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How to improve the immunogenicity of chemotherapy and radiotherapy

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Abstract Chemotherapy or radiotherapy could induce various tumor cell death modalities, releasing tumor-derived antigen as well as danger signals that could either be captured for triggering antitumor immune response or ignored. Exploring the interplay among therapeutic drugs, tumor cell death and the immune cells should improve diagnostic, prognostic, predictive, and therapeutic management of tumor. We summarized some of the cell death-derived danger signals and the mechanism for host to sense and response to cell death in the tumor microenvironment. Based on the recent clinical or experimental findings, several strategies have been suggested to improve the immunogenicity of cell death and augment antitumor immunity.

Keywords Cell death · DAMP · Tumor microenvironment · Immune cells

Abbreviations

DC	Dendritic cells
ALK	Anaplastic lymphoma kinase
ALCL	Anaplastic large cell lymphoma
DAMP	Damage-associated molecular patterns
HSP	Heat shock proteins
LysoPC	Lysophosphatidylcholine
HMGB1	High-mobility group box 1 protein
CRT	Calreticulin
ER	Endoplasmic reticulum
Tim	T cell immunoglobulin mucin
Mincle	Macrophage-inducible C-type lectin
SAP130	Spliceosome-associated protein 130
MDSCs	Myeloid-derived suppressor cells
DNAM-1	DNAX accessory molecule-1
SAP130	Spliceosome-associated protein 130
Treg	Regulatory T cells

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MDR	Multidrug resistance
CTX	Cyclophosphamide
TSC	Tumor stem cells
DLN	Draining lymph node
IDO	Indoleamine-pyrrole 2,3-dioxygenase

1 Immunogenicity of chemotherapy and radiotherapy

Chemotherapy and radiotherapy are commonly believed to kill cancer cells by apoptosis. This cell death modality is generally considered as a non-immunogenic. However, massive cell death might saturate the local capacity of silent corps removal, causing the accumulation of late-stage apoptotic cells. What is more important is that accumulating evidence suggest that certain chemotherapeutic agents and ionizing radiation could confer dying tumor cells immunogenic.

Irradiated tumor cells (cell lines or autologous dissociated tumor pieces) engineered to secrete GM-CSF are able to mobilize dendritic cells (DC), plasma cells, invariant NKT cells, and tumor-reactive $CD4^+$ and $CD8^+$ T cells, both in mice and in metastatic cancer patients [1]. This vaccine could promote tumor destruction, necrosis, and fibrosis correlating with humoral immune responses and favorable clinical outcome [2, 3]. In a lung metastasis model (B16F0 melanoma), irradiation of cutaneous melanomas prior to their resection resulted in more than a 20-fold reduction in lung metastases after systemic challenge with untreated melanoma cells. This study suggests that neoadjuvant irradiation of cutaneous melanoma tumors prior to surgical resection can stimulate an endogenous anti-melanoma immune response [4].

Our group has performed screening of anticancer compounds for their ability to induce immunogenic cancer cell death. In the absence of any adjuvant, subcutaneous inoculation of dying CT26 tumor cells pretreated with some chemotherapeutic agents could prevent tumor growth upon live CT26 cell rechallenge in immunocompetent Balb/c mice. Anthracyclines (daunorubicin, idarubicin, mitoxanthrone) were the most potent immunogenic cell death inducers not only in CT26 colon cancer but also in EL4 thymomas and MCA205 sarcomas [5, 6].

Furthermore, chemotherapy and immunotherapy could synergize. DNA-based vaccination targeting an oncogenic protein anaplastic lymphoma kinase (ALK) can immunize mice against anaplastic large cell lymphoma (ALCL) in a $CD8^+$ T cell- and IFN-γ-dependent manner and cure animals bearing advanced ALCL when combined with doxorubicin [7]. In a phase II trial launched in metastatic colon cancer, gemcitabine plus FOLFOX-4 (oxaliplatin, fluorouracil, and folinic acid) polychemotherapy in combination with GM-CSF and IL-2 could elicit tumor antigen-specific immune responses and induce very high objective response and disease control rates [8].

Many chemotherapeutic agents used to treat malignant diseases damage lymphocytes and consequently suppress cell-mediated immunity. More recently, new cancer treatment agents such as tyrosine kinase inhibitors, thalidomide and its derivatives, proteasome inhibitors, and interferons have been found to have diverse immunomodulatory activities blocking immune surveillance of the malignancy and permitting disease recurrence, or, favorably, by reprogramming immunity to increase autologous antitumor effects.

These findings suggest that certain chemotherapy could reset tumor immunogenicity and tumor cell death modalities triggered by specific antitumor response and act as cryptic vaccines [9].

2 Cell death-derived DAMP could induce sterile inflammation

Robust acute inflammation could be triggered by sterile cell death which induces damage-associated molecular patterns (DAMP) exposed on the plasma membrane or secreted extracellularly. These cell-derived DAMP, such as uric acid, DNA (specifically unmethylated CpG-rich regions), HMGB1, SAP130, S100 proteins, and heat shock proteins (HSP) [10–16] could stimulate an IL-1- and inflammasome-dependent response [17]. Interestingly, inflammations triggered by sterile cell death and microbial stimulus differ in their dependency of the IL-1R-Myd88 pathway [18]. Upon chemotherapy or radiotherapy, various cell death modalities occur, including apoptosis, necrosis, autophagy, mitotic catastrophe, and pyroptosis. Here, we will give a brief summary of the DAMP profile derived from dying tumor cells and sensors from host phagocytes, especially focusing on the immunogenic cell death modules.

