Immunological impact of cell death signaling driven by radiation on the tumor microenvironment

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Abstract

Therapeutic irradiation of the tumor microenvironment causes differential activation of pro-survival and pro-death pathways in malignant, stromal, endothelial and immune cells, hence causing a profound cellular and biological reconfiguration via multiple, non-redundant mechanisms. Such mechanisms include the selective elimination of particularly radiosensitive cell types and consequent loss of specific cellular functions, the local release of cytokines and danger signals by dying radiosensitive cells, as well as altered cytokine secretion by surviving radioresistant cells. Altogether, these processes create chemotactic and immunomodulatory cues for incoming and resident immune cells. Here, we discuss how cytoprotective and cytotoxic signaling modules activated by radiation in specific cell populations reshape the immunological tumor microenvironment.

Introduction

Radiation therapy (RT) is used with curative therapeutic or palliative intent in more than 50% of cancer patients. Traditionally, RT has been harnessed as a safe means to deliver ionizing energy to malignant cells, resulting in their permanent proliferative inactivation (cellular senescence) or demise{Galluzzi, 2018, 29362479}. In recent decades, considerable technological progress has fostered the widespread dissemination of modern RT variants that enable superior fractionation schedules and unprecedented anatomical precision, resulting in improved therapeutic outcomes in a number of localized oncological settings{Kuban, 2008, 17765406}. However, it has also become clear that – at least in some situations – the therapeutic efficacy of RT does not only reflect the inhibition of irradiated cancer cells, but also involves the initiation or potentiation of an adaptive, tumor-targeting immune response that operates locoregionally and systemically to control metastatic lesions{Ngwa, 2018, 29449659}. Such an out-of-field effect, commonly known as "abscopal response", is thought to originate from the ability of RT to (1) increase the local availability of antigens expressed by cancer cells and not covered by central tolerance (antigenicity), and (2) drive the release of immunostimulatory cytokines and danger signals in the tumor bed (adjuvanticity). This culminates in the recruitment and activation of antigen-presenting cells (APCs), which initiate anticancer immunity upon migrating to tumor-draining lymph nodes and cross-priming to $CD8^+$ cytotoxic T lymphocytes (CTLs){Ngwa, 2018, 29449659}. This implies that RT can only drive anticancer immunity when employed according to doses, fractionation schedules and target volumes that neither compromise the ability of the tumor microenvironment (TME) to generate chemotactic and immunostimulatory cues for APCs, nor alter the capacity of the latter to migrate to lymph nodes and cross-prime naïve T cells against tumor-associated antigens{Deutsch, 2019, 31364597}. Moreover, it suggests that RT can be employed (at least in some circumstances) to convert

an immunologically “cold” tumors (*i.e.*, a neoplasm that is poorly infiltrated by immune cells) into a “hot” lesions (*i.e.*, a neoplasm that displays robust immune infiltration){Demaria, 2016, 27774519}.

At the molecular level, irradiation damages nucleic acids, lipids and proteins in a dose-dependent manner, reflecting either direct ionization events or oxidative damage caused by RT-induced reactive oxygen species (ROS) and reactive nitrogen species (RNS){Baumann, 2016, 27009394}. These molecular alterations cause organellar damage initially coupled to the activation of responses aimed at the restoration of cellular homeostasis, including (but not limited to) the DNA damage response (DDR), the unfolded protein response (UPR) and autophagy{Galluzzi, 2018, 30305710}. When RT-dependent damage is limited, these processes ensure the survival of irradiated cells, potentially coupled to their re-entry into the cell cycle. Conversely, when damage cannot be resolved by repair mechanisms, the molecular mechanisms of adaptation to stress switch from a cytoprotective to a cytostatic or cytotoxic mode, generally culminating with cellular senescence or RCD, in one of its forms{Galluzzi, 2018, 29362479}. Importantly, cellular responses to stress – be they successful (*i.e.*, resulting in homeostasis restoration) or not (*i.e.*, leading to cellular senescence or RCD) – are intimately connected to the regulation of microenvironmental and systemic homeostasis as they control the emission of a plethora of bioactive factors with immunomodulatory properties, including not only chemokines and other cytokines, but also damage-associated molecular patterns (DAMPs), ions, metabolites and surface receptors{Galluzzi, 2018, 29362479}. Moreover, not all cellular components of the TME display the same radiosensitivity. Thus, while some cells including cancer-associated fibroblasts (CAFs) and tumor-associated macrophages (TAMs) exhibit remarkable radioresistance, others such as endothelial cells and natural killer (NK) cells are relatively more radiosensitive (although the sensitivity of any given cell type to RT varies with numerous factors including anatomical localization, proliferative state and degree of differentiation). Along similar lines, the extraordinary genetic, metabolic and functional heterogeneity

displayed by malignant cells considerably alters their intrinsic sensitivity to RT{Dagogo-Jack, 2018, 29115304}.

Here, we discuss the molecular and cellular mechanisms whereby cytoprotective and cytostatic or cytotoxic pathways influence the immunological configuration of the TME as they control the release of immunomodulatory factors by irradiated cells and determine the ultimate fate of both resident and newly recruited cell populations (**Figure 1**).

Cytoprotective signals elicited by radiation

Both malignant and non-malignant components of the TME survive sub-lethal RT doses by activating robust cytoprotective mechanisms that not only enable the restoration of cellular homeostasis, but also relay a signal of danger to the organism via the immune system{Galluzzi, 2018, 30305710} (**Box 1**). The activation of cytoprotective pathways in cancer cells and non-transformed cells of the TME differentially influence the immunological configuration of malignant lesions, not only as a consequence of differential cell survival, but also upon the modulation of core immunological processes, as described here below.

DNA damage response. RT causes a variety of DNA damage ranging from nucleotide lesions to single- and double-strand breaks (SSBs and DSBs), whose amount and complexity depends on dose as well as linear energy transfer (LET, a measure of the amount of energy that an ionizing wave-particle transfers to material){Ward, 2000, 12760053}. In the initial phases, the DDR largely delivers cytoprotective signals as it provides cells with time to repair damage by inducing a reversible cell cycle arrest{Scully, 2019, 31263220}. In this context, multiple components of early DDR signaling, including (but not limited to) ATM serine/threonine kinase (ATM), ATR serine/threonine kinase (ATR), checkpoint kinase 1 (CHEK1) and poly(ADP-ribose) polymerase 1 (PARP1), have been shown to inhibit type I interferon (IFN) secretion{Chabanon, 2019, 30589644;Dillon, 2019, 30770349}, hence limiting tumor infiltration by myeloid APCs and negatively impacting antitumor immunity. At least in part, this reflects the ability of a proficient DDR to limit the accumulation of cytosolic DNA fragments and to prevent the formation of micronuclei{Heijink, 2019, 30626869}, both of which potentially drive type I IFN secretion via cyclic GMP-AMP synthase (CGAS){Vanpouille-Box, 2018, 30216189}. Apparently at odds with these findings, ATM and PARP1 reportedly drive type I IFN secretion by activating transmembrane protein 173 (TMEM173, best known as STING) in a CGAS-independent manner{Dunphy, 2018, 30193098}.

Whether this pathway, which has been discovered in keratinocytes, is at play in the TME remains to be elucidated. Interestingly, a nuclear CGAS pool has recently been shown to suppress the DDR, suggesting the existence of a feedforward loop linking DNA damage to type I IFN secretion, which would be instrumental for the control of viral infection{Liu, 2018, 30356214}. PARP1 has also been shown to limit type I IFN induction by the RNA sensor DExD/H-box helicase 58 (DDX58, best known as RIG-I){Ghosh, 2018, 29590171}, but the precise molecular mechanisms remain to be fully elucidated. Importantly, several sensors of cytosolic nucleic acids other than CGAS and RIG-I have been shown to support type I IFN secretion by stressed or infected cancer cells, including adenosine deaminase RNA-specific (ADAR) and Z-DNA binding protein 1 (ZBP1) (Ref. {Vanpouille-Box, 2019, 31554927}). The actual role of these systems in type I IFN secretion downstream of DDR inhibition, however, remains obscure.

A fraction of tumors exhibits alterations in multiple components of the DDR{Pilie, 2019, 30356138}. This not only favors genomic instability, but also confers sensitivity to immunotherapy with immune checkpoint blockers, potentially as a consequence of superior type I IFN signaling in the TME{Heijink, 2019, 30626869;Turajlic, 2019, 30918367}. Conversely, tumors with a proficient DDR as conferred by the overexpression of various DDR-relevant kinases exhibit increased radioresistance downstream of robust DNA repair coupled to limited type I IFN signaling. Along similar lines, proficient DDR signaling via ATR and CHEK1 favors the upregulation of CD274 (best known as PD-L1) on the surface of irradiated cells, ultimately promoting the exhaustion of cytotoxic T lymphocytes via programmed cell death 1 (PDCD1, best known as PD-1){Sato, 2017, 29170499;Vendetti, 2018, 29952768}. Altogether, these observations suggest that the antiapoptotic phase of the DDR not only limits tumor infiltration by APCs and their *in situ* maturation, but also supports the inhibition or exhaustion of tumor-infiltrating $CD8^+$ T cells.

The DDR driven by RT can also mediate immunostimulatory effects{Wilkins, 2019, 30632153}. Indeed, irradiated cells expose a variety of NK cell-activating ligands (NKALs) on their surface as a consequence of ROS generation and activation of DDR kinases including ATM, ATR and CHEK1{Gasser, 2005, 15995699;Soriani, 2009, 19098271}. Upon binding to killer cell lectin-like receptor K1 (KLRK1, best known as NKG2D) or CD226 (best known as DNAM-1) on the surface of NK cells, membrane-bound NKALs (but not their soluble counterparts) support antigen-independent NK cell activation against irradiated cells, especially when the latter express low amounts of MHC Class I molecules{Lopez-Soto, 2017, 28810142}. Numerous tumor types overexpress matrix metalloproteases that cleave NKALs as a mechanism to avoid NK cell cytotoxicity{Lambrecht, 2018, 30242265}. Robust NK cell activation can reconfigure both the primary and metastatic TME via at least three distinct mechanisms. First, NK cells are well known for their ability to limit metastatic dissemination and seeding{Lopez-Soto, 2017, 28810142}. Second, NK cells are particularly active against cancer stem cells (CSCs){Grossenbacher, 2016, 27096096}, which are intrinsically radioresistant due to a robust DDR{Vitale, 2017, 28475867}. Third, activated NK cells secrete high levels of C-C motif chemokine ligand 5 (CCL5), X-C motif chemokine ligand 1 (XCL1) and fms-related tyrosine kinase 3 ligand (FLT3LG) to recruit conventional type I dendritic cells (cDC1) to the tumor microenvironment{Bottcher, 2018, 29429633}. Of note, RT also favors the expression of NK cell-inhibitory MHC Class I molecules{Son, 2016, 27671170}. However, whether MHC Class I upregulation by RT directly depends on the DDR remains unclear. Irrespective of this uncertainty, the ultimate ability of the DDR driven by RT to activate NK cells in the TME at least in part originates from the balance between NKALs, NKAL-specific metalloproteases and MHC Class I molecules.

