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Molecular determinants of immunogenic cell death elicited by radiation therapy

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Abstract

Cancer cells undergoing immunogenic cell death (ICD) can initiate adaptive immune responses against dead cell-associated antigens, provided that (1) said antigens are not perfectly covered by central tolerance (antigenicity), (2) cell death occurs along with the emission of immunostimulatory cytokines and damage-associated molecular patterns (DAMPs) that actively engage immune effector mechanisms (adjuvanticity), and (3) the microenvironment of dying cells is permissive for the initiation of adaptive immunity. Finally, ICD-driven immune responses can only operate and exert cytotoxic effector functions if the microenvironment of target cancer cells enable immune cell infiltration and activity. Multiple forms of radiation, including non-ionizing (ultraviolet) and ionizing radiation, elicit *bona fide* ICD as they increase both the antigenicity and adjuvanticity of dying cancer cells. Here, we review the molecular determinants of ICD as elicited by radiation as we critically discuss strategies to reinforce the immunogenicity of cancer cells succumbing to clinically available radiation strategies.

Keywords

ATP; calreticulin; HMGB1; immune checkpoint inhibitors; type I IFN; PD-L1

Introduction

Dying cells can initiate antigen-specific immune responses, admitting that cell death occurs in immunocompetent syngeneic hosts, a process that has been dubbed “immunogenic cell death” (ICD).[1–3] The ability of regulated cell death (RCD) to elicit adaptive immunity

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coupled with effector functions and immunological memory relies upon three critical determinants: (1) antigenicity, *i.e.*, the fact that dying (cancer) cells express antigens that are not perfectly covered by central tolerance, implying that T cell clones specific for such antigens are available in the mature T cell repertoire of the host;[4] (2) adjuvanticity, *i.e.*, the ability of stressed and dying cells to secrete chemokines and cytokines as well as immunostimulatory damage-associated molecular patterns (DAMPs) that overall recruit and activate professional antigen-presenting cells (APCs) such as dendritic cells (DCs) to sites of cell death;[5,6] and (3) microenvironmental features that not only are permissive for the recruitment and activation of APCs or precursors thereof by ICD-associated cytokines and DAMPs (at sites of cell death), but also enable antigen-specific immune effector cells, notably $CD8^+$ cytotoxic T lymphocytes (CTLs) primed by said APCs to reach their targets and execute antigen-specific immune responses (Figure 1).[7–10] Importantly, none of these requirements is truly intrinsic to dying cells.[1,11] Indeed, not only the composition of the mature T cell repertoire, but also the productive recognition of cytokines and DAMPs as well as the microenvironment of the initiators (dying cells) or target (living cells resisting death) of adaptive immunity is largely dictated by the host.[1,11,12] Moreover, the immunogenicity of dying cells is not necessarily dictated by RCD modality.[13] Indeed, while caspase-dependent apoptosis generally occurs in an immunologically silent or even tolerogenic manner,[14–17] multiple instances of apoptosis have been shown to constitute *bona fide* cases of ICD.[18]

DAMP emission by dying cells is generally elicited in the context of failing adaptation to stress.[3] Thus, cells initially respond to ICD-inducing conditions by activating a panoply of cytoprotective mechanisms aimed at restoring cellular homeostasis, including (but not limited to): (1) the unfolded protein response (UPR) in the context of the so-called “integrated stress response” (ISR),[19–22] (2) the DNA damage response,[23–25] (3) autophagy,[26–28] and (4) the mitochondrial stress response.[29–31] Once exposed on the surface of dying cells or released in their extracellular microenvironment, DAMPs are detected by pattern recognition receptors (PRRs) expressed by immune effector cells including APCs and precursors thereof, other myeloid cells and both innate and adaptive lymphoid cell populations.[5,32–34]

A number of conditions have been shown to elicit *bona fide* ICD especially (but not exclusively) in cancer cells. These include: (1) numerous intracellular pathogens, notably oncolytic viruses,[35,36] (2) a wide panel of conventional chemotherapeutics,[37–39] (3) targeted anticancer agents,[40–45] (4) oncolytic peptides,[46–49] as well as (5) numerous physical stressors encompassing (but not limited to) high hydrostatic pressure,[50,51] extracorporeal photochemotherapy,[52–54] photodynamic therapy,[55–57] nanopulse stimulation,[58] near-infrared photoimmunotherapy,[59–62] as well as various forms of ionizing and non-ionizing radiation.[63–65]

