Published in final edited form as:

Radiat Res. 2018 March ; 189(3): 219–224. doi:10.1667/RR14941.1.

Cross Talk between Radiation and Immunotherapy: The Twain Shall Meet

Swaminathan P. Iyer^a, Clayton R. Hunt^b, and Tej K. Pandita^{b,1}

^aDepartment of Hematology, Houston Methodist Cancer Center, Weil Cornell Medical College, Houston Texas 77030

^bDepartment of Radiation Oncology, The Houston Methodist Research Institute, Weil Cornell Medical College, Houston Texas 77030

Abstract

There has been increased interest in the immune stimulatory properties of ionizing radiation based on several preclinical models and recently completed clinical studies performed in combination with checkpoint inhibitors. This is a paradigm shift in that it considers the role of radiation beyond its direct cytotoxic effects, however, the factors that promote or limit radiation-induced immunogenicity are still unclear. Here we review the role of radiation in modulating the various aspects of the tumor immune microenvironment and discuss in particular the direct effects of radiation on the DNA damage response and its immediate consequences to neighboring cells. The latter “danger response” in particular can enhance recruitment of dendritic and macrophage cells to the tumor microenvironment, which in turn can activate or diminish subsequent T-cell priming. Identification of the critical factors that modulate the interaction between radiation-induced cell damage and the immune system will allow for rational combinational therapy design and the development of biomarkers that predict effective immune responses.

INTRODUCTION

In the last few years, the conceptual framework for radiotherapy has expanded. For the greater part of the previous century, the main focus of scientific research was on the cytotoxic effects, with the goal of curing, or providing chemo-sensitization or palliation of symptoms. There is increasing recognition of the benefits of radiotherapy for its ability to alter the components of the microenvironment that can potentially overcome immune tolerance. In keeping with the rapidly evolving developments in targeted tumor immunotherapy, we need to consider the immunotherapeutic potential of radiation based on its ability to induce local and abscopal immune responses to contain tumor growth. The abscopal effect is a rare phenomenon first described by Mole in 1953 as “...local irradiation of one tissue involved in a response in another or similar tissue remote from the irradiated site (1, 2).” Since then, there has been tremendous interest in and attention toward

¹Address for correspondence: Houston Methodist Research Institute, 6550 Fannin St., Houston TX 77030; spiyer@houstonmethodist.org or tpandita@houstonmethodist.org.

understanding the mechanism of this phenomenon, even though only a few case reports of its effects have been well documented (3).

IONIZING RADIATION CAN ACTIVATE THE IMMUNE SYSTEM THROUGH DNA DAMAGE AND INTERFERON PATHWAYS

Interferons (IFNs) play a critical role in skewing the effect of ionizing radiation to augment the anti-tumor response. Type I IFNs (IFN- α and IFN- β which use a common IFN- α/β receptor), are well known for antiviral responses and critical mediators that bridge innate responses to adaptive immunity. While endogenous I interferons (IFN) have been shown to play a role in immune-editing in a tumor model, they are also important factors that promote dendritic cell cross priming of CD8 cells and generation of the anticancer cytotoxic T lymphocyte (CTL) response (4). Moreover, while radiation increases IFN- β , its effects are diminished in an IFN- α/β receptor knockout murine model (4). Stimulator of interferon genes (STING), an endoplasmic reticulum adaptor, was first shown to be critical for the induction of type I interferon by non-CpG intracellular DNA species produced by various infectious DNA pathogens such as the DNA virus herpes simplex virus 1 (HSV-1) or bacteria *Listeria monocytogenes* (5). DNA is sensed in the cytosol by cGMP-AMP (cGAMP) synthase cGAS, which binds to double-stranded DNA irrespective of the sequence (6). The activated cGAS catalyzes the conversion of GTP and ATP into 2'3'-cGAMP, which acts as a second messenger to bind and activate STING. Furthermore, STING, in turn, activates the protein kinases IKK and TBK1, leading to further activation of the transcription factors NF- κ B and IRF3, respectively, inducing transcription of a series of immune and inflammatory gene products, including type I IFNs (7).