NF-κB signaling. One of the major cytoprotective pathways driven by RT culminates with the nuclear translocation of active NF-κB dimers{Taniguchi, 2018, 29379212} (**Box 2**). NF-κB is sensitive to a variety of intracellular conditions imposed by RT, including DNA damage – via DDR kinases{He, 2017, 28586028} as well as cytosolic CGAS-STING activation{Ishikawa, 2008, 18724357} – and oxidative stress{Nakajima, 2013, 23792277}. Moreover, NF-κB signaling in the irradiated TME can be initiated downstream of the RT-driven secretion of cytokines including tumor necrosis factor (TNF) and interleukin 1 beta (IL1B){Janus, 2018, 29476964}. The transcriptional programs coordinated by NF-κB exhibit considerable heterogeneity depending on both activation pathway (**Box 2**) and cell type{Taniguchi, 2018, 29379212}. That said, the core signaling modules initiated by NF-κB can be identified in: (1) an anti-apoptotic module encompassing BCL2 apoptosis regulator (BCL2) and CASP8 and FADD like apoptosis regulator (CFLAR), (2) an immunostimulatory module involving numerous cytokines (*e.g.*, IL6, TNF) and chemokines (*e.g.*, IL18, CXCL10), (3) an inflammatory module linked to prostaglandin-endoperoxide synthase 2 (PTGS2, best known as COX2) and nitric oxide synthase 2, inducible (NOS2), as well as (4) a cellular adhesion module involving intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion molecule 1 (VCAM1), amongst other components{Taniguchi, 2018, 29379212}.

Besides favoring radioresistance in malignant cells, transactivation of anti-apoptotic BCL2 family members by NF-κB supports the survival of tumor-infiltrating CTLs and $CD4^+CD25^+FOXP3^+$ regulatory T (T_{REG}) cells{Opferman, 2003, 14668867}. Moreover, anti-apoptotic BCL2 family members promote oxidative phosphorylation over glycolysis in a variety of cell types{Alavian, 2011, 21926988}. This may influence the immunological configuration of the TME not only because oxidative phosphorylation is required for optimal T cell memory responses{Bantug, 2018, 28944771}, but also because a decreased flux though glycolysis results in reduced secretion of lactate, hence de-inhibiting anticancer CTL responses{Corbet, 2017, 28912578}. Of note, canonical NF-κB signaling (**Box 2**) in the TME also

increases the radioresistance of cancer cells by favoring ATM upregulation via IL6{Chen, 2015, 26572130}.

Globally, the immunomodulatory factors synthesized and released downstream of NF-κB activation facilitate the establishment of an immunostimulatory TME dominated by mature DCs, T_H1 $CD4^+$ T cells, and robust cytotoxic lymphoid functions{Taniguchi, 2018, 29379212}. Moreover, canonical NF-κB signaling contributes to the establishment of immunological memory upon antigenic challenges, promotes optimal NK cell activation, favors the polarization of macrophages towards an M1-like immunostimulatory profile, limits the generation of tolerogenic DCs, and supports type I IFN secretion by irradiated DCs{Hou, 2018, 30170810;Taniguchi, 2018, 29379212}. Conversely, non-canonical NF-κB signaling (**Box 2**) appears to support metastatic tumor dissemination{Bakhoum, 2018, 29342134} and to suppress type I IFN secretion by irradiated DCs, largely reflecting a reduced occupancy of the *IFNB1* promoter by RELA proto-oncogene, NF-kB subunit (RELA){Hou, 2018, 30170810}. Intriguingly, a robust immunosuppressive function has recently been attributed to REL proto-oncogene, NF-kB subunit (REL), reflecting its key importance for the development and function of T_{REG} cells{Grinberg-Bleyer, 2017, 28886380}. Similar observations, however, could not be made for RELA{Grinberg-Bleyer, 2017, 28886380}, suggesting that REL may mediate immunosuppressive effects in the TME independent of canonical NF-κB signaling. Taken together, these findings suggest that RT-driven NF-κB signaling has a complex impact on the immunological configuration of the TME.

Autophagy. Macroautophagy (herein referred to as autophagy) is a cytoprotective mechanism that involves the sequestration of dispensable or cytotoxic cytoplasmic material by double-membraned organelles (so-called autophagosomes) that transport such material to lysosomes for degradation{Galluzzi, 2017, 28596378}. Autophagy can be activated by numerous perturbations of

cellular homeostasis, including RT (at least in part linked to ATM activation by ROS in the context of the DDR){Guo, 2010, 20966255}. In malignant cells, proficient autophagic responses support radioresistance by limiting ROS generation by mitochondria, boosting antioxidant defenses via nuclear factor, erythroid 2 like 2 (NFE2L2, best known as NRF2), and enhancing the ability of irradiated cells to repair DNA{Rybstein, 2018, 29476153}. Along similar lines, autophagy is critical for the survival and functions of various immune cell populations of the TME, including DCs, T cells, NK cells and TAMs{Clarke, 2019, 30531943}.

At the same time, proficient autophagic responses limit inflammatory signaling in the TME by multiple mechanisms. First, autophagy efficiently disposes of CGAS-activating micronuclei and DNA fragments emerging after RT{Lan, 2014, 25284779;Bartsch, 2017, 29016854}. Second, STING is targeted by autophagic responses initiated by one of its signal transducers, *i.e.*, TANK binding kinase 1 (TBK1), in the context of negative regulatory feedback{Prabakaran, 2018, 29496741}. Third, STING can be inactivated upon phosphorylation by unc-51 like autophagy activating kinase 1 (ULK1), an autophagy activator that can also be stimulated by CGAS{Konno, 2013, 24119841}. Fourth, the selective degradation of mitochondria permeabilized by RT restrains the cytoplasmic availability of mitochondrial DNA (mtDNA) and ROS, which would otherwise activate the NLRP3 inflammasome to stimulate IL1B and IL18 secretion{Nakahira, 2011, 21151103}, as well as CGAS-STING signaling{Sliter, 2018, 30135585}. Thus, successful autophagic responses to RT not only preserve cellular viability, but maintain the TME in a largely immunosuppressed status.

Of note, cancer cells and CAFs mounting proficient autophagic responses can release a large panel of nutrients that support cellular viability as they rewire the nutritional network of the TME. These nutrients include ATP, alanine, arginine, lactate, and ketone bodies{Rybstein, 2018, 29476153}. Although attention has previously been centered on malignant cells as the recipients of autophagy-derived

nutritional support, it is now clear that immune cells populating the TME must subsist in the context of a fierce metabolic competition{Bantug, 2018, 28944771}. Thus, increased nutrient availability downstream of autophagy activation in cancer cells and CAFs is expected to benefit, at least to some extent, some cellular compartments of the TME other than malignant cells. Autophagic CAFs have also been reported to favor pancreatic tumor progression as a consequence of IL6 release{Endo, 2017, 28126348}. Whether this pathway can be actually initiated by RT remains unclear.

In summary, while early DDR signaling, NF-κB-dependent transcription, and successful autophagic responses all share robust anti-apoptotic effects, they may have a differential impact on the immunological configuration of the TME (**Table 1**). This largely reflects the diametrically opposed influences of NF-κB and autophagy on the inflammatory TME milieu, as well as the capacity of a proficient DDR to limit the accumulation of immunostimulatory DNA in the cytosol of irradiated cells (**Figure 2**).

Cytotoxic signals elicited by radiation

Over time, cellular TME compartments experiencing irreparable damage upon irradiation initiate an irreversible cascade of events leading to their demise, most often in the context of apoptotic caspase activation (**Box 3**). Importantly, the signaling pathways that promote apoptotic RCD elicited by RT can relay signals to the extracellular milieu to restructure the TME{Galluzzi, 2018, 30305710}.

Failing DDR. Irreparable DNA damage drives apoptosis largely upon the stabilization of tumor protein p53 (TP53, best known as p53) (**Box 4**), resulting in a series of transcriptional and post-transcriptional events that drive irreversible mitochondrial outer membrane permeabilization (MOMP) or cellular senescence{Hafner, 2019, 30824861}. It has been suggested that the extent of DNA damage caused by RT dictates the ultimate fate (*i.e.*, apoptosis *versus* senescence) of cells experiencing DDR-driven p53 activation. Malignant cells generally differ from non-transformed components of the TME as they generally manifest *TP53* mutations or other alternations of p53 signaling that render them relatively insensitive to RT{Hafner, 2019, 30824861}. However, multiple other factors appear to be at play in this context, including (but not limited to) the status of other genes involved in cellular senescence such as RB transcriptional corepressor 1 (*RB1*, encoding a key negative regulator of the G_1-S transition) and cyclin dependent kinase inhibitor 2A (CDKN2A, encoding two major cell cycle inhibitors, *i.e.*, $p16^{Ink4a}$ and $p14^{Arf}$), which are lost in some tumors{Pei, 2006, 16965958}, as well as autophagic competence{Dou, 2015, 26524528}. Irrespective of this unresolved question, p53 activation in irradiated cells not only determines their fate, but also underlies the emission of multiple signals that impinge on the immunological configuration of the TME{Munoz-Fontela, 2016, 27667712}.

p53-orchestrated transcriptional programs exhibit considerable variation across cell type, but can involve the upregulation of: (1) immunostimulatory cytokines involved in the so-called senescence-associated secretory phenotype (SASP), such as C-C motif chemokine ligand 2 (CCL2){Acosta, 2013, 23770676}; (2) components of the type I IFN signaling pathway, like IFN regulatory factor 5 (IRF5), IRF9, eukaryotic translation initiation factor 2 alpha kinase 2 (EIF2AK2, best known as PKR), and ISG15 ubiquitin like modifier (ISG15){Mori, 2002, 11973653}; (3) NKALs including UL16 binding protein 1 (ULBP1) and ULBP2{Iannello, 2013, 24043758}; (4) MHC Class I molecules{Wang, 2013, 23965983}; (5) pro-phagocytic and immunomodulatory receptors such as V-set immunoregulatory receptor (VSIR, best known as VISTA){Yoon, 2015, 26228159}; (6) Fas cell surface death receptor (FAS), which is involved in CTL-dependent cancer cell killing{Fulda, 1998, 10203687}. Altogether, these proteins cooperate to establish an inflammatory TME that supports macrophage-dependent phagocytosis but also increases the sensitivity of cancer cells to NK cell- or CTL-dependent lysis{Munoz-Fontela, 2016, 27667712}. Importantly, the same does not hold true for baseline p53 activity in non-transformed tissues, which exerts major anti-inflammatory effects to support tissue homeostasis{He, 2015, 26565902;Lujambio, 2013, 23562644}. Moreover, basal p53 activation inhibits autophagy{Rybstein, 2018, 29476153} and supports bioenergetic metabolism to limit ROS production{Kruiswijk, 2015, 26122615}. Taken together, these observations suggest that the activity of p53 shifts from largely anti-inflammatory (in support of cellular homeostasis) to cytotoxic and pro-inflammatory (in support of systemic homeostasis) upon activation beyond a threshold level. Further complicating the picture, *TP53* can be transactivated by type I IFN signaling{Kim, 2006, 16513254}, at least in non-transformed cells of the TME that most likely exhibit intact p53 signaling. However, in the absence of adequate post-translational modifications that drive full-blown p53 activation, increased p53 levels may not be sufficient to initiate pro-inflammatory responses.