Among these physical agents, radiation therapy (RT) has been consistently investigated for its potential to elicit ICD in cancer cells and hence synergize with commonly employed immunotherapeutics such as immune checkpoint inhibitors (ICIs).[66–69] This reflects clinical considerations linked to RT safety[12,70] and availability,[71] as well as the ability of RT to elicit ICD and other immunostimulatory effects, at least when delivered focally and

according to specific dose and fractionation schedules.[69,72,73] In line with this notion, mouse cancer cells exposed to multiple forms of RT (including RT with charged particles) *ex vivo* can be successfully employed to establish prophylactic immunity upon inoculation into immunocompetent syngeneic hosts.[63,74,75] Moreover, RT has been shown to synergize with various forms of immunotherapy including immune checkpoint inhibitors (ICIs) in a variety of preclinical tumor models, resulting not only in the inhibition of irradiated lesions, but also in (at least some degree) of immunological control of distant, non-irradiated tumors (the so-called abscopal response).[76]

Here, we review molecular determinants of ICD as driven by RT and other radiation strategies as we critically discuss potential approaches to boost the immunogenicity of irradiated cancer cells.

CALR

Calreticulin (CALR) is an endoplasmic reticulum (ER) chaperone involved in many biological processes that include (among others) the regulation of calcium homeostasis, the folding of newly synthesized glycoproteins and the trafficking of properly loaded MHC I molecules.[77,78] Anthracyclines as well as many other chemical and physical ICD inducers elicit the rapid translocation of CALR to the outer leaflet of the plasma membrane, a process that occurs prior to the apoptosis-related (and generally tolerogenic) externalization of phosphatidylserine.[19,38] Surface-exposed CALR operates as a potent pro-phagocytic signal upon binding to LDL receptor related protein 1 (LRP1, best known as CD91) on the surface of immature APCs.[79–81] Moreover, externalized CALR has been shown to promote natural killer (NK) cell activation both directly, through natural cytotoxicity triggering receptor 1 (NCR1, best known as NKp46),[82] and indirectly, via a mechanism that involves $CD11c^{+}CD14^{high}$ myeloid cells trans-presenting interleukin 15 (IL15).[83–86] Supporting the key role of CALR exposure in the immunogenicity of RCD, various genetic and pharmacological strategies blocking CALR exposure and/or preventing its interaction with CD91 have been shown to limit the ability of cancer cells succumbing to ICD inducers to generate prophylactic immunity upon inoculation in immunocompetent syngeneic hosts.[3]

CALR exposures as elicited by ionizing irradiation *in vitro* has been documented in mouse mammary adenocarcinoma TS/A cells (RT dose: 2–20 Gy),[64] mouse colorectal carcinoma (CRC) CT26 cells (RT dose: 75 Gy),[63] mouse melanoma B16 and S91 cells (RT dose: 5Gy),[75] human osteosarcoma U2OS cells (RT dose: 5 Gy),[75] human prostate cancer LNCaP cells (RT dose: 10 Gy),[87] human triple negative breast cancer (TNBC) MD-MBA-231 cells (RT dose: 10 Gy),[87] and human lung adenocarcinoma H522 cells (RT dose: 10 Gy).[87] Importantly, the ability of irradiated CT26 cells to protect immunocompetent BALB/c (syngeneic) mice from the subsequent inoculation of living cells of the same type could be abrogated by the RNA interference (RNAi)-mediated knockdown of CALR, an effect that could be rescued with recombinant CALR absorption.[63]

Intriguingly, CALR exposure on the surface of irradiated LNCaP and MD-MBA-231 was accompanied not only by eIF2α phosphorylation as a marker of an ongoing ISR, but also by the upregulation of multiple components of the antigen-presenting machinery including

CALR itself as well as low molecular mass protein 2 (LMP2), LMP7 and transporter 2, ATP binding cassette subfamily B member (TAP2), ultimately rendering cancer cells surviving irradiation more susceptible to lysis by CTLs.[87,88] While technically more challenging, the ability of RT to drive CALR exposure has also been documented *in vivo*, both in LNCaP tumors established in immunodeficient mice upon the delivery of a single RT dose of 10 Gy,[87] and in patients with metastatic renal cell carcinoma (RCC) receiving stereotactic body radiotherapy (SBRT) in a single fraction of 15 Gy.[89]