The DNA structure-specific endonuclease MUS81, which cleaves DNA structures at stalled replication forks, can mediate the STING-dependent activation of innate immune signaling. This cleavage of genomic DNA along with PARP-dependent DNA repair pathways leads to the accumulation of cytosolic DNA in prostate cancer cells (8). Also, defects in the DNA repair kinase ATM restrain spontaneous type I IFNs in humans and mice. ATM-deficient mice accumulate cytoplasmic DNA upon DNA damage. This loss of ATM primes the type I IFN System via STING pathway and leads to robust antimicrobial host responses (9). During double-strand break (DSB) repair, RAD51 catalyzes the core reactions of homologous recombination, including strand breakage in the duplex DNA and eventually the pairing of homologous DNA strands, enabling strand exchange. Defects in RAD51 result in accumulation of cytosolic DNA, which in turn stimulates the STING pathway (10). A recently published study also demonstrated that in irradiated cells that have undergone a subsequent cell division, there is increased accumulation of DNA containing micronuclei (11). These micronuclei are efficient inducers of STING and induce the cell to secrete paracrine factors that can activate STING. In mice, transplantation of irradiated tumor cells followed by CTLA4 treatment produced an abscopal effect on pre-existing tumors with the improved control being dependent on STING and CTLA4 treatment (11). It has been previously reported that, in a mouse model, the efficacy of anti-CTLA4 treatment in controlling metastatic cancer was influenced by the mode of radiation delivery, in that fractionated doses were more effective than an equivalent single dose (12). This may be

related to a more extended production of antigens to immune activation. Taken together, all these results illustrate that a key antitumor effect of radiation is mediated by IFN pathways and indicate the potential for STING-targeting to enhance radiation-induced adaptive anticancer immunity.

ANTIGEN PRESENTATION

Generation of antigenic peptides displayed on major histocompatibility (MHC) class I molecules is the cumulative result of different proteolytic processes along with chaperone complexes called the antigen processing machinery (APM). The concept of immunogenic modulation is dependent on antigen presentation by the surviving cells leading to the activation of CTLs. The majority of intracellular protein degradation is processed by the ubiquitin-proteasome pathway, and generation of antigenic peptides by proteasomes seems to be a stochastic process that may generate or destroy epitopes by cleavage within epitopes (13, 14). Exposure to IFN- α/β and/or IFN- γ changes the classical proteasome configuration to an immunoproteasome (15). Proteasome inhibitors have also been shown to block most of this antigen presentation of peptides by MHC class I (16). Studies have also shown that the proteasomes usually generate longer precursor peptides that need further processing to get to the correct size required for binding to MHC class I molecules (17–19). Whereas proteasomes perform the C-terminus trimming from the longer precursors, there is evidence that these peptides escape from the proteasome into the endoplasmic reticulum ER with the aid of transporter associated with antigen processing (TAP) proteins. Some of TAPs mature, and longer peptides go to subcellular compartments of the endoplasmic reticulum (ER) for N-terminal trimming by an aminopeptidase called ER aminopeptidase (ERAP1) (20). Immunoproteasomes are found constitutively in professional antigen-presenting cells (APCs), which are dendritic cells (18, 21, 22), and in most cell types after exposure to IFN- γ . The effect of increasing the peptide pool postirradiation can: 1. enhance degradation of the existing proteins; and 2. generate new antigens that can be presented through the APM for T-cell recognition. This was demonstrated by directed irradiation that could eradicate murine adenocarcinoma (23). How and when radiation consistently modulates the APMs is not clear and requires further attention, given its therapeutic implications.

ROLE OF INFLAMMASOME

Ionizing radiation exposure induces cell and tissue damage causing local and systemic inflammatory responses. However, it is evident now that the type of cell death that the cells undergo has clear implications not only for the dying cells but also the immediate microenvironment. Immunogenic cell death (ICD) is strictly defined by a cascade of developments in a spatiotemporal manner either from chemotherapy or radiation that leads to facilitation of the antigen presentation through intermediary markers that represent endoplasmic reticulum stress, and plasma membrane penetration that can elicit immune responses. ICD triggers what is known as the inflammasome pathway and danger-associated molecular patterns (DAMPs) (24). Inflammasomes are molecular pathways activated upon cellular infection or stress that trigger the maturation of pro-inflammatory cytokines such as interleukin-1 beta to engage innate immune defenses. This was described in a mouse model wherein exposure induces a dose-dependent increase in inflammasome activation in cells

like macrophages, dendritic, NK, T and B cells as judged by cleaved caspase 1 detection (25).