Failing autophagy and UPR. Irradiated cells activate both the UPR and autophagy to cope with genetic and metabolic stress. While successful autophagic responses are known to mediate robust anti-inflammatory effects in the TME (see above), little is known about the immunological correlate of a successful UPR. Conversely, it is clear that both autophagy and the UPR are required for lethally irradiated cancer cells to emit DAMPs that recruit APCs to the TME and activate them, culminating in the initiation of adaptive anticancer immunity{Galluzzi, 2017, 27748397}. For instance, cancer cells succumbing to immunogenic cell death (ICD) driven by RT and other forms of irradiation release high amounts of ATP, which operates not only as a chemoattractant of APCs or their precursors upon binding to purinergic receptor P2Y2 (P2RY2){Ma, 2013, 23562161;Michaud, 2011, 22174255} but also as an immunostimulatory cue upon binding to purinergic receptor P2X 7 (P2RX7), resulting in IL1B secretion{Ghiringhelli, 2009, 19767732}. Importantly, activation of (ultimately unsuccessful) autophagic responses in the course of ICD is key for the cellular ATP pool to be preserved and to be available for release in the final steps of cellular demise{Michaud, 2011, 22174255}. Extracellular ATP can rapidly be degraded by the sequential activity of ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1, best known as CD39) and 5'-nucleotidase ecto (NT5E, best known as CD73), resulting in the accumulation of immunosuppressive adenosine{Di Virgilio, 2018, 30006588}. This implies that the original configuration of the TME in terms of CD39 and CD73 expression has a major impact on the ultimate immunological effects of ATP release in the TME.

RT-driven ICD is also associated with the exposure of various endoplasmic reticulum (ER) chaperones on the cell surface, where they promote the phagocytic activity of APCs{Golden, 2014, 25071979}. Exposure of ER chaperones such as calreticulin (CALR) on the surface of cancer cells undergoing RT-driven ICD requires the phosphorylation of eukaryotic translation initiation factor 2 subunit alpha (EIF2S1, best known as eIF2α) by EIF2AK3 (best known as PERK), which is one the key events of the UPR in mammalian cells{Panaretakis, 2009, 19165151}. Intriguingly, it seems that other signaling

events normally involved in the UPR, including the activation of transcriptional programs driven by activating transcription factor 4 (ATF4) ATF6, and mature X-box binding protein 1 (XBP1) are dispensable for ICD-associated CALR exposure{Bezu, 2018, 29358668}. To what extent such a dissociation of the UPR influences the irradiated TME, as yet, remains unclear. Importantly, activation of the UPR in DCs and T lymphocytes have been shown to mediate robust immunosuppressive effects by a metabolic effect{Song, 2018, 30305738;Cubillos-Ruiz, 2015, 26073941}. It remains to clarified whether DCs and T cells mount robust UPRs upon irradiation.

Caspase activation. Although caspases have been traditionally subdivided into a purely inflammatory group (CASP1, CASP4, CASP5, CASP11) and an exquisitely apoptotic group (CASP2, CASP3, CASP6, CASP7, CASP8, CASP9, CASP10, CASP12), it is now clear that such as a distinction must be abandoned{Galluzzi, 2016, 26885855}. On the one hand, inflammatory caspases have been mechanistically involved in the execution of non-apoptotic RCD by pyroptosis. On the other hand, apoptotic caspases have been shown to regulate key immunological manifestations of cell death{Galluzzi, 2016, 26885855}. For instance, activation of CASP8 downstream of death receptor signaling, which is one of the apoptotic pathways driven by RT in the TME, mediates robust immunostimulatory effects by favoring CALR exposure on the membrane of dying cells{Panaretakis, 2009, 19165151} and by promoting the activation of the NLRP3 inflammasome (and hence supporting IL1B and IL18 secretion){Van Opdenbosch, 2019, 31216460}. Apparently at odds with this notion, CASP8 also prevents the activation of necroptosis, a particularly inflammatory RCD modality (**Box 5**), by catalyzing the cleavage of receptor interacting serine/threonine kinase 3 (RIPK3){Kang, 2013, 23260196}. However, the contribution of necroptosis to RT-driven RCD seems to be marginal (see

below), implying that CASP8 activation in the radiosensitive compartment of an irradiated TME is to be considered mostly pro-inflammatory.

Conversely, the MOMP-dependent activation of CASP3 and other executioner caspases has major anti-inflammatory effects. In particular, CASP3 activation is responsible for the release of lysophosphatidylcholine by dying cells and the exposure of phosphatidylserine (PS) on their surface, which cumulatively recruit macrophages as they favor the PS-dependent clearance of cell corpses in the context of immunosuppressive signals{Nagata, 2018, 29400998}. Moreover, CASP3 limits type I IFN secretion by cells undergoing RT-driven RCD{Rodriguez-Ruiz, 2019 #277}, most likely because it accelerates the terminal inactivation of cells experiencing MOMP-dependent CGAS-STING signaling{Buqué, 2019 #278;White, 2014, 25525874} and proteolytically inactivates CGAS{Ning, 2019, 30878284}. CASP3 activation in irradiated cancer cells also supports the biosynthesis of prostaglandin E_2, which not only mediates robust immunosuppressive effects{Zelenay, 2015, 26343581}, but also favors the proliferation of radioresistant cells{Huang, 2011, 21725296} and neoangiogenesis{Feng, 2015, 26431328}, as well as the proteolytic inactivation of the DAMP IL33{Luthi, 2009, 19559631}. Recent data indicate that CASP3 activation by some chemotherapeutics can also mediate immunostimulatory effects as it cleaves gasdermin E (GSDME) to drive pyroptosis{Wang, 2017, 28459430} (**Box 5**). It remains to be determined whether this pathway is relevant for the reconfiguration of the TME by RT.

In summary, failing DDR, UPR and autophagic responses ultimately leading to apoptotic RCD are key for lethally irradiated cancer cells to relay danger signals to the TME via membrane-exposed and secreted factors (**Table 2**). In this setting, while CASP8 appears to support RT-driven inflammation, CASP3

mediates robust immunosuppressive functions and hence stands out as a potential target for the development of RT regimens with improved immunological activity (**Figure 3**).

Non-apoptotic cell death signals driven by radiation

At least in some settings, non-apoptotic signaling pathways culminating in RCD, including mitotic catastrophe (MC), mitochondrial permeability transition (MPT)-driven regulated necrosis, necroptosis, ferroptosis, pyroptosis and parthanatos (**Box 5**), may be involved in the cytotoxic effects of RT. Each of these RCD-related processes is associated with the release of bioactive factors that can alter the configuration of the TME.

Mitotic catastrophe. Although MC cannot be considered a form of RCD *sensu stricto*, cancer cells receiving RT at therapeutic doses often undergo MC as a prelude to their terminal fate, especially in the presence of p53 defects{Vitale, 2011, 21527953}. Depending on a variety of factors including the duration of mitotic arrest imposed by RT or its genotoxic effects, such fate encompasses apoptotic RCD, necrotic RCD, cellular senescence as well as the recovery of proliferation{Vitale, 2011, 21527953}. In this latter scenario, cancer cells surviving MC generally acquire extensive karyotypic abnormalities (*i.e.*, aneuploidy, hyperploidy) often coupled to chromosomal instability (CIN){Vitale, 2011, 21072053}, which is generally accompanied by robust tumor infiltration by cytotoxic T cells. At least in part, this reflects the ability of micronuclei formed in the context of RT-driven MC to activate robust CGAS-STING signaling via TBK1 and IRF3, and hence favor the abundant secretion of type I IFN{Mackenzie, 2017, 28738408;Harding, 2017, 28759889}. In this context, both three prime repair exonuclease 1 (TREX1), a cytosolic exonuclease activated by high RT doses{Vanpouille-Box, 2017, 28598415}, and autophagy (reflecting the ability of autophagy to dispose of micronuclei){Bartsch, 2017, 29016854} limit the immunogenic potential of MC. Hyperploid cells escaping MC secrete IL2 and spontaneously expose NKALs and CALR on the plasma membrane, thus being more prone to immunosurveillance than their euploid counterparts{Senovilla, 2012, 23019653;Acebes-Huerta, 2016, 27057443}. Conversely,

aneuploid cancer cells tend to exhibit increased metastatic potential as a consequence of non-canonical NF-κB signaling downstream of indolent CGAS activation{Bakhoum, 2018, 29342134} together with a poorly immunostimulatory phenotype{Davoli, 2017, 28104840}. Altogether, these observations suggest that MC is associated with the establishment of an immunostimulatory TME that favors the eradication of cells with mitotic issues. In line with this notion, cancer cells escaping MC and resuming proliferation as aneuploid entities exhibit increased immunosuppressive potential, at least in part reflecting a selection process imposed by the TME.

MPT-driven regulated necrosis. Cancer cells succumbing to MPT-driven RCD present necrotic morphological features coupled to the release of pro-inflammatory factors including ROS in their microenvironment{Vanden Berghe, 2014, 24452471}. Initial findings based on the chemical MPT inhibitor cyclosporin A appeared to link the MPT-related loss of intracellular Ca^{2+} compartmentalization with inflammasome activation and consequent IL1B secretion{Murakami, 2012, 22733741}. However, the deletion of peptidylprolyl isomerase F (*Ppif*), coding for the only key regulator of MPT-driven RCD identified so far, fails to inhibit inflammasome activation in bone marrow-derived macrophages{Allam, 2014, 24990442}. Along similar lines, MPT was initially suggested to underlie the accumulation of CGAS-activatory mtDNA in the cytosol{Nakahira, 2011, 21151103;Patrushev, 2004, 15583871}. However, mtDNA herniation though BCL2 associated X, apoptosis regulator (BAX) and BCL2 antagonist/killer 1 (BAK1) oligomers appears to constitute the major mechanism for the CGAS activation seen after mitochondrial permeabilization{McArthur, 2018, 29472455}, although a role for secondary MPT in this context remains plausible{Riley, 2018, 30049712}. MPT is expected to favor the release of numerous other mitochondrial components potentially operating as DAMPs, including ATP and

cardiolipin{Galluzzi, 2012, 23175281}. However, the relatively rapid nature of MPT-driven regulated necrosis may compromise, at least in part, the global immunogenicity of the process{Buqué, 2019 #278}.

At least theoretically, MPT can be activated by numerous intracellular conditions associated with irradiation, notably ROS accumulation and cytosolic Ca^{2+} overload{Vanden Berghe, 2014, 24452471}. Moreover, the DDR-driven activation of p53 has been linked to the induction of MPT in some settings{Vaseva, 2012, 22726440}. However, virtually no reports mechanistically link RT-driven RCD to MPT-driven necrosis, suggesting that MPT-driven necrosis may not play a major role in the ability of RT to reshape the TME.