Intriguingly, CALR may also modulate the intrinsic radiosensitivity of cancer cells. Indeed, the transgene-enforced overexpression of CALR has been shown to sensitize radioresistant human glioblastoma U251MG and T98G to ionizing radiation, at least partially via a mechanism that involves reduced pro-survival signaling via AKT serine/threonine kinase 1 (AKT1) coupled with disruption of intracellular Ca^{2+} homeostasis.[90–94]

Taken together, these observations suggest that irradiated cancer cells may experience perturbations of reticular homeostasis coupled with the activation of an adaptive response culminating with the upregulation of CALR and its exposure to the cell surface in support of increased adjuvanticity. Moreover, CALR appears to promote intrinsic radiosensitivity, at least in some experimental settings.

ATP

While ATP exists intracellularly at concentrations of 1–10 μM, extracellular ATP levels in healthy tissues are extremely low, at least in part owing to the existence of plasma membrane-associated enzymes that catalyze the sequential conversion of ATP into the immunosuppressive molecule adenosine, including ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1, best known as CD39) and 5’-nucleotidase ecto (NT5E, best known as CD73).[95–97] At odds with adenosine, extracellular ATP mediates potent chemotactic and immunostimulatory effects (which culminate with NLRP3 inflammasome activation and consequent IL1B and IL18 secretion) upon binding to purinergic receptor P2Y2 (P2RY2)[98–100] and purinergic receptor P2X 7 (P2RX7),[101] respectively, on the surface of APCs or their precursors. Importantly, the ICD-associated release of ATP in the extracellular microenvironment appears to require proficient pre-mortem autophagic responses, as these enable dying cells to preserve ATP stores which ultimately are released via a dual mechanism involving lysosomal exocytosis and pannexin 1 (PANX1) channels.[26,102–104]

In line with the ability of autophagy-dependent ATP release to support danger signaling in the context of ICD, CT26 cells overexpressing CD39 or depleted of key components of the molecular machinery for autophagy including autophagy related 5 (ATG5), ATG7, and beclin 1 (BECN1) fails to provide immunological protection to BALB/c mice when used as a prophylactic vaccine upon exposure to ICD-inducing chemotherapeutics.[26,105] Similarly, ATG5-deficient CT26 tumors growing in immunocompetent BALB/c mice partially lose their ability to respond to ICD-inducing chemotherapeutics such as mitoxantrone,[26] as well as to RT (delivered as a single dose of 8 Gy),[106] two therapeutic approaches that been shown to trigger ATP release from various human and murine cancer cell lines *in vitro*

and/or *in vivo*.[26,64,87] Importantly, such a defect could be rescued by the concomitant administration of a CD39 inhibitor, suggesting it indeed reflected limited ATP release downstream of defective autophagy.[106,107] Further corroborating the importance of this pathway for ICD as driven by RT, systemic autophagy activation by alternate-day feeding or caloric restriction,[108–112] has been shown to considerably improve the ability of a single RT dose of 6–8 Gy to limit local disease progression and metastatic dissemination of mouse TNBC 4T1 and 67NR lesions established in immunocompetent BALB/c mice.[113,114] Altogether, these findings suggest that autophagy activation may support ICD induction by RT.

However, it is important to note that autophagy mediates considerable cytoprotective effects on malignant cells, hence rendering them less sensitive to the cytostatic and cytotoxic activity of RT, as demonstrated in a multitude of *in vitro* experimental tumor models as well as *in vivo*, in immunodeficient mice bearing human or mouse malignant lesions.[115] Moreover, proficient autophagic responses have been shown to: (1) limited oxidative stress and hence impair ICD-associated CALR exposure as driven by photodynamic therapy,[116,117] (2) promote the lysosomal degradation of MHC Class I molecules by cancer cells, hence rendering them poorly visible by the adaptive immune system,[118] and (3) inhibit type I interferon (IFN) by malignant cells undergoing ICD in response to RT (see below).[74] In line with this notion, genetic signatures of proficient autophagy in diagnostic biopsies correlate with inhibited type I IFN and interferon gamma (IFNG) signaling as well as with poor disease outcomes in patients with breast cancer.[74,119] That said, the vast majority of clinical trials testing lysosomal inhibitors such as chloroquine and hydroxychloroquine (which potently inhibit autophagy) along with standard-of-care (SOC) chemotherapy or RT failed to document a clinical benefit for combinatorial regimens over SOC only.[115,120] While the reasons underlying these largely negative clinical observations remain to be fully elucidated, it is tempting to speculate that systemic autophagy inhibition may not represent an optimal therapeutic strategy in view of the fact that autophagy is required for the optimal function of many immune cell types, including (but not limited to) DCs, NK cells and CTLs.[121]