Sub lethal radiation exposure of several breast, lung and prostate cell lines induced secretion of some of these DAMPs, like HMGB1 and calreticulin (discussed below), along with upregulation of the APM (26). A nuclear chromatin-binding factor, HMG1 promotes protein assembly on specific DNA targets. It is also secreted outside the cell and is a potent mediator of inflammation (27). In fact, in both murine and human studies, it is the interaction of HMGB1 with Toll-like receptor 4 (TLR4) expressed by dendritic cells along with its adaptor MyD88 that is required for efficient processing and cross presentation of antigen from dying tumor cells (28). When in a complex with CpG-containing DNA, HMGB1 can also induce the synergistic interaction and activation of the receptor for advanced glycosylation end products (RAGE) and Toll-like receptor 9 (TLR9) through Myd88 pathways and cause immunity (29). Thus, how cells die after irradiation and chemotherapy determines the subsequent role of the immune system in effectively removing them.

THE ROLE OF MACROPHAGES

Macrophages are crucial for clearing apoptotic cells generated in injured tissue and for host defense against infection and cancer. Indeed, there are mechanisms allowing phagocytes to recognize apoptotic cells as “unwanted self” and often they are uncoupled from inflammatory responses so that they do not induce unwanted “autoimmunity”. It appears that differential clearance of these “dying cells” through anti-inflammatory triggers may depend on the state of the dying cell, the phagocyte receptors engaged and the induction of suppressive TGF- β , which can turn this potentially inflammatory response instead into a quiet process.

There are two main groups of macrophages, designated as M1 and M2. M1 macrophages are activated by LPS and IFN- γ and secrete high levels of IL-12 and low levels of IL-10. M2 are resident tissue macrophages, which produce elevated levels of IL-10, TGF- β and low levels of IL-12. Indeed, tumor-associated macrophages (TAM), which have characteristics of wound healing, and regulatory macrophages are abundant in the tumor microenvironment. TAMs are associated with a poor prognosis in cancers of cervix, breast and bladder (30, 31). So, TAM combines most of the functions of M2 by assisting in remodeling, immune regulation and efficient phagocytic activity promoting tumor growth. Many preclinical models have shown improvement in the response of tumors to irradiation by depleting TAMs from tumors or by suppressing their polarization from an M1 to an M2 phenotype (32).

DENDRITIC CELLS AS “ANTIGEN PRESENTING”

Observations suggest that bone-marrow-derived dendritic cells can ingest apoptotic cells and present antigens derived from the ingested cells to T cells, thus initiating immune responses mediated by cytotoxic lymphocytes. In the absence of maturation signals, the uptake by “immature” dendritic cells of self-components packaged in apoptotic cells might reinforce tolerance to self-tissue, so that when tissue injury does occur, T cells likely to respond to self-antigens can be anergized or deleted. In the context of radiation, DAMPs can provide

this maturation signal and convert the dendritic cells to professional antigen-presenting cells (APCs) specialized for efficiently presenting the ingested antigen to lymphocytes. CD8 T cells recognize antigen presented by MHC class I molecules. Access to antigens to the MHC class I presentation pathway was initially thought to be restricted to endogenous proteins, i.e., those expressed in the cytoplasm of the cell presenting the antigen. It has subsequently been shown, however, that under these circumstances professional APCs, such as dendritic cells, can present exogenous antigens in the class I pathway; this process is referred to as “cross-presentation”.

HEAT SHOCK CHAPERONES AS DAMPS

The heat shock or stress response is one of the most highly conserved adaptive responses in nature. The stress response confers tolerance to a variety of stresses including radiation and other perturbations. Heat-shock proteins (HSPs) are highly conserved, function as molecular chaperones for a large panel of “client” proteins and have strong cytoprotective properties due to their roles in the synthesis, folding and degradation of proteins (33–35). Radiation can induce a “danger signal” by releasing HSPs like gp96, HSP70 or calreticulin, and this can produce a remarkable range of immune effects. In fact, HSP70 released from dying tumor cells or injected as part of a vaccine induces powerful pro-inflammatory signaling cascades leading to APC activation. Tumor-derived HSPs can transport the “chaperoned” antigenic peptides as cargo across the membranes of APCs for delivery to MHC class I molecules. Another advantage of cross presentation with HSP is that proteins from tumors that do not activate dendritic cells can still be processed in the MHC class I pathway of such professional APCs, allowing them to prime naive CD8 T cells, a process termed “cross-priming” (33).