Necroptosis. Although cancer cells often express CASP8 (which inhibits necroptosis) and reduced levels of RIPK3 and MLKL (**Box 5**), necroptosis stands out as a prominently pro-inflammatory RCD modality{Galluzzi, 2018, 29362479}, potentially culminating with increased tumor infiltration by both myeloid cells and CTLs{Snyder, 2019, 31227597}. This is not linked to rapid plasma membrane permeabilization, which is expected to mediate immunosuppressive effects upon a surge of extracellular potassium{Eil, 2016, 27626381}, but reflects the ability of RIPK1 and RIPK3 to favor CALR exposure and ATP release from dying cancer cells{Aaes, 2016, 27050509}, and that of RIPK1 to activate NF-κB-dependent inflammation (independent of its kinase activity){Yatim, 2015, 26405229}. Depending on the experimental setting, however, NF-κB activation by the necroptotic machinery can also favor the establishment of an immunosuppressive TME as a consequence of PGE_2 and CXCL1 secretion, resulting in the expansion of the TAM and myeloid-derived suppressor cell (MDSC) compartment{Yan, 2018, 30012671;Wang, 2018, 30423296}. Of note, RIPK1 and RIPK3 have also been suggested to promote inflammasome activation, at least in the context of CASP8 defects and supraphysiological stimulation of

the pathway (with IAP inhibitors){Kang, 2013, 23260196}. To which extent this pro-inflammatory circuitry is operational in physiopathological settings remains to be determined.

Experimental evidence demonstrating that necroptosis participates in the cytotoxic activity of RT is scarce and largely based on the relatively non-specific RIPK1 inhibitor Nec-1{Nehs, 2011, 22136818;Das, 2016, 26684801}. In a majority of cancer cells, indeed, caspase activation by RT is expected to prevent necroptosis as a consequence of CASP8-dependent RIPK3 degradation{Kang, 2013, 23260196}. In line with this notion, chemical pan-caspase inhibitors have been shown to convert RCD driven by RT from an apoptotic to a largely necroptotic response associated with abundant release of DAMPs and immunostimulatory cytokines{Werthmoller, 2015, 25973681}. Moreover, multiple components of the molecular machinery for necroptosis have been suggested to promote radioresistance by favoring cytoprotective NF-κB-dependent transcriptional programs{Liu, 2016, 26959742}. As a standalone exception, genetic data pointed to necroptosis as the major RCD mechanism in non-small cell lung carcinoma (NSCLC) cells expressing high RIPK3 levels subjected to ablative hypofractionated radiation therapy{Wang, 2018, 29619976}. Thus, despite the largely immunostimulatory profile of necroptosis, whether it significantly contributes to the reconfiguration of the irradiated TME remains unclear. As a note, interferon gamma (IFNG), the major effector of CTL-dependent anticancer immunity, reportedly mediates necroptosis when cancer cells lack CASP8{Chen, 2019, 31217278}, which is common in tumors under robust immunosurveillance{Martincorena, 2017, 29056346}, but does so by promoting PS exposure{Chen, 2019, 31217278}. Ultimately, this may compromise the immunogenicity of the process. In this context, pharmacological caspase inhibitors may convert RCD elicit by RT from an apoptotic to a necroptotic response, hence increasing its immunogenicity.

Ferroptosis. Ferroptotic cell death has recently been associated with the release of DAMPs that had been previously implicated in RT-driven ICD, including the non-histone chromatin-binding protein high mobility group box 1 (HMGB1){Golden, 2014, 25071979}, via a mechanism that depends on an intact autophagic machinery{Wen, 2019, 30686534}. Moreover, it seems that part of the effector functions of $CD8^+$ CTLs against cancer cells reflects the ability of IFNG to trigger ferroptosis upon the inhibition of cystine uptake{Wang, 2019, 31043744}. However, chemical inhibition of system x_c by erastin (a prototypic trigger of ferroptosis) also results in *PTGS2* upregulation{Yang, 2014, 24439385}, potentially setting the stage for compensatory PGE_2-dependent immunosuppression in the TME.

Recent data indicate that RT promotes ferroptosis by inhibiting cystine uptake by solute carrier family 7 member 11 (SLC7A11), resulting in cytotoxic lipid peroxidation in cancer cells that can be further exacerbated by IFNG{Lang, 2019, 31554642}. Moreover, erastin has been reported to radiosensitize NSCLC cells{Pan, 2019, 30854078}, potentially as a consequence of synergistic ROS production{Basit, 2017, 28358377}. Along similar lines, some components of the DDR machinery including ATM, FA complementation group D2 (FANCD2) and BRCA1 associated protein 1 (BAP1) appear to interface with ferroptotic signaling based on their ability to modulate iron metabolism{Song, 2016, 27773819} and cystine uptake{Zhang, 2018, 30202049}. Conversely, the precise role of p53 in ferroptosis remains a matter of debate, with some reports suggesting an activatory function{Jiang, 2015, 25799988} and others for an inhibitory one{Xie, 2017, 28813679}. In summary, the data are still too immature to understand whether the ability of RT to reconfigure the immunological TME involves ferroptosis.

Pyroptosis. RT is a potent activator of the NLRP3 and AIM2 inflammasomes downstream of ROS production and cytosolic DNA accumulation{Fernandes-Alnemri, 2009, 19158676}, which not only drives the secretion of IL1B and IL18, but also primes the cellular machinery for pyroptotic RCD upon

gasdermin D (GSDMD) cleavage by inflammatory caspases{Jorgensen, 2017, 28138137}. Resident and bone marrow derived macrophages are very sensitive to pyroptosis induction by low-dose RT{Hu, 2016, 27846608;Liu, 2017, 28151471}, consistent with the ability of RT to skew the TAM configuration from a largely anti-inflammatory (M2-like) to a mostly pro-inflammatory (M1-like) profile{Klug, 2013, 24209604}. In line with this notion, RT-driven pyroptosis has been implicated in the hematopoietic toxicity of RT{Hu, 2016, 27846608}. Moreover, pyroptosis-associated inflammasome activation has been shown to limit CGAS-STING signaling in DCs and TAMs{Corrales, 2016, 26927800}, potentially linked to the genotoxic effects of ROS production (which is exacerbated in the context of inflammasome signaling as a consequence of mitochondrial disruption){Yu, 2014, 25313054}. Of note, cancer cells are relatively resistant to pyroptosis, perhaps with the exception of a CASP3- and GDSME-dependent form of pyroptosis triggered by DNA-damaging chemotherapeutics{Wang, 2017, 28459430}. Whether RT drives the CASP3-mediated cleavage of GSDME and consequent pyroptotic response in cancer cells has not been tested yet.

Parthanatos. Parthanatos-related PARP1 hyperactivation has been associated with (1) decreased glycolytic activity (and thus lactate secretion) upon NAD^+ depletion and consequent hexokinase 1 (HXK1) inhibition{Fouquerel, 2014, 25220464}, (2) abundant HMGB1 release, at least in part linked to altered HMGB1 acetylation{Yang, 2014, 25392528}, (3) decreased PD-L1 exposure on the plasma membrane{Jiao, 2017, 28167507}, as well as (4) limited type I IFN downstream of CGAS and RIG-I signaling, largely reflecting the role of PARP1 in the DDR{Chabanon, 2019, 30589644;Ghosh, 2018, 29590171}. Thus, parthanatos drives the HGMB1-dependent, Toll-like receptor 4 (TLR4)-mediated activation of the myeloid TME compartment{Apetoh, 2007, 17704786}, and supports the derepression of CTLs downstream of lactate shortage and limited PD-1 signaling, but may at the same time limit type

I IFN-dependent immunostimulation. Adding another layer of complexity to the system, CGAS and TREX1 promotes and inhibits, respectively, the genoprotective functions of PARP1 by physically interacting with the latter{Liu, 2018, 30356214;Christmann, 2010, 20511593}.

Of note, DNA-alkylating agents are the best characterized activators of parthanatos{Wang, 2016, 27846469}, whereas little is known on the actual implication of parthanatos in RT-driven RCD. PARP1 inhibitors are currently approved for the therapy of breast cancer with *BRCA1* or *BRCA2* mutations{Lord, 2016, 26775620}, and chemical inhibition of PARP1 has been extensively investigated as a strategy for radiosensitization{Michmerhuizen, 2019, 31413177}, largely reflecting the key role of PARP1 in the DDR. However, the robust immunomodulatory functions PARP1 suggest that, at least in some settings, parthanatos activation may enable superior RT efficacy. This possibility remains clinically unexplored.

In summary, it must be stressed that different variants of RCD may co-exist in the irradiated TME, which constitutes a prominent obstacle for the precise dissection of immunostimulation versus immunosuppression *in vivo*.

Differential activation of senescence and cell death by RT in the tumor microenvironment

One of the major mechanisms through which RT reconfigures the immunological TME reflects the differential radiosensitivity of its cellular components.

Cancer cells. The intrinsic radiosensitivity of malignant cells exhibits considerable degree of intra- and inter-cancer variability{Yard, 2016, 27109210}, which depends not only on cellular features, but also on microenvironmental factors{Good, 2013, 23850153}. The former encompass the ability of cancer cells to mount efficient DDR and UPR upon irradiation, their autophagic competence, their global capacity to buffer ROS, as well as their intrinsic propensity to undergo MOMP upon stress{Czabotar, 2014, 24355989}. This latter parameter, which is also known as the "apoptotic threshold" reflects the availability of anti-apoptotic members of the BCL2 family (**Box 1**) and their baseline occupancy by BH3 proteins{Czabotar, 2014, 24355989} (**Box 3**). One of the major cell-extrinsic factors influencing radiosensitivity is partial oxygen tension. Thus, tumor areas in which the vasculature is poorly organized tend to exhibit increased radioresistance as compared to near-to-normoxic zones{Hill, 2017, 27416998}.

Obviously, RT dose has a major influence on the intrinsic radiosensitivity of malignant cells. Thus, while high doses tend to cause a robust cytotoxic effects, low doses are prone to cause cellular senescence coupled with SASP acquisition{Faget, 2019, 31235879}. Although senescence involves a permanent proliferative arrest that may appear as a desirable therapeutic objective, multiple SASP components quench anticancer immunity as they favor T cell exclusion, such as transforming growth factor beta 1 (TGFB1) (Refs. {Mariathasan, 2018, 29443960;Tauriello, 2018, 29443964}), or promote the recruitment of myeloid cells that can acquire an immunosuppressive phenotype, such as CCL2 (Ref. {Coppe, 2010,

20078217}). Other components of the SASP act as mitogens, such as insulin like growth factor 1 (IGF1){Faget, 2019, 31235879}. Thus, at least in some settings, the SASP reconfigures the TME so that radioresistant cancer cells may resume proliferation. In line with this notion, removal of senescent cells improves the therapeutic response of malignant cells responding to DNA damaging agents *in vivo*{Demaria, 2017, 27979832}. However, whether senolysis can be employed as a strategy to boost radiosensitivity remains to be elucidated.