In summary, autophagy appears to influence the immunogenicity of cancer cells succumbing to RT (and other ICD inducers) in a context-dependent manner. The precise reasons underlying such apparently discrepant observations may relate to features of the tumor microenvironment (TME) potentially including baseline infiltration by specific immune cells and/or the expression levels of ATP receptors, extracellular ATP-degrading enzymes and other components of the type I IFN signaling machinery. Additional work is required to deconvolute the contribution of ATP secretion (which is generally promoted by autophagy) vs MHC Class I presentation and type I IFN signaling (which are inhibited by autophagy) in the immunogenicity of RT in specific oncological settings.

HMGB1

High mobility group box 1 (HMGB1) is a non-histone chromatin-binding protein that translocates first from the nucleus to the cytoplasm and then from the cytoplasm to the extracellular microenvironment in the context of multiple RCD instances, including

ICD.[122] Depending on oxidation status, extracellular HMGB1 can exert mostly chemotactic effects (fully reduced form), upon forming a complex with C-X-C motif chemokine ligand 12 (CXCL12) and binding to C-X-C motif chemokine receptor 4 (CXCR4), mostly immunostimulatory effects (partially oxidized form), upon binding to advanced glycosylation end-product specific receptor (AGER) or Toll-like receptor 4 (TLR4), or be virtually inactive or even tolerogenic (fully oxidized form).[123–126] That said, the TLR4-dependent activation of MYD88 innate immune signal transduction adaptor (MYD88) appears to represent the most relevant signaling pathways elicited by HMGB1, ultimately resulting in DC maturation and increased antigen processing and cross-presentation to CTLs.[125,127] Indeed, while HMGB1-driven AGER signaling has been implicated in DC activation,[128] the perception of anthracycline-driven RCD as immunogenic is largely compromised in *Tlr4*$^{-/-}$ hosts.[125] Moreover, pharmacological TLR4 activation with dendrophilin restores at least some degree of immune control against mouse CRCs and fibrosarcomas expressing low HMGB1 levels.[129,130]

Akin to CALR exposure and ATP secretion, HMGB1 release has been documented to occur in a dose-dependent when TS/A cells are exposed to ionizing radiation *in vitro*.[64] Similar results have been obtained in human breast and prostate cancer cell lines exposed to a single RT dose of 10 Gy *in vitro*,[87] as well as in a panel of mouse and human cancer cell lines subjected to carbon ion RT in a single dose of 5 Gy.[75] Suggesting a relevance for this mechanism in the therapeutic activity of RT, CT26 CRCs as well as mammary TS/A lesions established subcutaneously in immunocompetent BALB/c mice have been shown to exhibit reduced sensitivity to a single RT dose of 10 Gy when developing in *Tlr4*$^{-/-}$ vs wild-type hosts.[125] However, blocking extracellular HGMB1 with a neutralizing antibody failed to influence the control of mouse CRC MC38 lesions subcutaneously developing in immunocompetent syngeneic C57BL/6 mice as enabled by a single RT dose of 20 Gy.[131] Similar results were obtained upon the establishment of MC38 tumors in *Myd88*$^{-/-}$ mice as well as in mice lacking the alternative TLR signal transducer TIR domain containing adaptor molecule 1 (TICAM1, best known as TRIF).[131] Whether such an apparent discrepancy relates to tumor type, RT dose or other variables remains to be clarified.

