid tissues and reduce the growth of the primary tumor or distant metastasis (2). $CD8^+$ T cells utilize T-cell receptors to recognize tumor derived antigens which bind to MHCI, and mount cytolytic attack to tumor cells. Radiation could increase the peptide repertoire and MHC class I expression on tumor cells, and boosts the efficacy of adoptive CTL immunotherapy *in vivo* (3). These studies propose the essential role of $CD8^+$ T cell response in RT.

Type I IFN optimizes anti-tumor adaptive immunity after radiation

Type I IFNs, comprising IFNα and IFNβ proteins, are known for their unique role in inhibiting viral infection through ISG genes and critical mediators bridging innate response to adaptive immune response. Type I IFN can promote the activation and cross-presentation of DC, which is crucial to initiate the adaptive immunity. IFN-α/β directly promotes the activation, expansion and differentiation of T cell. Besides, type I IFN increases NK cell cytotoxicity by modulating the surface expression of activating and inhibitory receptors. Recently, the critical role of type I IFN in tumor immunity is gradually understood. Type I IFN can directly induce the apoptosis of tumor cell and inhibit the proliferation. Through promoting the antigen expression in neoplastic cells, type I IFN also increases the immunogenicity of tumor. Administration of exogenous IFNα have been used to treat tumors such as acute myeloid leukemia (4).

Moreover, Burnette *et al*. found that the anti-tumor efficacy of radiation depends on both the generation of host adaptive immune response and innate type I IFN response (5). However, the molecular mechanisms of type I IFN induction and details of type I IFN bridging radiation-mediated tumor damage to immune response are still unclear.

cGAS-STING sensing dying tumor DNA mediates type I IFN induction after RT

Emerging data suggest that the efficacy of various tumor therapy modalities including Ab, RT and chemotherapy depends on generation of adaptive immune response. One of critical questions is what danger signalings are triggered

by these therapy modalities and how they are recognized to initiate the adaptive response. The sensing of infection and injury can be mediated by pattern-recognition receptors (PRRs), which recognize pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) (6-8). TLRs, as one kind of important PRRs, were reported to sense HMGB1 induced by anti-her2 or anthracycline chemotherapy, and were essential for optimal tumor control (9,10). Surprisingly, Deng *et al*. found that TLR sensing is dispensable for the RT, because the deficiency of MyD88 or TRIF as TLR adaptors did not impair the tumor control. Furthermore, the fact that tumor regression could be induced by radiation after blocking of HMGB-1 indicates existing of other critical danger signaling sensing. DNA breaks generated after irradiation could be the main danger signals. Recently, STING-mediated cytosolic DNA sensing cascade was demonstrated to be the major mechanism of type I IFN induction after viral infection, which is TLR independent. Using *Tmem173*$^{-/-}$ mice (STING-deficient mice), Deng *et al*. first found that absence of STING impaired radiation-mediated tumor regression.

Radiation treatment could induce elevated type I IFN expression in tumor microenvironment. However, radiation induced type I IFN upregulation was impaired if STING is deficient in the host but not tumor cells *per se*. Further qPCR experiments showed that CD11c^{+} DC cells were the main producer of type I IFN after radiation, which was impaired most significantly by STING deficiency compared with other cell populations in tumor. DCs bridge innate immunity and adaptive immunity due to its cross-priming ability. Deng *et al*. further explored that STING mediated type I IFN production in DC was essential to cross-prime CD8^{+} T cells. STING-mediated type I IFN production needs the engagement with TBK1 to direct IRF3 activation. IRF3-deficient DC also showed impaired cross-priming ability. Exogenous type I IFN treatment could rescue the cross-priming ability of STING deficient DC.

These results suggest that STING signaling-dependent DNA sensing is essential to trigger the adaptive immune response after radiation. It raises the question that what is the sensor of irradiation induced damaged DNA. The previous identified intracellular DNA sensors include IFN16, DAI, AIM2, DDx41 and RNA polymerase III. However, these sensors function differently and specifically depend on the cell type, expression level or DNA source. Until recently, Cai *et al*. identified cyclic-GMP-AMP (cGAMP) synthase (cGAS) as a universal cytosolic DNA sensor for STING activation (11). In this research, Deng *et al*. found that cGAS knockout or silenced by siRNA impaired the cross-priming ability of DC similar as STING deficiency. cGAS deficient DC produce significantly reduced type I IFN compared to WT DC cultured with irradiated tumor cells. This suggested that the cGAS-STING-IFN signaling is required for DC's cross-priming of irradiated tumor.

Potential questions and translational perspectives

One interesting question is how DNA from irradiated tumor cells transport into DC to trigger cytosolic DNA sensing pathway. Deng *et al*. found that DNA delivery in a cell contact-dependent manner but not in the free soluble form is essential to the cross-priming activity of DC. Burnette *et al*. have demonstrated that radiation induced DC infiltration into tumor, which was activated by type 1 IFN to enhance cross-priming capability (5). It raises one interesting scenario: irradiation induces tumor apoptosis and DC recruitment. DC may discriminate and approach apoptotic tumor cell to initiate phagocytosis for exogenous DNA harvest. Recently, Vacchelli *et al*. found that chemotherapy induced dying cells to release ANXA1, which promote stable contact of DCs with tumor cells through FPR1 for antigen capturing and processing (12). Whether a similar mechanism exists in the RT model needs to be explored further. Another interesting question is whether DCs activate local CD8^{+} T cells for tumor control or need to migrate into draining LN to prime *de novo* T cell. Does the local pre-activated CD8^{+} T cells in tumor switch to a tolerant status? If so, it will be a more efficient tumor therapy strategy to combine irradiation with anti-immune checkpoint antibody.

The studies by Deng *et al*. suggest that activating of STING signaling in DC to promote cross-priming could be one efficient strategy for tumor therapy. The 2'3'-cGAMP is synthesized by cGAS recognizing cytosolic double-stranded DNA as a secondary messenger to trigger STING signaling. Deng *et al*. found that exogenous 2'3'-cGAMP treatment could significantly improve the tumor therapeutic effect of radiation, with about 70% of mice completely rejected the tumors. Recently, Corrales *et al*. generated synthetic cyclic dinucleotides which could activate both murine and human STING, and showed impressive therapeutic effect in diverse tumor models through intratumoral injection (13). In Deng's research, cGAMP treatment alone has no therapeutic effect. Improving the ability of targeting and entering tumor will be promising for cyclic dinucleotides-

STING based tumor therapy.

It must be pointed out that, one of recent researches showed that anthracycline chemotherapeutic drug induces production of type I IFN in tumor cells through TLR3-mediated RNA sensing pathway, which promotes tumor cells to release CXCL10 to attract $CD8^+$ T cells and further tumor eradiation (14). Thus, in some instances, the effect of type I IFN response in tumor cells could not be ignored. It should be taken into consideration of targeting type I IFN signaling in tumor cells to attract $CD8^+$ T cell for DC priming to optimize tumor control.

In this study, Deng *et al.* demonstrate a novel mechanism that the cytosolic DNA sensing for type I IFN is induced via radiation treatment for tumor control. In another study, Deng *et al*. did a combination of radiation with immune checkpoint inhibitor, anti-PD-L1, which synergistically generated durable anti-tumor responses in the mouse models (15). So far, the detailed mechanisms of this combination is still not clear. To translate these discoveries into practice, it will be necessary to further determine the toxicity and synergy of radiation with nucleic acid-sensing agonists, as well as to develop effective immunotherapies that further improve RT.

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Footnote

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