EFFECTS OF RADIATION ON T-CELL IMMUNE SELECTION AND HOMEOSTASIS

Thus far we have seen that radiation has the potential to release tumor-associated antigens and stress-related danger signals that can prime T cells. Many of these T cells are present as part of an immune infiltration, called tumor infiltrating lymphocytes, in the tumor or traffic into the tumor microenvironment after chemokine signaling. Whatever the source, these cells are generally sensitive to radiation and can demonstrate expansion or reduction in numbers through DNA damage response and efficient repair or apoptosis. Immune homeostasis preserves the equilibrium in T-cell diversity, allowing it to return to a normal steady state after such perturbations. This dynamic event is mediated by a particular form of T-cell apoptosis called apoptosis-induced cellular death (AICD), which achieves the necessary contraction at the end of an immune response and maintains peripheral tolerance. DNA DSBs can be induced in quiescent and proliferating lymphocytes after irradiation even though the phosphorylation of H2AX and apoptosis are more sensitive in proliferating lymphocytes. Another effect of radiation is on the important subpopulation of regulatory T cells or Treg cells, characterized by expression of the forkhead box P3 (Foxp3) transcription factor and high levels of CD25. Treg cells, which play a crucial role in mediating immune homeostasis, are relatively resistant to radiotherapy. Moreover, radiation can increase the

production of Treg cells and their recruitment to the TME. While memory and effector T cells can counteract cancer progression, they invariably fail. Some reasons for this include: 1. The clonal deletion of relatively lower numbers of T-cell receptors for tumor antigens; 2. Poor immunogenicity of the solid tumors to induce priming and boosting of the immune response; 3. Immunosuppressive factors such as the expression of inhibitory checkpoint signals like CTLA4 and PD1/PDL1; and 4. Inhibitory signals from T regulatory cells. It is therefore imperative for combination therapy with radiation to address some of these aspects with the overall effect of enhancing the immune response. Zhang *et al.* showed that in treatment with tumor antigen-specific T cells, nonirradiated tumors often recurred, whereas irradiated tumors exhibited complete eradication (36). This immune upregulation may indicate that the rare phenomenon of abscopal effect is clinically significant. Another study combined intratumoral injection of the TLR9 agonist CpG PF-3512676 in two phase I clinical studies, one in low-grade B-cell lymphoma and another in cutaneous lymphoma along with localized low-dose radiotherapy. Systemic anti-lymphoma responses were documented in both studies, along with induction of tumor-reactive CD8 T cells (37, 38). Blocking immune checkpoints like CTLA4 and B7-H1 or PD-1 in preclinical radiotherapy models increased the rate of tumor regression (39, 40). Park *et al.* demonstrated that in mouse models, tumor-specific CD8⁺ T-cell immunity could be induced by the combination of PD-1 blockage and radiotherapy (39). Specifically, in the PD-1-deficient mice, there was a much higher number of radiation-induced, tumor-specific effector T cells that infiltrated into distant tumor sites. This abscopal effect likely derives from a combination of the local immune response of removing the inhibition on CD8⁺ cytotoxic T cell (CTL) and the facilitation of antitumor effects by the systemic cytokine patterns on the CTL (4, 40, 41). Such synergy between local radiation and immune checkpoint inhibitors has also been demonstrated in melanoma patients treated with radiation and CTLA-4 inhibitor ipilimumab (42–45). Although specific radiation treatment intervals are uncertain, most reports agree that timing is likely crucial to immune checkpoint augmentation of abscopal effects. In their meta-analysis, Reynders *et al.*, reviewed perceived abscopal effect in 23 case reports (40) with radiotherapy alone or in combination with immune therapy. The study showed a median abscopal response time of five months with a wide range of 1–24 months (12). Grimaldi *et al.* observed the abscopal effect in 11 out of 21 patients treated with ipilimumab followed by radiotherapy (42). Ipilimumab was administered 4–8 months before radiotherapy, and the abscopal effect was observed with a median time of one month and range of one to four months after radiotherapy.