Lending additional support to the clinical relevance of these findings, patients with breast cancer carrying a loss-of-function *TLR4* allele experience inferior disease outcome on ICD-inducing chemotherapy or RT than individual carrying wild-type *TLR4*.[125] Along similar lines, circulating HGMB1 levels have been linked with improved disease outcome and/or signs of ongoing anticancer immune responses in patients with breast cancer, rectal cancer, head and neck squamous cell carcinoma (HNSCC) and esophageal squamous cell carcinoma (ESCC) receiving RT alone and/or combined with chemotherapy.[132–136] That said, circulating and/or intratumoral levels of HMGB1 have also been associated with poor disease outcome upon irradiation in a variety of clinical cohorts, including patients with bladder carcinoma,[137] nasopharyngeal cancer,[138] CRC,[139] hepatocellular carcinoma,[140] HNSCC,[141] prostate carcinoma,[142] and ESCC.[143] In this latter setting, it was found that the RNAi-mediated depletion of HMGB1 increases the radiosensitivity of human ESCC cell lines, both *in vitro* and *in vivo* (upon establishment in immunodeficient hosts).[143] At least partially, this may reflect the ability of HMGB1 to elicit radioprotective autophagic responses[143,144]

In conclusion, RT has been consistently shown to drive the relocation of HMGB1 from the nucleus to the cytoplasm and ultimately the extracellular space of cancer cells, a process that is required for RCD to be perceived as immunogenic but may also elicit cytoprotective autophagic responses that limit cell-intrinsic radiosensitivity.

Type I IFN

In human, type I IFN is encoded by a large family of homologous genes encompassing 13 genes coding for IFNα (*IFNA1*, *IFNA2*, *IFNA4*, *IFNA5*, *IFNA6*, *IFNA7*, *IFNA8*, *IFNA10*, *IFNA13*, *IFNA14*, *IFNA16*, *IFNA17* and *IFNA21*), as well as individual genes coding for IFNβ (*IFNB1*), IFNε (*IFNE*), IFNκ (*IFNK*) and IFNω (*IFNW1*).[145] Besides playing a central role in antiviral immune immunity,[146] type I IFN secretion is crucial for cancer cells succumbing to chemotherapy or RT to be perceived as immunogenic.[147] In the former setting, type I IFN is initiated downstream of double-stranded RNA (dsRNA) sensing by TLR3, resulting in the abundant secretion of the T cell chemoattractant CXCL10 by cancer cells.[148] In the latter setting instead, type I IFN responses appear to be largely mediated by cyclic GMP-AMP synthase (CGAS) and stimulator of interferon response cGAMP interactor 1 (STING1), upon recognition of micronuclear[149–151] or mitochondrial DNA (mtDNA),[74] both via cancer cell intrinsic mechanisms,[74,149,150] or upon the uptake of dying cells or extracellular vesicles therefrom by cross-presenting basic leucine zipper ATF-like transcription factor 3 (BATF3)-dependent DCs.[131,152–154]

Regardless of source, type I IFN mediates potent immunostimulatory effects upon binding to ubiquitously expressed, generally heterodimeric receptors composed of interferon alpha and beta receptor subunit 1 (IFNAR1) and IFNAR2.[155] In particular, type I IFN promotes DC cross-priming,[156] boosts the cytotoxic functions of NK cells[157–159] promotes the functional competence of naïve T cells,[160] triggers the secretion of pro-inflammatory mediators by macrophages[161,162] and inhibits the immunosuppressive functions of $CD4^+CD25^+FOXP3^+$ regulatory T (T_{REG}) cells.[163,164]

Supporting the clinical relevance of ICD-associated type I IFN signaling, a type I IFN-related transcriptional signature has been shown to predict disease outcome in breast cancer patients treated with neoadjuvant anthracycline-based chemotherapy.[148] Similarly, single nucleotide polymorphisms (SNPs) in *IFNAR1* have been associated with poor clinical outcome in patients with glioma receiving SOC temozolomide-based chemoradiation.[165] That said, transcriptional signature of type I signaling signatures have also been correlated with resistance to chemotherapy and RT in patients with breast carcinoma[166–168] and melanoma,[169] potentially reflecting the ability of weak, indolent and non-resolving type I IFN responses, as opposed to their robust, acute and resolving counterparts,[170,171] to promote cancer stemness and suppress anticancer immunity.[155,172,173]