Endothelial cells. Single-fraction RT doses >5-10 Gy provoke vascular dysfunction associated with endothelial cell death{Maeda, 2017, 27816364}. At least partially, this originates from the limited capacity of the tumor endothelium to undergo RT-driven senescence as efficiently as its normal counterpart{Lafargue, 2017, 28431961;Wang, 2016, 27387862}, but does not appear to involve DDR-driven apoptotic RCD{Moding, 2014, 25036710}. Conversely, single-fraction RT doses < 5-10 Gy generally loosen inter-endothelial cell junctions, resulting in increased vessel permeability{Park, 2012, 22229487}. Moreover, low RT doses (< 1Gy) have been associated with endothelial cell activation and neoangiogenesis in support of tumor growth and metastatic dissemination{Sofia Vala, 2010, 20574535}. However, neoangiogenesis driven by low RT doses may not originate from the response of endothelial cells to irradiation only, but may involve cytokine signaling from other cellular components of the TME, including TAMs{Lerman, 2010, 20631377}.

These observations have at least two major implications for the configuration of the irradiated TME. On the one hand, RT delivered in low doses (but high enough not to drive neoangiogenesis) is expected to improve the ability of circulating immune cells to infiltrate the TME{Guipaud, 2018, 29630386}, potentially supporting RT-driven anticancer immunity. Moreover, endothelial cells activated by (but not succumbing to) low-dose RT express increased levels of adhesion molecules that favor immune cell

extravasation and activation, including ICAM1 and VCAM1{Liao, 2013, 23485580}. On the other hand, high-dose RT is expected to mediate a robust vascular dysfunction leading to nutrient shortage, and hypoxia, but also reduced infiltration by immune cells{Guipaud, 2018, 29630386} and their limited activation (which is normally supported by endothelial cells){Hendry, 2016, 28066431}. In this setting, while nutrient shortage favors the therapeutic activity of RT, as demonstrated *in vivo*, in human xenograft models concomitantly receiving RT and angiogenesis inhibitors{Fokas, 2012, 22452803}, hypoxia and limited immune activation may (at least partially) offset it. These considerations support the emerging notion that the optimal therapeutic efficacy may involve the use of doses and fractionation schedules that are compatible with the engagement of circulating immune cells{Deutsch, 2019, 31364597} while exploiting the beneficial effects of RT against the tumor vasculature. Of note, endothelial cells undergo a robust UPR upon irradiation{Kim, 2014, 24456547}, but whether this culminates in DAMP emission is unclear. Lymphatic endothelial cells can also respond to RT by upregulating ICAM1 and VCAM1, or cause transient lymphedema that impinges on leukocyte trafficking to the TME, but the precise immunological consequences of these processes remain uninvestigated{Rodriguez-Ruiz, 2017, 28068246}.

CAFs. CAFs are the most abundant cells of the TME, and generally support disease progression via nutritional and immunological mechanisms{Kalluri, 2016, 27550820}. CAFs are extraordinarily resistant to RT delivered at clinically relevant doses, largely reflecting their limited tendency to proliferate, their ability to mount solid cytoprotective responses to radiation (including a proficient DDR), and their high apoptotic threshold{Kalluri, 2016, 27550820}. Thus, while CAFs receiving low-dose RT undergo a reversible cell cycle arrest that is instrumental to repair damage and elude RCD{Tommelein, 2018, 29217764}, high RT doses (> 12 Gy) predominantly drive CAFs into cellular

senescence (and less so RCD){Hellevik, 2012, 22500976}. Importantly, CAFs undergoing cellular senescence in response to RT largely preserve their tumor-supporting functions{Gorchs, 2015, 26029659}. In particular, senescent CAFs secrete high levels of TGFB1{Arshad, 2015, 25635683}, hence favoring the establishment of an immunosuppressive TME mediating T cell exclusion{Mariathasan, 2018, 29443960;Tauriello, 2018, 29443964}. Moreover the SASP of irradiated CAFs strongly promotes disease progression as it sustains cancer cell radioresistance, proliferation and metastatic potential by (1) stimulating cancer cell proliferation with cytokines and growth factors encompassing CXCL1, CXCL12, IGF1 and heparin binding growth factor (HDGF){Wang, 2019, 31101063}, (2) supporting the acquisition of migratory capacity by tumor cells via the so-called epithelial-to-mesenchymal transition (EMT){Arshad, 2015, 25635683}, and (3) favoring cytoprotective autophagic responses in malignant cells{Wang, 2017, 28258923}. Of note, the recruitment of bone marrow-derived mesenchymal stem cells to the bed of irradiated tumors appears to be instrumental for CAFs to acquire full-blown immunosuppressive functions{Shi, 2017, 27811929}. In line with this observations, CAF-related transcriptional signatures have been linked to radioresistance in patients with rectal carcinoma{Isella, 2015, 25706627}. In summary, CAFs stand out as major drivers in the establishment of an immunosuppressive TME upon irradiation, hence constituting a promising target for the development of novel strategies for radiosensitization{Vitale, 2019, 30998941}.

Lymphocytes. Circulating lymphocytes are exquisitely radiosensitive, which is linked to their high propensity to activate RCD in response to DNA damage{Schaue, 2012, 23050243}. However, the radiosensitivity of different lymphocyte subsets can vary significantly, linked not only to cellular identity, but also to differentiation and activation status{Schaue, 2012, 23050243}. Thus, while very low RT doses (0.1-0.5 Gy) drive RCD in a significant fraction of B lymphocytes and NK cells (largely as a consequence

of DDR-driven p53 activation){Moreno-Villanueva, 2019, 31083348}, other lymphocyte subsets including memory T cells, NKT cells and T_{REG} cells reportedly exhibit greater radioresistance{Yao, 2011, 21930972}. Along similar lines, $CD8^+$ T cells reportedly display higher radiosensitivity than their T_H1 $CD4^+$ counterparts{Bogdandi, 2010, 20726712}. At least in part, the increased radioresistance of antigen-experienced ($CD44^{Hi}$) and effector memory T cells as compared to their naïve ($CD44^{Lo}$) counterparts originates from activation-dependent alterations in DDR proficiency{Heylmann, 2018, 30323167}, chromatin accessibility{Pugh, 2014, 24990082}, and BCL2-dependent cytoprotection{Yao, 2011, 21930972}. Along similar lines, $CD4^+CD25^+FOXP3^+$ T_{REG} cells are less sensitive to RT than their $FOXP3^-$ counterparts owing to a comparatively higher apoptotic threshold{Qu, 2010, 20095846;Liu, 2015, 26807310}. Of note, it has been proposed that radiosensitive B and NK cells succumb to RT via apoptosis, while T cells do so by manifesting biochemical features of necrosis{Falcke, 2018, 30428512}. However, whether any specific form of regulated necrosis is mechanistically involved in the demise of irradiated T cells remains unclear.

Irrespective of this conundrum, mild to high RT doses have originally been suggested to deplete (at least some subsets of) tumor-infiltrating lymphocytes that may negatively impact therapeutic efficacy. Such an imbalance can be further aggravated by the differential capability of specific lymphocyte subsets (e.g., effector *versus* T_{REG} cells) to reconstitute their pools, although data in this respect remain contradictory{Belka, 1999, 10368044;Zheng, 2015, 26475064}. Along similar lines, the actual radiosensitivity of tumor-resident T cells has recently been questioned{Arina, 2019, 31477729}. Thus, as newly recruited T cells appear to exhibit superior sensitivity to RT, intensive fractionation schedules may not constitute an optimal therapeutic choice{Deutsch, 2019, 31364597}. Moreover, even in the context of optimal dosimetry and delivery volumes, normal tissues adjacent to malignant lesions receive a fraction of the RT dose due to scattering, which may be particularly detrimental for anticancer immune responses if tumor-draining lymph nodes are involved{Deutsch, 2019, 31364597}.

DCs. DCs are a rather scarce immunological component of the TME, but have a key role in the initiation of anticancer immunity{Mulder, 2019, 30967658}. Mature DCs are relatively radioresistant, at least in part owing to constitutive ATM activation in support of a proficient DDR{Bauer, 2011, 22160723}. According to some reports, irradiated DCs secrete increased amounts of pro-inflammatory cytokines including IL1B and IL12{Persa, 2018, 30110907;Chun, 2012, 23983665} and lower quantities of anti-inflammatory cytokines like IL10{Chun, 2012, 23983665}. However, DCs have also been reported to respond to RT by producing elevated amounts of anti-inflammatory cytokines at the expense of their pro-inflammatory counterparts{Merrick, 2005, 15812550}. Such an apparent discrepancy is likely to originate from (1) the use of highly heterogeneous experimental settings; (2) the use of different RT doses, as well as (3) the elevated biological heterogeneity of distinct DC populations{Alcantara-Hernandez, 2017, 29221729}. The same holds true for reports investigating the expression of maturation markers such as CD80, CD86 and MHC Class II molecules on the surface of irradiated DCs{Shigematsu, 2007, 17192700;Jahns, 2011, 21376737}. That said, DCs are quintessential for the ability of RT to mount a robust anticancer immune response with systemic outreach{Vanpouille-Box, 2017, 28598415}, implying that (at least at RT doses and fractionation schedule compatible with the initiation of abscopal responses) DCs not only survive irradiation, but also mature and migrate to lymph nodes and engage in cross-presentation. In this context, a major role is played by the ability of tumor-infiltrating DCs to take up DNA-containing exosomes released by irradiated cancer cells and produce type I IFN{Diamond, 2018, 29907693}.

TAMs. TAMs can constitute up to >50% of the immune tumor infiltrate, and can adopt a spectrum of functional states ranging from mostly immunostimulatory and tumoricidal (M1-like) to largely

immunosuppressive and tumor-supportive (M2-like){Cassetta, 2018, 30361552}. TAMs are generally considered as a rather radioresistant population of the TME, especially when they are polarized towards an M2-like state{Leblond, 2017, 29069812}. This largely reflects the ability of M2-like TAMs to mount a robust DDR and proficient anti-oxidant responses{Vitale, 2019, 31269428}. Of note, the irradiated TME produces multiple chemoattractants for circulating TAM precursors, including (but not limited to) CXCL12 and colony stimulating factor 1 (CSF1){Xu, 2013, 23418320;Wang, 2013, 23940516}.