CONCLUSIONS

Our understanding of the mechanisms underlying the recognition and elimination of dying cancer cells by the immune system in the context of radiation exposure has considerably advanced, as summarized in Fig. 1. The start of this dynamic process includes radiation-induced immune stimulation by APM-presented tumor antigens along with the “danger signal”. Downstream components in the TME, such as macrophages and dendritic cells, may suppress or support the eventual immune activation. When the latter occurs, it translates the local radiation treatment into the systemic response of the abscopal effects. Antibodies targeting the checkpoint, such as CTLA4 and PD1/PDL1, have been able to skew these

results and improve the rate of abscopal effects. While this phenomenon shows much promise, there are still several unanswered questions about how radiation induces a change in the immunological microenvironment. Translational research is needed to determine the type of radiation, the timing of immunotherapy and the safety profile of such a combination in future clinical studies.

Acknowledgments

The authors are supported by the Houston Methodist Research Institute and National Institutes of Health (grant nos. RO1 CA129537 and RO1GM109768).

References

1. Mole RH. Whole body irradiation; radiobiology or medicine? *Br J Radiol.* 1953; 26:234–241. [PubMed: 13042090]
2. Andrews JR. Dose-time relationships in cancer radiotherapy. A clinical radiobiology study of extremes of dose and time. *Am J Roentgenol Radium Ther Nucl Med.* 1965; 93:56–74.
3. Papac RJ. Spontaneous regression of cancer. *Cancer Treat Rev.* 1996; 22:395–423. [PubMed: 9134002]
4. Burnette BC, Liang H, Lee Y, Chlewicki L, Khodarev NN, Weichselbaum RR, et al. The efficacy of radiotherapy relies upon induction of type I interferon-dependent innate and adaptive immunity. *Cancer Res.* 2011; 71:2488–96. [PubMed: 21300764]
5. Ishikawa H, Barber GN. STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. *Nature.* 2008; 455:674–8. [PubMed: 18724357]
6. Ablasser A, Goldeck M, Cavlari T, Deimling T, Witte G, Rohl I, et al. cGAS produces a 2′–5′-linked cyclic dinucleotide second messenger that activates STING. *Nature.* 2013; 498:380–4. [PubMed: 23722158]
7. Barber GN. STING: infection, inflammation and cancer. *Nat Rev Immunol.* 2015; 15:760–70. [PubMed: 26603901]
8. Ho SS, Zhang WY, Tan NY, Khatoor M, Suter MA, Tripathi S, et al. The DNA structure-specific endonuclease MUS81 mediates DNA sensor STING-dependent host rejection of prostate cancer cells. *Immunity.* 2016; 44:1177–89. [PubMed: 27178469]
9. Hartlova A, Erttmann SF, Raffi FA, Schmalz AM, Resch U, Anugula S, et al. DNA damage primes the type I interferon system via the cytosolic DNA sensor STING to promote anti-microbial innate immunity. *Immunity.* 2015; 42:332–43. [PubMed: 25692705]
10. Bhattacharya S, Srinivasan K, Abdisalaam S, Su F, Raj P, Dozmorov I, et al. RAD51 interconnects between DNA replication, DNA repair and immunity. *Nucleic Acids Res.* 2017; 45:4590–605. [PubMed: 28334891]
11. Harding SM, Benci JL, Irianto J, Discher DE, Minn AJ, Greenberg RA. Mitotic progression following DNA damage enables pattern recognition within micronuclei. *Nature.* 2017; 548:466–70. [PubMed: 28759889]
12. Dewan MZ, Galloway AE, Kawashima N, Dewyngaert JK, Babb JS, Formenti SC, et al. Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti-CTLA-4 antibody. *Clin Cancer Res.* 2009; 15:5379–88. [PubMed: 19706802]
13. Kisselev AF, Akopian TN, Woo KM, Goldberg AL. The sizes of peptides generated from protein by mammalian 26 and 20 S proteasomes. Implications for understanding the degradative mechanism and antigen presentation. *J Biol Chem.* 1999; 274:3363–71. [PubMed: 9920878]
14. Toes RE, Nussbaum AK, Degermann S, Schirle M, Emmerich NP, Kraft M, et al. Discrete cleavage motifs of constitutive and immunoproteasomes revealed by quantitative analysis of cleavage products. *J Exp Med.* 2001; 194:1–12. [PubMed: 11435468]
15. Rock KL, York IA, Saric T, Goldberg AL. Protein degradation and the generation of MHC class I-presented peptides. *Adv Immunol.* 2002; 80:1–70. [PubMed: 12078479]