Importantly, type I IFN secretion as elicited by RT is under negative control by a number of inducible mechanisms. Specifically, the RT-driven cytosolic accumulation of double-stranded DNA (dsDNA) is actively counteracted by autophagy, which actively disposes of permeabilized and hence mtDNA-spilling mitochondria,[74] as well as by the dose-dependent upregulation of three prime repair exonuclease 1 (TREX1), which degrades dsDNA.[174]

Moreover, the rapid execution of apoptosis by caspase 9 (CASP9) and CASP3 also prevent mtDNA-driven type I IFN secretion in cancer cells by converting dying cells, which retain metabolic functions, into terminally inactive cell corpses.[166,175] In line with this notion, various signatures of apoptotic proficiency have been correlated with poor disease outcome in patients with breast cancer.[166] In the same setting, though, proficient type I IFN signaling was also linked to detrimental disease outcome,[166] pointing to a type I IFN-independent impact of apoptotic defects on the survival of patients with breast cancer.

Taken together, these observations suggest that type I IFN production by irradiated malignant cells and/or tumor-infiltrating immune cells is crucial for the initiation of innate and adaptive anticancer immunity through RT-driven ICD. However, RT also elicits immunosuppressive pathways that need to be targeted to maximize its immunogenicity, as discussed below.

Strategies to boost RT-driven ICD

As amply discussed above, the immunogenicity of RT-driven cell death relies on antigenicity, adjuvanticity and microenvironmental features, all of which are dictated by dying cells as well as by their host.[13] This implies that defects in any of these features at least a priori limit the ability of RT to elicit adaptive anticancer immunity via ICD. That said, cancer cells tend to express *per se* a number of neoantigens not covered by central tolerance, be them genetically encoded or emerging post-transcriptionally/post-translationally.[176,177] Moreover, RT is known to boost MHC Class I exposure on cancer cells,[178,179] promote the expression of genes encoding neoantigenic determinants,[180] and aggravate stress conditions generally linked to the generation of posttranslational neoantigens, such as oxidative stress.[181,182] Thus, the immunogenicity of RT-driven ICD is generally limited at the levels of cancer cell adjuvanticity and microenvironment. Accumulating preclinical evidence has defined a number of translationally relevant strategies to circumvent such defects and hence enable superior immune responses to RT (Figure 2).

For example, defective phagocytosis of irradiated cells has been efficiently targeted with monoclonal antibodies specific for CD47, which potently suppresses pro-phagocytic signals delivered by CD91.[183–185] More specifically, CD47-targeting antibodies have been shown to synergize with RT at the induction of systemic anticancer immunity in mouse models of CRC (RT dose: 5 Gy × 3), an effect that could be potentiated by programmed cell death 1 (PDCD1, best known as PD-1) inhibitors,[186,187] glioblastoma, along with an inhibitor of the PD-L1 ligand CD274 (best known as PD-L1),[188,189] and small cell lung cancer.[190]

Poor extracellular ATP accumulation can be efficiently targeted with CD73-specific monoclonal antibodies, which have been shown to improve both the local and the abscopal efficacy of RT in immunocompetent mouse models of rectal cancer (RT dose: 4Gy × 3)[191] and mammary adenocarcinoma (RT dose: 8Gy × 3),[192] as well as with a CD39 inhibitor, which has been shown to improve the efficacy of RT in immunocompetent mouse models of CRC (RT dose: 8Gy x1).[106] Along similar lines, superior ATP (and HMGB1) release after irradiation has been documented in human lung cancer and osteosarcoma cell lines exposed

in vitro to a single RT dose of 5 Gy in the presence of ATR serine/threonine kinase (ATR) inhibitors, an effect that (for ATP only) was maximized by caspase inhibition.[193] Moreover, short-course (but not prolonged) ATR inhibition has been shown to improve tumor-targeting immunity as elicited by 2 RT fractions of 2 Gy each in mouse immunocompetent models of CRC.[194]

Limited type I IFN signaling in response to RT has been efficiently restored with TLR3 agonists administered i.t. in wild-type C57BL/6 or BALB/c mice baring subcutaneous MC38, B16 or TS/A lesions (RT dose: 8Gy × 3),[195] as well as with TLR9 agonists delivered i.t. in preclinical models of colorectal and lung cancer (RT dose: 12Gy × 3). Importantly, a similar therapeutic strategy has been assessed in patients with lymphoma who were allocated to a single RT fraction of 4 Gy in combination with an intratumorally administered TLR9 agonists, an approach that was safe, elicited systemic signs of anticancer immunity and was associated with at least some efficacy.[196–198]