On the one hand, RT can drive a robust wave of TAM infiltration in the context of hypoxia (see above), which is known to promote M2 polarization and exacerbate the ability of TAMs to establish local immunosuppression{Vitale, 2019, 31269428}. This is especially true for high single-fraction RT doses (which are also potent inducers of hypoxia){Maeda, 2017, 27816364}. Thus, high RT doses favor TAM-driven circuitries that elevate radioresistance, at least in part as a consequence of vascular endothelial growth factor A (VEGFA)-driven neoangiogenesis{Hughes, 2015, 26269531} and a global rewiring in the metabolic profile of the TME{Wenes, 2016, 27773694} associated with the abundant release of immunosuppressive metabolites including kynurenin{Labadie, 2019, 30377198}. Of note, the capacity of high-dose RT to drive the polarization of TAMs toward an M2-like state appears to be insensitive to fractionation{Tsai, 2007, 17398016}. Consistent with these observations, chemical inhibitors of CSF1 signaling, which prevent the recruitment of TAM precursors to the irradiated TME and favor M2-to-M1 TAM repolarization, have been shown to exert exquisite radiosensitizing effects *in vivo*{DeNardo, 2019, 30718830}. On the other hand, low and intermediate RT doses have been shown to favor the repolarization of M2-like TAMs into their M1-like counterparts, correlating with prominent therapeutic effects (at least in mice){Klug, 2013, 24209604}. Intriguingly, this may reflect (at least in part) the ability of repolarized TAMs to influence endothelial cell biology and angiogenesis via RNS{Nadella, 2018, 30035346}.

In summary, the relative radioresistance of CAFs and TAMs coupled to the comparatively higher radiosensitivity of endothelial cells, lymphocytes and DCs supports the establishment of a hypoxic and immunosuppressive TME upon high-dose RT. This places great emphasis on dose and fractionation as dials to control the immunological outcome of RT-driven cell death signaling in the TME.

Concluding remarks

Both cytoprotective and cytotoxic signals elicited by RT in different compartments of the TME can have a variety of immunomodulatory effects. As an additional layer of complexity, cell death signaling within malignant lesions varies considerably with multiple factors, including (but not limited to) tumor type, disease burden, vascularization and genetic make-up. Each of these variables can alter cytoprotective and cytotoxic signals elicited by RT not only in a direct manner (*e.g.*, malignant cells overexpressing BCL2 display increased radioresistance){Galluzzi, 2018, 29362479}, but also by influencing the composition of the TME (*e.g.*, *CTNNB1* hyperactivation favors T-cell exclusion){Luke, 2019, 30635339}. Considering the complex biology of the irradiated TME, multiple novel therapeutic avenues can be envisioned to foster the immunogenicity of RT in support of local and systemic efficacy. The ability of RT to alter the immunological configuration of the TME largely originates from the differential activation of cytoprotective and cytotoxic signal transduction pathways in malignant, stromal, endothelial and immune cells. This not only culminates in the release of chemoattractants and immunomodulatory factors by both radiosensitive and radioresistant cells, but also with the elimination of particularly radiosensitive cells, which altogether redefine the cellular and secretory profile of the tumor bed. Importantly, numerous biological processes are involved in this process. As a standalone example, RT is known for its ability to release bioactive TGFB1 from the extracellular matrix, which has major implications for the establishment of local immunosuppression and T cell exclusion and occurs irrespective of cell death signaling{Travis, 2014, 24313777}. For decades, considerable efforts have been dedicated towards the development of radiosensitizing agents that would simply increase the intrinsic sensitivity of malignant cells to DDR-driven apoptosis{Wang, 2018, 29224916}. The extensive involvement of immune cells in radioresistance suggests that altering the immunological consequence of RT-driven RCD may constitute an alternative and perhaps superior approach to clinical

radiosensitization. As preclinical data in support of these notions have begun to accumulate{Rodriguez-Ruiz, 2019 #277}, renewed attention should be given to the consequences of RT-driven cell death signaling on the immunological TME.

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Box 1. Major anti-apoptotic mechanisms in irradiated cells.

Besides preventing the activation of apoptosis by mounting a proficient DNA damage response (DDR) that enables DNA repair in the context of a reversible cell cycle arrest, both normal and malignant cells can resist the cytotoxic effects of radiation by harnessing core anti-apoptotic mechanisms that operate when the DDR is already promoting cell death. A key position in this setting is occupied by anti-apoptotic members of the Bcl-2 protein family, including BCL2 apoptosis regulator (BCL2), BCL2 like 1 (BCL2L1, best known as BCL-X_L) and MCL1 apoptosis regulator, BCL2 family member (MCL1). BCL2, BCL-X_L and MCL1 not only engage in inhibitory physical interactions with their pro-apoptotic counterparts to inhibit MOMP (see also **Box 3**) but also support bioenergetic metabolism and Ca^{2+} homeostasis, *de facto* mediating multipronged cytoprotective effects. CASP8 and FADD like apoptosis regulator (CFLAR, best known as c-FLIP) also supports the survival of irradiated cells, largely reflecting its ability to interrupt lethal signals originating from death receptors. Indeed, although the latter are not systematically involved in regulated cell death (RCD) driven by radiation therapy (RT), TNF receptor superfamily member 1A (TNFRSF1A) has been reported to support the cytotoxicity of RT downstream of tumor necrosis factor (TNF) secretion by irradiated cells. Finally, irradiated cells can prevent (at least to some degree) the potentially cytolytic activation of the caspase cascade harnessing various proteins of the baculoviral IAP repeat containing (BIRC) family, including (but not limited to) BIRC2, BIRC3, and X-linked inhibitor of apoptosis (XIAP). Besides directly binding to (and hence inhibiting the proteolytic activity of) caspases, BIRC proteins favor canonical NF-κB signaling downstream of TNFRSF1A activation (see also **Box 2**), which amongst other targets, involves the upregulation of both BCL2 and CFLAR{Singh, 2019, 30655609;Galluzzi, 2018, 29362479}.

Box 2. Principles of NF-κB signaling.

NF-κB activation can be initiated by two non-mutually exclusive signal transduction cascades, which are commonly referred to as "canonical" and "non-canonical". Canonical NF-κB signaling is demarcated by the activation of mitogen-activated protein kinase kinase kinase 7 (MAP3K7, best known as TAK1) and consequent engagement of a heterotrimeric complex involving component of inhibitor of nuclear factor kappa B kinase complex (CHUK, best known as IKKα), inhibitor of nuclear factor kappa B kinase subunit beta (IKBKB, best known as IKKβ) and inhibitor of nuclear factor kappa B kinase subunit gamma IKBKG (best known as NEMO), which is cumulatively known as IKK. Upon TAK1-dependent phosphorylation of IKKβ, IKK acquires the ability to catalyze the phosphorylation of NFKB inhibitor alpha (NFKBIA; best known as IκB), culminating in IκB proteasomal degradation and consequent derepression of heterodimers consisting of mature nuclear factor kappa B subunit 1 (NFKB1; also known as p50) and either RELA proto-oncogene, NF-kB subunit (RELA; also known as p65) or REL proto-oncogene, NF-kB subunit (REL). Mature p50 and REL can also exist in the cytoplasm in complex with the p50 precursor p105, which undergoes partial proteasomal breakdown upon IKK activation to favor the emergence of transcriptionally active p50/REL and p50/p50 dimers. Irrespective of binding partner (i.e., p65, REL or p50), p50 orchestrates canonical NF-κB transcriptional programs (prototypic target: tumor necrosis factor, *TNF*). Non-canonical NF-κB signaling is initiated by MAP3K14 (best known as NIK), which stimulates the catalytic activity of IKKα dimers. This drives the IKKα-dependent phosphorylation of nuclear factor kappa B subunit 2 (NFKB2; best known as p100) complexed with RELB proto-oncogene, NF-kB subunit (RELB), followed by partial p105 degradation by the proteasome and consequent activation of non-canonical NF-κB transcriptional programs by p52/RELB heterodimers (prototypic target: C-X-C motif chemokine ligand 13, *CXCL13*){Sun, 2017, 28580957}.

Box 3. Major pro-apoptotic mechanisms in irradiated cells.

Normal as well as malignant cells exposed to radiation therapy (RT) accumulate DNA lesions that – above a minimal threshold for detection – initiate repair via the DNA damage response (DDR). When such lesions exceed the repair capacity of the cell, however, the DDR switches from a mostly cytoprotective mechanism aimed at the DNA repair and the recovery of cellular homeostasis to a signal transduction pathway driving apoptotic cell death. In this setting, the DDR kinases ATM serine/threonine kinase (ATM) and ATR serine/threonine kinase (ATR) acquire the ability to phosphorylate checkpoint kinase 2 (CHEK2) and CHEK1, respectively, hence initiating the stabilization of tumor protein p53 (TP53, best known as p53) (see also **Box 4**). Active p53 mediates pro-apoptotic functions largely by orchestrating a transcriptional program involving the overexpression of various pro-apoptotic Bcl-2 family members, including (but not limited to) BCL2 associated X, apoptosis regulator (BAX), BCL2 binding component 3 (BBC3, best known as PUMA), and phorbol-12-myristate-13-acetate-induced protein 1 (PMAIP1, best known as NOXA). Altogether, these proteins offset the cytoprotective functions of anti-apoptotic Bcl-2 proteins (see also **Box 1**) to mediate mitochondrial outer membrane permeabilization (MOMP) and the consequent release of potentially cytotoxic proteins into the cytosol. In particular, cytosolic cytochrome c, somatic (CYCS) drives the dATP-dependent assembly of a supramolecular platform involving apoptotic peptidase activating factor 1 (APAF1) and caspase 9 (CASP9), which is commonly known as the apoptosome. In the molecular context provided by the apoptosome, CASP9 undergoes proximity-induced activation, hence acquiring the ability to proteolytically activate CASP3 and setting off a series of irreversible proteolytic cascades that precipitate apoptotic cell death{Tait, 2010, 20683470}. Importantly, at least in mammals, apoptotic caspases are not strictly required for cells to undergo regulated cell death (RCD) in response to damage, but largely control

the timing and microenvironmental (including the immunological) manifestations of the process{Galluzzi, 2015, 25236395}.

Box 4. Principles of p53 signaling.

In healthy cells, tumor protein p53 (TP53, best known as p53) is constitutively degraded by the proteasome downstream of MDM2 proto-oncogene (MDM2)-dependent poly-ubiquitination. A variety of stress conditions, including DNA damage, favors the activation of signal transduction cascades that support *TP53* transactivation and reduce the affinity of p53 for MDM2, hence promoting its accumulation. One of the best characterized of these signaling pathways is initiated by DNA double-strand breaks, resulting in the ATM serine/threonine kinase (ATM)-driven activation of checkpoint kinase 2 (CHEK2) and consequent CHEK2-dependent stabilizing phosphorylation of p53. That said, many other kinases can catalyze the phosphorylation-dependent stabilization of p53, including CHEK1 (which is also activated by DNA damage). Moreover, several post-translational p53 modifications other than phosphorylation have been shown to limit its affinity for MDM2 (and hence promote its stabilization), or to regulate its biological activity. These include (but are not limited to): acetylation, methylation, mono-ubiquitination, and ADP-ribosylation. On stabilization, p53 generates transcriptionally competent tetramers that accumulate in the nucleus to regulate the expression of numerous genes involved in both the adaptive (cytoprotective) and misadaptive (cytotoxic) phase of stress responses, including several genes involved in temporary cell arrest, cellular senescence and apoptosis (see also **Box 3**). Moreover, p53 has been shown to mediate cytotoxic effects via transcription-independent mechanisms, for the most part linked to the capacity of mono-ubiquitinated p53 to exit the nuclear compartment and directly interact with mitochondria to favor their permeabilization. That said, the actual contribution of non-transcriptional over transcriptional mechanisms to p53-driven regulated cell death (RCD) remains to be clarified in a majority of settings{Hafner, 2019, 30824861}.