16. Rock KL, Gramm C, Rothstein L, Clark K, Stein R, Dick L, et al. Inhibitors of the proteasome block the degradation of most cell proteins and the generation of peptides presented on MHC class I molecules. *Cell*. 1994; 78:761–71. [PubMed: 8087844]
17. Kessler JH, Beekman NJ, Bres-Vloemans SA, Verdijk P, van Veelen PA, Kloosterman-Joosten AM, et al. Efficient identification of novel HLA-A(*0201-presented cytotoxic T lymphocyte epitopes in the widely expressed tumor antigen PRAME by proteasome-mediated digestion analysis. *J Exp Med*. 2001; 193:73–88. [PubMed: 11136822]
18. Morel S, Levy F, Burlet-Schiltz O, Brasseur F, Probst-Keppler M, Peitrequin A-L, et al. Processing of some antigens by the standard proteasome but not by the immunoproteasome results in poor presentation by dendritic cells. *Immunity*. 2000; 12:107–17. [PubMed: 10661410]
19. Lucchiari-Hartz M, van Endert PM, Lauvau G, Maier R, Meyerhans A, Mann D, et al. Cytotoxic T lymphocyte epitopes of HIV-1 Nef: Generation of multiple definitive major histocompatibility complex class I ligands by proteasomes. *J Exp Med*. 2000; 191:239–52. [PubMed: 10637269]
20. Serwold T, Gonzalez F, Kim J, Jacob R, Shastri N. ERAAP customizes peptides for MHC class I molecules in the endoplasmic reticulum. *Nature*. 2002; 419:480–3. [PubMed: 12368856]
21. Macagno A, Gilliet M, Sallusto F, Lanzavecchia A, Nestle FO, Groettrup M. Dendritic cells up-regulate immunoproteasomes and the proteasome regulator PA28 during maturation. *Eur J Immunol*. 1999; 29:4037–42. [PubMed: 10602014]
22. Gileadi U, Moins-Teisserenc HT, Correa I, Booth BL Jr, Dunbar PR, Sewell AK, et al. Generation of an immunodominant CTL epitope is affected by proteasome subunit composition and stability of the antigenic protein. *J Immunol*. 1999; 163:6045–52. [PubMed: 10570292]
23. Reits EA, Hodge JW, Herberts CA, Groothuis TA, Chakraborty M, Wansley EK, et al. Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. *J Exp Med*. 2006; 203:1259–71. [PubMed: 16636135]
24. Karki R, Man SM, Kanneganti TD. Inflammasomes and cancer. *Cancer Immunol Res*. 2017; 5:94–99. [PubMed: 28093447]
25. Stoecklein VM, Osuka A, Ishikawa S, Lederer MR, Wanke-Jellinek L, Lederer JA. Radiation exposure induces inflammasome pathway activation in immune cells. *J Immunol*. 2015; 194:1178–89. [PubMed: 25539818]
26. Gameiro SR, Jammeh ML, Wattenberg MM, Tsang KY, Ferrone S, Hodge JW. Radiation-induced immunogenic modulation of tumor enhances antigen processing and calreticulin exposure, resulting in enhanced T-cell killing. *Oncotarget*. 2014; 5:403–16. [PubMed: 24480782]
27. Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature*. 2002; 418:191–5. [PubMed: 12110890]
28. Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A, et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nat Med*. 2007; 13:1050–9. [PubMed: 17704786]
29. Tian J, Avalos AM, Mao SY, Chen B, Senthil K, Wu H, et al. Toll-like receptor 9-dependent activation by DNA-containing immune complexes is mediated by HMGB1 and RAGE. *Nat Immunol*. 2007; 8:487–96. [PubMed: 17417641]
30. Bingle L, Brown NJ, Lewis CE. The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. *J Pathol*. 2002; 196:254–65. [PubMed: 11857487]
31. Wang R, Zhang J, Chen S, et al. Tumor-associated macrophages provide a suitable microenvironment for non-small lung cancer invasion and progression. *Lung Cancer*. 2011; 74:188–96. [PubMed: 21601305]
32. Chen HM, Ma G, Gildener-Leapman N, et al. Myeloid-derived suppressor cells as an immune parameter in patients with concurrent sunitinib and stereotactic body radiotherapy. *Clin Cancer Res*. 2015; 21:4073–85. [PubMed: 25922428]
33. Srivastava P. Interaction of heat shock proteins with peptides and antigen presenting cells: chaperoning of the innate and adaptive immune responses. *Annu Rev Immunol*. 2002; 20:395–425. [PubMed: 11861608]