While not immediately translatable to clinical settings (see above), both autophagy inhibitors and post-mitochondrial caspase blockers have also been shown to increase the ability of cancer cells to elicit systemic anticancer immunity upon irradiation (RT dose: 8Gy × 3) in immunocompetent mouse models of breast carcinoma.[74,166,175] Along similar lines, TREX1 inhibition holds promise as a combinatorial partner for RT-driven RCD to exert maximal immunostimulatory effects,[174] but to the best of our knowledge no pharmacological TREX1 inhibitors are currently available to formally address this possibility.

Importantly, a number of preclinical approaches have been successfully tested for their ability to restore microenvironmental conditions permissive for the perception of RT-driven RCD as immunogenic as well as for the execution of the consequence adaptive immune responses. These approaches include (but are not limited to): (1) monoclonal antibodies targeting transforming growth factor beta (TGF-β), as shown in mouse models of TNBC (RT dose: 6Gy × 5 or 8 Gy × 3),[199–201] an effect that could be further potentiated with PD-1 blockers plus tumor necrosis factor receptor superfamily, member 9 (TNFRSF9, best known as CD137) agonists;[202,203] (2) TNFRSF4 (best known as OX40) agonists, as demonstrated in BALB/c mice bearing syngeneic 4T1 cells (RT dose: 8Gy × 3), which could also be boosted by PD-1 blockade;[204,205] as well as (3) conventional, FDA-approved ICIs targeting cytotoxic T lymphocyte-associated protein 4 (CTLA4) and PD-1 signaling, as demonstrated in a wide panel of immunocompetent tumor models.[68,206–210] Importantly, while multiple randomized clinical trials combining RT with FDA-approved ICIs have been completed (and many others are ongoing), results have been disappointing in some instances, calling for the careful reconsideration of conventional RT approaches in support of improved cooperativity with ICIs.[69,211]

Despite these and other obstacles against the rapid implementation of preclinical findings into the clinical practice, multiple strategies that can be harnessed for restoring or reinforcing RT-driven ICD exist, including a large number of approaches with direct translational relevance.

Concluding remarks

In summary, RT is a potent inducer of ICD, an immunostimulatory cell death modality with clinical implications extending largely beyond radiation oncology.[212] However, RT (especially when delivered according to standard fractional schedules and to conventional target volumes) can also elicit a number of immunosuppressive mechanisms that ultimately counteract ICD-driven immunostimulation.[67,213,214] In line with this notion, while some randomized clinical trials testing RT in combination with FDA-approved ICIs documented a good cooperativity in the absence of unexpected side effects,[215–218] many other randomized clinical studies failed to highlight superior therapeutic effects for RT/ICI combinations as compared to SOC RT-based therapeutic regimens.[219–222] In this setting, it will be crucial not only to adapt conventional RT approaches to limit local and systemic immunosuppression, but also identify novel, therapeutically relevant targets to extend the intrinsic immunostimulatory effects of RT. Additional work is therefore required to fully harness the ability of RT to elicit ICD for the development of novel, safe and efficient therapeutic strategies against cancer.

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Competing interests.

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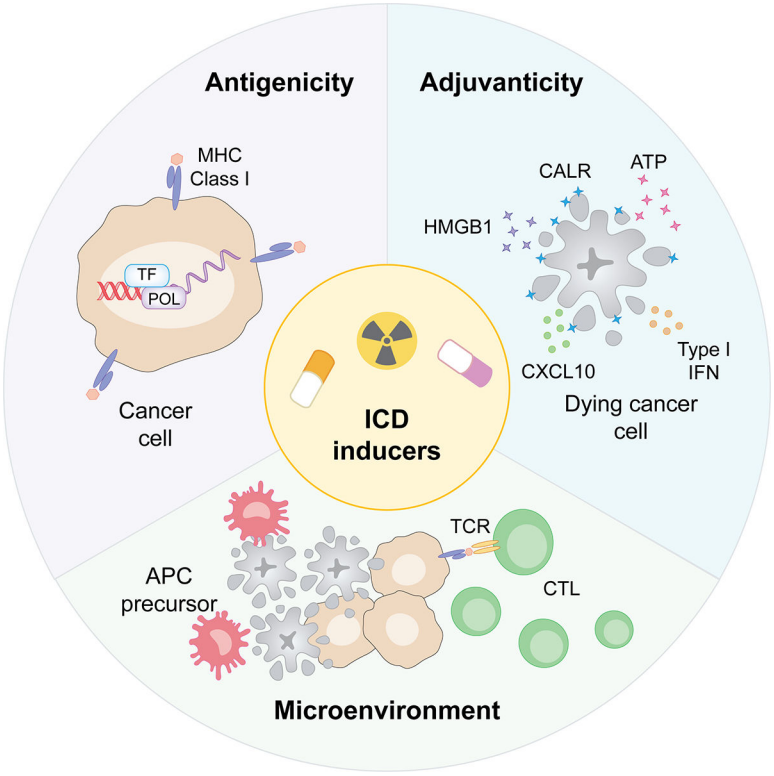