Box 5. Non-apoptotic variants of RCD.

Apoptosis, which is currently defined as a variant of cellular demise mechanistically involving caspase activation, has long been considered as the only form of regulated cell death (RCD). However, it is now clear that cells can undergo RCD upon activation of numerous, intimately interconnected mechanisms, including signal transduction cascades that ultimately manifest with a necrotic morphology. Considerable efforts have been devoted to defining each of these mechanisms based on (precisely quantifiable) biochemical processes, as opposed to (hardly quantifiable and prone to misinterpretation) morphological features. Mitochondrial permeability transition (MPT)-driven regulated necrosis occurs downstream of permeabilization of the inner mitochondrial membrane by a hitherto elusive supramolecular complex regulated by peptidylprolyl isomerase F (PPIF, best known as CYPD). Necroptosis is mediated with the assembly of a supramolecular complex containing receptor interacting serine/threonine kinase 3 (RIPK3) and optionally RIPK1 (the so-called necrosome) that catalyzes the phosphorylation-dependent activation of mixed lineage kinase domain like pseudokinase (MLKL), which unleashes its capacity to form pores in the plasma membrane. Of note, RIPK3 can also be activated by Toll-like receptor (TLR3) upon endosomal nucleic acid detection. Ferroptosis is an iron-dependent variant of RCD characterized by extensive lipid peroxidation and under tonic inhibition by glutathione peroxidase 4 (GPX4). Pyroptosis is driven by the ability of inflammatory caspases activated in the context of inflammasome signaling to cleave gasdermin family members, hence unleashing their ability to form oligomers that permeabilize the plasma membrane. Finally, parthanatos is a form of RCD that is initiated by alkylating DNA damage, and mechanistically involves the hyperactivation of poly(ADP-ribose) polymerase 1 (PARP1) and the bioenergetic catastrophe resulting from PARP1-dependent NAD^+ depletion. Of note, mitotic catastrophe (MC) is currently not viewed as a form of RCD *sensu stricto*, but as an oncosuppressive mechanism for the terminal inactivation of cells experiencing mitotic issues. Indeed, cells experiencing MC can undergo

cellular senescence, which is not associated with the irreversible loss of all cellular functions that characterize cell death, or even resume proliferation upon mitotic slippage. Importantly, other signal transduction pathways culminating in the death of normal and malignant cells have been described, including autophagy-dependent cell death and lysosome-dependent cell death. However, the immunological consequences of these processes and their relevance for RT-driven RCD remain virtually unexplored{Galluzzi, 2018, 29362479}.

Legends to Figures

Figure 1. **Principles of TME reshaping by RT-driven stress signaling.** Both normal and malignant components of the tumor microenvironment (TME) respond to the genotoxic and oxidative stress imposed by radiation therapy (RT) with the activation of cytoprotective mechanisms that support repair and restoration of cellular homeostasis. When damage is excessive, however, such signal transduction pathways switch to a cytotoxic/cytostatic mode culminating with regulated cell death (RCD) or cellular senescence. In both its cytoprotective and cytotoxic phase, adaptation to stress is intimately connected to microenvironmental homeostasis, largely reflecting the emission of cytokines, metabolites and other bioactive factors with paracrine and endocrine activities by radioresistant cells (surviving irradiation), as well as by their radiosensitive counterparts (succumbing to it). Moreover, malignant, endothelial, stromal and immune cells exhibit variable degrees of radiosensitivity, depending not only on specific cell (sub)type, but also on activation state and precise anatomical localization. Thus, not all cellular components of TME are equally able to persist after irradiation. Cumulatively, these constitute the main principles underlying the ability of RT to reconfigure the TME. CAF, cancer-associated fibroblast; DAMP, damage-associated molecular pattern; DC, dendritic cell; DDR, DNA damage response; EC, endothelial cell; LYM, lymphocyte; SASP, senescence-associated secretory phenotype; TAM, tumor-associated macrophage; UPR, unfolded protein response.

Figure 2. **Effects of anti-apoptotic signaling in irradiated cells on the TME.** Both normal and malignant cells survive radiation therapy (RT) owing to the activation of robust cytoprotective mechanisms including the DNA damage response (DDR), autophagy and NF-κB signaling. Alongside, the mechanisms of adaptation to RT-driven stress control the emission of signals that alter the composition and functional configuration of the tumor microenvironment (TME). These signals encompass immunomodulatory proteins exposed on the cell surface such as PD-L1, MHC Class I

molecules, and NK cell-activating ligands (NKALs), adhesion molecules like ICAM1 and VCAM1, metabolites including ATP, lactate, amino acids (AAs) and ketone bodies (KBs), cytokines like IL1B, IL6, IL18, CXCL10, TNF and type I interferon (IFN), pro-oxidants like nitric oxide (NO) and reactive oxygen species (ROS), as well as bioactive lipids such as prostaglandins (PGs). See also **Table 1**.

Figure 3. **Effects of pro-apoptotic signaling in irradiated cells on the TME.** Non-transformed and transformed cells exposed to cytotoxic doses of radiation therapy (RT) undergo regulated cell death (RCD) in the context of failing adaptation to stress, generally as a consequence of p53-initiated, caspase (CASP)-mediated apoptosis. The signal transduction cascades activated by RT also modulate the emission of molecules that can modify the composition and functional activity of the tumor microenvironment (TME). These molecules include cell surface-exposed endoplasmic reticulum chaperones such as calreticulin (CALR), cell surface-associated immunomodulatory proteins like VISTA, MHC Class I molecules, and NK cell-activating ligands (NKALs), death receptors such as FAS, plasma membrane phospholipids like phosphatidylserine (PS), cytokines such as IL1B, IL18, IL33, CCL2 and type I interferon (IFN), metabolites (*e.g.*, ATP), and bioactive lipids like prostaglandin E_2 (PGE_2) and lysophosphatidylcholine (LPC). UPR, unfolded protein response. See also **Table 2**.

Table 1. Main bioactive factors produced by cells resisting to irradiation.

Stress pathway	Factor	Class	Effect of RT	Immunomodulation	Ref.
Autophagy	Alanine	Nutrient	Increased secretion	Nutritional support	{Rybstein, 2018, 29476153}
	Arginine	Nutrient	Increased secretion	Nutritional support	{Rybstein, 2018, 29476153}
	ATP	Nucleotide	Increased secretion	Nutritional support	{Rybstein, 2018, 29476153}
	Cytosolic (mt)DNA	Nucleic acid	Inhibited accumulation	Inhibited type I IFN, IL1B and IL18 secretion	{Lan, 2014, 25284779;Bartsch, 2017, 29016854;Prabakaran, 2018, 29496741;Konno, 2013, 24119841;Nakahira, 2011, 21151103;Sliter, 2018, 30135585}
	Ketone bodies	Nutrient	Increased secretion	Nutritional support	{Rybstein, 2018, 29476153}
	Lactate	Nutrient	Increased secretion	Nutritional support	{Rybstein, 2018, 29476153}
	ROS	Oxidative species	Increased generation	Cytosolic mtDNA accumulation	{Nakahira, 2011, 21151103}
DDR	Cytosolic DNA	Nucleic acid	Inhibited accumulation	Inhibited type I IFN secretion	{Heijink, 2019, 30626869;Chabanon, 2019, 30589644;Dillon, 2019, 30770349}
	Cytosolic RNA	Nucleic acid	Inhibited accumulation	Inhibited type I IFN secretion	{Ghosh, 2018, 29590171}
	MHC Class I molecules	Surface proteins	Cell surface exposure	Antigen presentation and NK cell inhibition	{Son, 2016, 27671170}
	NKALs	Surface proteins	Cell surface exposure	NK cell activation	{Gasser, 2005, 15995699;Soriani, 2009, 19098271}
	PD-L1	Surface protein	Cell surface exposure	T cell exhaustion	{Sato, 2017, 29170499}
NF-κB signaling	CXCL10	Chemokine	Increased secretion	T cell recruitment	{Taniguchi, 2018, 29379212}
	ICAM1	Surface protein	Surface protein	T cell recruitment	{Taniguchi, 2018, 29379212}
	IL18	Cytokine	Increased secretion	Inflammation	{Taniguchi, 2018, 29379212}
	IL6	Cytokine	Increased secretion	Paracrine radioresistance	{Chen, 2015, 26572130}
	Lactate	Nutrient	Inhibited secretion	T cell derepression	{Corbet, 2017, 28912578}
	NO	Oxidative species	Increased secretion	Inflammation	{Taniguchi, 2018, 29379212}
	Prostaglandins	Bioactive lipid	Increased secretion	Inflammation	{Taniguchi, 2018, 29379212}
	TNF	Cytokine	Increased secretion	Inflammation	{Taniguchi, 2018, 29379212}
	VCAM1	Surface protein	Cell surface exposure	T cell recruitment	{Taniguchi, 2018, 29379212}

Abbreviations: DDR, DNA damage response; IFN, interferon; mtDNA, mitochondrial DNA; NK, natural killer; NKAL, NK cell-activating ligand; NO, nitric oxide; ROS, reactive oxygen species; RT, radiation therapy.

Table 2. Main bioactive factors produced by cells succumbing to irradiation.

Stress pathway	Factor	Class	Effect of RT	Immunomodulation	Ref.
CASP3 activation	IL33	Cytokine	Degradation	Inhibited T cell recruitment and activation	{Luthi, 2009, 19559631}
	LPC	Bioactive lipid	Increased secretion	Macrophage recruitment and tolerogenic phagocytosis	{Nagata, 2018, 29400998}
	PGE_2	Bioactive lipid	Increased secretion	Cancer cell proliferation, neoangiogenesis and inhibited T cell activation.	{Zelenay, 2015, 26343581;Huang, 2011, 21725296;Feng, 2015, 26431328}
	PS	Phospholipid	Cell surface exposure	Macrophage-dependent (tolerogenic) phagocytosis	{Nagata, 2018, 29400998}
	Type I IFN	Cytokine	Inhibited secretion	Inhibited myeloid cell recruitment and activation	{Rodriguez-Ruiz, 2019 #277;White, 2014, 25525874;McArthur, 2018, 29472455;Ning, 2019, 30878284}
CASP8 activation	CALR	ER chaperone	Cell surface exposure	DC-dependent (immunogenic) phagocytosis	{Panaretakis, 2009, 19165151}
	IL18	Cytokine	Increased secretion	Inflammation	{Van Opdenbosch, 2019, 31216460}
	IL1B	Cytokine	Increased secretion	Inflammation	{Van Opdenbosch, 2019, 31216460}
Failing autophagy	ATP	Nucleotide	Increased secretion	Myeloid cell recruitment and activation, IL1B secretion and potential adenosine accumulation	{Ma, 2013, 23562161;Michaud, 2011, 22174255;Ghiringhelli, 2009, 19767732;Di Virgilio, 2018, 30006588}
Failing DDR	CCL2	Chemokine	Increased secretion	Myeloid cell recruitment and paracrine induction of senescence	{Acosta, 2013, 23770676}
	FAS	Surface protein	Cell surface exposure	Increased cancer cell sensitivity to T cells	{Fulda, 1998, 10203687}
	MHC Class I molecules	Surface protein	Cell surface exposure	Antigen presentation and NK cell inhibition	{Wang, 2013, 23965983}
	NKALs	Surface protein	Cell surface exposure	NK cell activation	{Iannello, 2013, 24043758}
	VISTA	Surface protein	Cell surface exposure	Macrophage-dependent (tolerogenic) phagocytosis	{Yoon, 2015, 26228159}
	Type I IFN	Cytokine	Increased secretion	Myeloid cell recruitment and activation	{Mori, 2002, 11973653}
Failing UPR	CALR	ER chaperone	Cell surface exposure	DC-dependent (immunogenic) phagocytosis	{Golden, 2014, 25071979;Panaretakis, 2009, 19165151}

Abbreviations: DC, dendritic cell; ER, endoplasmic reticulum; IFN, interferon; LPC, lysophosphatidylcholine; NK, natural killer; NKAL, NK cell activating ligand; PGE_2, prostaglandin E_2; PS, phosphatidylserine; RT, radiation therapy.