34. Bansal H, Bansal S, Rao M, Foley KP, Sang J, Proia DA, et al. Heat shock protein 90 regulates the expression of Wilms tumor 1 protein in myeloid leukemias. *Blood*. 2010; 116:4591–9. [PubMed: 20651072]
35. Bansal H, Yihua Q, Iyer SP, Ganapathy S, Proia DA, Penalva LO, et al. WTAP is a novel oncogenic protein in acute myeloid leukemia. *Leukemia*. 2014; 28:1171–4. [PubMed: 24413322]
36. Zhang B, Bowerman NA, Salama JK, Schmidt H, Spiotto MT, Schietinger A, et al. Induced sensitization of tumor stroma leads to eradication of established cancer by T cells. *J Exp Med*. 2007; 204:49–55. [PubMed: 17210731]
37. Brody JD, Ai WZ, Czerwinski DK, Torchia JA, Levy M, Advani RH, et al. In situ vaccination with a TLR9 agonist induces systemic lymphoma regression: a phase I/II study. *J Clin Oncol*. 2010; 28:4324–32. [PubMed: 20697067]
38. Kim SB, Pandita RK, Eskiocak U, Ly P, Kaisani A, Kumar R, et al. Targeting of Nrf2 induces DNA damage signaling and protects colonic epithelial cells from ionizing radiation. *Proc Natl Acad Sci U S A*. 2012; 109:E2949–55. [PubMed: 23045680]
39. Park SS, Dong H, Liu X, Harrington SM, Krco CJ, Grams MP, et al. PD-1 restrains radiotherapy-induced abscopal effect. *Cancer Immunol Res*. 2015; 3:610–9. [PubMed: 25701325]
40. Reynders K, Illidge T, Siva S, Chang JY, De Ruyscher D. The abscopal effect of local radiotherapy: using immunotherapy to make a rare event clinically relevant. *Cancer Treat Rev*. 2015; 41:503–10. [PubMed: 25872878]
41. Lim JY, Gerber SA, Murphy SP, Lord EM. Type I interferons induced by radiation therapy mediate recruitment and effector function of CD8(+) T cells. *Cancer Immunol Immunother*. 2014; 63:259–71. [PubMed: 24357146]
42. Grimaldi AM, Simeone E, Giannarelli D, Muto P, Falivene S, Borzillo V, et al. Abscopal effects of radiotherapy on advanced melanoma patients who progressed after ipilimumab immunotherapy. *Oncoimmunology*. 2014; 3:e28780. [PubMed: 25083318]
43. Hiniker SM, Chen DS, Reddy S, Chang DT, Jones JC, Mollick JA, et al. A systemic complete response of metastatic melanoma to local radiation and immunotherapy. *Transl Oncol*. 2012; 5:404–7. [PubMed: 23323154]
44. Postow MA, Callahan MK, Barker CA, Yamada Y, Yuan J, Kitano S, et al. Immunologic correlates of the abscopal effect in a patient with melanoma. *N Engl J Med*. 2012; 366:925–31. [PubMed: 22397654]
45. Starnell EF, Wolchok JD, Gnjjatic S, Lee NY, Brownell I. The abscopal effect associated with a systemic anti-melanoma immune response. *Int J Radiat Oncol Biol Phys*. 2013; 85:293–5. [PubMed: 22560555]

FIG. 1.

How radiation impacts tumor and immune cells to modulate the immunological response. Right side: Tumor cell (brown) interacts with T cell (blue circle) to suppress response, but radiation-induced cell death releases DAMPs and CRT/gp96, which activate macrophages (irregular blue cells) to produce IFN- γ and IL-12-stimulating CD8 cells, thereby increasing cytotoxic T lymphocytes (CTL, round blue cells). Radiation damage can also directly stimulate macrophage IFN- γ production as well as suppress Treg cells (green cells). Radiation damage to the tumor cell can also initiate DNA and new antigen release. Left side: Antigen processing pathway from immunoproteosome production of peptides to cell surface display for immune recognition.