Figure 1. Core determinants of ICD.

The immunogenicity of regulated cell death (RCD), i.e., the ability of dying cells to elicit antigen-specific immunity coupled with effector and memory functions (as opposed to mere inflammation), relies on three core determinants: (1) antigenicity, i.e., dying cells must express antigenic determinants that can be recognized by circulating T cells; (2) adjuvanticity, i.e., dying cells must emit chemotactic and immunostimulatory signals that enable antigen-presenting cell (APC) recruitment, activation and migration to lymphoid organs for T cell cross-priming; and (3) a permissive microenvironment, i.e., cells must die in an environment that enables APC recruitment and functions. Moreover, cells targeted by immunogenic cell death (ICD)-driven immunity must reside in a microenvironment that is permissive for cytotoxic T lymphocyte (CTL) infiltration and effector functions. CALR, calreticulin; CXCL10, C-X-C motif chemokine ligand 10; DAMP, damage-associated molecular pattern; HMGB1, high mobility group box 1; IFN, interferon.

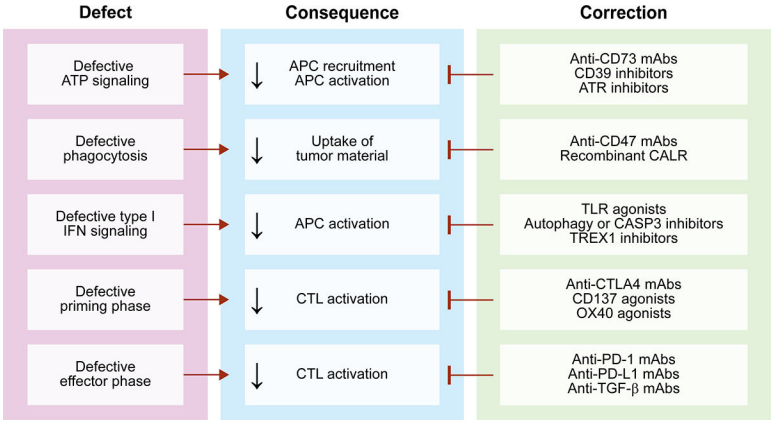

Figure 2. Strategies to enhance immunogenic cell death induced by RT.

Depending on multiple variables, radiation therapy (RT) may kill cancer cells in the context of suboptimal immunostimulation, resulting in a variant of regulated cell death (RCD) with limited immunogenicity. A number of strategies have been investigated to circumvent these defects and restore superior immunogenic cell death (ICD)-driven adaptive immune responses against non-irradiated or radioresistant cancer cells. APC, antigen-presenting cell; ATR, ATR serine/threonine kinase; CD39 (official name: ENTPD1), ectonucleoside triphosphate diphosphohydrolase 1; CASP3, caspase 3; CD73 (official name: NT5E), 5’-nucleotidase ecto; CD137 (official name: TNFRSF9), tumor necrosis factor receptor superfamily, member 9; CTL, cytotoxic T lymphocyte; CTLA4, cytotoxic T lymphocyte-associated protein 4; mAb, monoclonal antibody; OX40 (official name: TNFRSF4), tumor necrosis factor receptor superfamily, member 4; PD-1 (official name: PDCD1), programmed cell death 1, PD-L1 (official name: CD274); TGF-β, transforming growth factor beta; TLR, Toll-like receptor; TREX1, three prime repair exonuclease 1.