Annotation to References

Ref. {Chabanon, 2019, 30589644}

This is an elegant demonstration that PARP1 inhibition has immunostimulatory effects that originate from accrued type I IFN secretion by cancer cells downstream of CGAS-STING signaling.

Ref. {Liu, 2018, 30356214}

These authors were the first to report that the knockdown of CGAS suppresses DNA damage and inhibits tumor growth, both *in vitro* and *in vivo,* as a consequence of improved PARP1 functions and hence increased genomic stability.

Ref. {Sato, 2017, 29170499}

This study demonstrates that BRCA2 limits the ability of CHEK1 activation by radiation therapy to promote the exposure of PD-L1 on the membrane of cancer cells, hence preventing them from driving T cell exhaustion.

Ref. {Gasser, 2005, 15995699}

This is the first report demonstrating that cells responding to DNA damage expose increased amounts of NKG2D ligands on their surface, and thus are prone to activate NK cells.

Ref. {Bottcher, 2018, 29429633}

These authors identify the ability of NK cells to recruit conventional type I DCs to the tumor bed by secreting the chemoattractants CCL5 and XCL1.

Ref. {Taniguchi, 2018, 29379212}

This is comprehensive review on the multifaceted roles of NF-κB-dependent immunomodulation in cancer.

Ref. {Bakhoum, 2018, 29342134}

This is the first report demonstrating that indolent CGAS signaling caused by chromosomal instability favors non-canonical NF-κB activation in support of tumor progression and metastatic dissemination.

Ref. {Bartsch, 2017, 29016854}

These authors demonstrate that the deletion of RNase H2 favors the formation of CGAS-activating micronuclei that are efficiently disposed of by autophagy.

Ref. {Sliter, 2018, 30135585}

This elegant study highlights the ability of mitochondrial autophagy to prevent excessive, potentially detrimental CGAS-STING signaling.

Ref. {Lujambio, 2013, 23562644}

These authors report that p53-dependent cellular senescence in non-transformed hepatic stellate cells is instrumental for the maintenance of tissue homeostasis and the prevention of liver oncogenesis.

Ref. {Michaud, 2011, 22174255}

This study elegantly demonstrates that robust autophagic responses are required for dying cancer cells to release ATP in amounts that are compatible with the recruitment and activation of DC precursors.

Ref. {Panaretakis, 2009, 19165151}

This is the first mechanistic characterization of the signal transduction modules involved in the exposure of CALR on the surface of cancer cells undergoing ICD.

Ref. {Rodriguez-Ruiz, 2019 #277}

This article is the first to demonstrate that apoptotic caspases limit the ability of RT to drive potent immune responses with systemic outreach as they inhibit type I IFN secretion by irradiated cancer cells.

Ref. {Huang, 2011, 21725296}

These authors delineate a CASP3-dependent mechanism through which radiosensitive cancer cells succumbing to RT release PGE_2, ultimately favoring tumor repopulation by radioresistant cells.

Refs. {Mackenzie, 2017, 28738408;Harding, 2017, 28759889}

These articles demonstrate that micronuclei accumulating in the course of mitotic catastrophe driven by RT are the major drivers of CGAS-STING signaling and consequent type I IFN secretion.

Ref. {Senovilla, 2012, 23019653}

This is an elegant demonstration that hyperploid cancer cells are under preferential immunosurveillance, as compared to their euploid counterparts, at least in part reflecting constitutive CALR exposure on the membrane.

Ref. {McArthur, 2018, 29472455}

These authors provide compelling data in support of the ability of CGAS to detect mitochondrial DNA herniating into the cytosol across BAX/BAK1 oligomers upon MOMP.

Refs. {Aaes, 2016, 27050509;Yatim, 2015, 26405229}

These articles were the first to demonstrate that necroptotic RCD can be perceived as immunogenic and hence drive antigen-specific immune responses in the absence of external adjuvants.

Ref. {Wang, 2019, 31043744}

This is the first report about the ability of $CD8^+$ T cells to mediate ferroptosis in cancer cells as a consequence of system x_c inhibition by IFNG.

Ref. {Klug, 2013, 24209604}

These authors highlight the ability of low-dose RT to drive the repolarization of M2-like TAMs into their M1-like counterparts in support of adaptive anticancer immunity.

Refs. {Mariathasan, 2018, 29443960;Tauriello, 2018, 29443964}

These reports demonstrate that the immunosuppressive effects of TGFB1 also involve the generation of a stromal reaction that prevents tumor infiltration by T cells.

Ref. {Alcantara-Hernandez, 2017, 29221729}

This study offers the first high-dimensional phenotypic characterization of human DCs, revealing considerable degrees of inter-individual variation and tissue specialization.

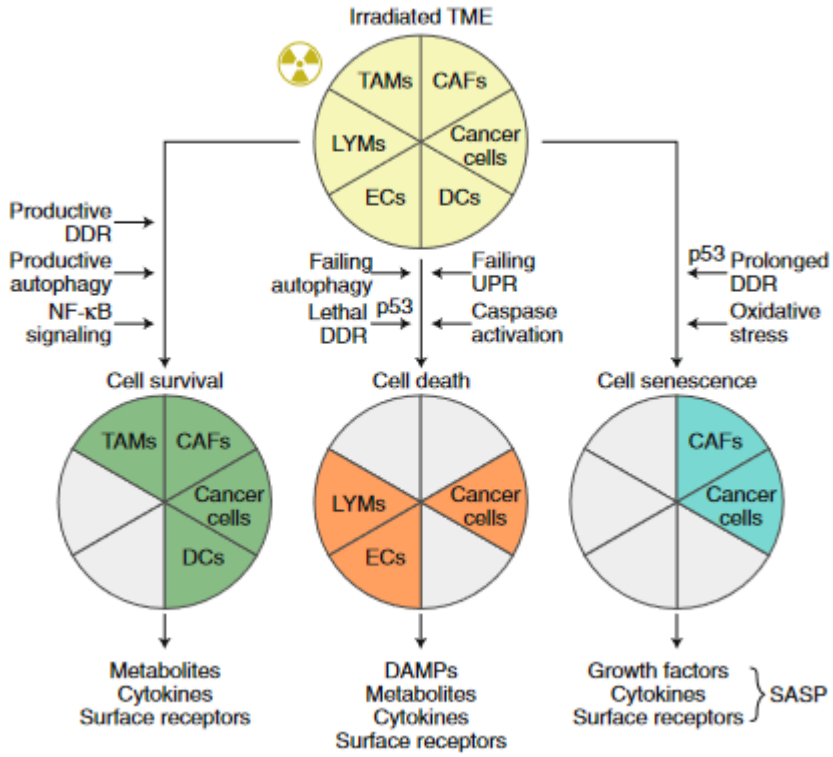

Fig. 1 |Principles of tME reshaping by Rt-driven stress signaling.

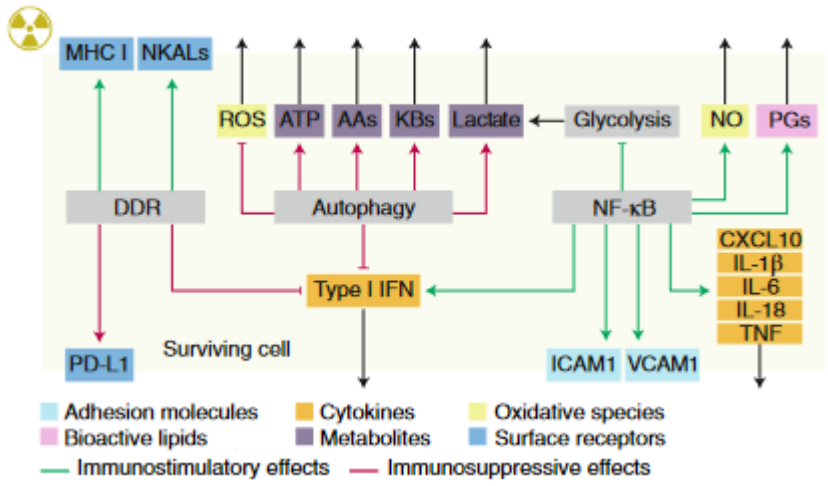

Fig. 2 |Effects of anti-apoptotic signaling in irradiated cells on the tME.

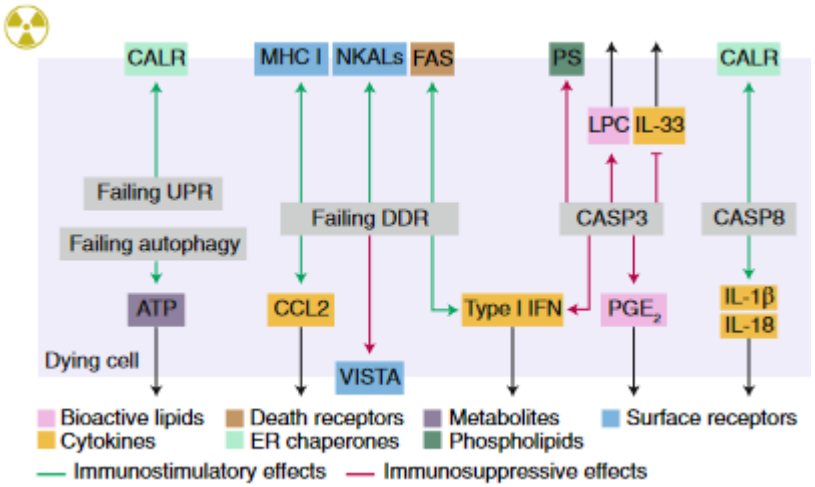

Fig. 3 |Effects of pro-apoptotic signaling in irradiated cells on the tME.