2.1 “Find me” DAMP

Dying cells could recruit phagocytic cells owing to the release of various “find me” signals. Apoptotic microparticles could transfer chemokine receptors and arachidonic acid between cells, activate complement, promote leukocyte rolling, and stimulate the release of pro-inflammatory mediators [19]. Lysophosphatidylcholine (lysoPC), but none of the lysoPC metabolites or other lysophospholiplids, represents the essential apoptotic attraction signal able to trigger a chemotactic response through phagocyte receptor G2A [20, 21]. The prototypical DAMP high-mobility group box 1 protein (HMGB1) is released with sustained autophagy, late apoptosis, and

necrosis. HMGB1 could act as chemotactic and/or activating factors for macrophages, neutrophils, and DC [22–24]. ATP and UTP released by apoptotic or necrotic cells play a non-redundant role in dying cell clearance by monocytes/macrophages that express the purinergic receptor P2RY2. Forced expression of CD39 (NTPDase-1, an ecto-apyrase responsible for the degradation of NTP) could abrogate the chemoattractant activity of apoptotic cells [25]. Both pan-sphingosine kinase (SphK) inhibitor and chemotherapeutic drug doxorubicin could induce apoptosis and upregulate sphingosine kinase 1 expression, allowing the release of sphingosine-1-phosphate which causes cytoskeletal rearrangements and chemoattraction of macrophages, even at low nanomolar ranges [26]. Despite emerging "find me" signals identified, future research should also dissociate the factors recruiting antitumor *versus* pro-tumor effectors.

2.2 "Eat me" and "don't eat me" signals

Calreticulin (CRT) is a highly conserved Ca^{2+}-binding protein mainly located in the lumen of the endoplasmic reticulum (ER) and also in the nucleus and cytoplasm [27]. Cancer cells treated with UV expose larger amounts of ecto-CRT (CRT on the plasma membrane), which is redistributed in the form of "patches" and pre-dominantly co-localized with phosphatidylserine (PS) [28]. Cytotoxic treatment, such as anthracyclines, oxaliplatin, UVC, and γ-radiation, could induce apoptotic cancer cell death and exposure of ecto-CRT, which became receptive for engulfment by DC [6, 29, 30]. Ecto-CRT exposure was found to be a pre-apoptotic event accompanied by the co-translocation of ERp57. Both Ecto-CRT and ERp57 have been proven to determine the immunogenicity of cell death [31]. ER stress, and more specifically the PERK/eIF2α arm of the unfolded protein response pathway, plays an important role in ecto-CRT/ERp57 translocation [32]. Myeloid leukemia, migrating hematopoietic progenitors, and also solid tumors were found to overexpress CD47, resulting in a reduced uptake by SIRPα-expressing macrophages. Thus, CD47 could act as a "don't eat me" signal that prevents the recognition and removal of apoptotic cells by professional and nonprofessional phagocytes.

Tumors have been shown to overexpress HSP, probably due to the stressful tumor microenvironment [33]. Intracellular overexpression of HSP could inhibit apoptosis and exhibit cytoprotective activity [34], while membrane expression of HSP could be potently immunostimulatory [35]. Ecto-HSP70 and HSP90 could determine the immunogenicity of stressed or dying tumor cells due to their ability to interact with a number of antigen-presenting cells (APC) surface receptors, such as CD91, LOX1, and CD40, and facilitate cross-presentation of tumor antigens [36–38]. Furthermore, HSP could promote DC maturation [39] and activate NK cells [40, 41] and act as an immunoadjuvant. Interestingly, stress tissue-derived HSP are more immunostimulatory than recombinant HSP [42]. Large stress protein (e.g., HSP110 and GRP170) chaperoned protein antigen could induce a superior antitumor response compared with peptide antigen [43]. These findings will provide a rational chaperoning-based antitumor vaccine designing for clinical investigation.

2.3 Sensors of cell death on phagocytes

Several receptors expressed on phagocytes could act as sensors of cell death. Tim (T cell immunoglobulin mucin) family member (Tim4 and Tim1) expression on professional phagocytes, such as resident peritoneal macrophages and splenic dendritic cells, or semiprofessional, non-myeloid phagocytes could specifically bind PS and are critical for the efficient apoptotic cell clearance [44]. Liver X receptor [45], MerTK [46, 47], MFG-E8 [48, 49], and BAI1 [50] are also important phagocyte receptors responsible for apoptotic cell clearance. Blocking these signal pathways may evoke extensive tumor cell apoptosis, revert immunotolerance [51], trigger efficient pro-inflammatory cytokine secretion and cross-presentation of dying tumor cells [48, 52, 53]. Mincle (macrophage-inducible C-type lectin) expression is induced after exposure to various stimuli and stresses on macrophages. It detects dead cell-derived spliceosome-associated protein 130 (SAP130) and acts as the sensor of non-homeostatic cell death for inflammatory response [12, 54].

3 Tumor antigen presentation: APC and mechanisms

APC provide a critical link between tumor cell death and adaptive immunity. Kenneth Murphy's group discovered Batf3 as the transcription factor for CD8α^+ DC, and they proved the critical role of CD8α^+ DC-mediated cross-presentation in tumor rejection using Batf3 knockout mice [55]. By targeting $CD8^+$ and $CD8^-$ DC using chimeric monoclonal antibodies for CD205 and 33D1, respectively, Dudziak et al. [56] showed that these two DC populations are specialized in presenting antigen on major histocompatibility complex (MHC) class I and class II, respectively. As these two populations could capture comparable amounts of soluble and bead-associated antigen, the possible explanation may be that CD8α^+ DC are equipped with a specialized machinery for cross-presentation [57]. Both $CD8^+$ and $CD8^-$ DC are effective at cross-presenting HA tumor antigen, but they may differ in the expression of co-stimulatory receptor, thus contributing to either induction or regulation of tumor-specific responses [58].

Langerin (CD207), a C-type lectin that is sufficient to induce the formation of Birbeck granules, is expressed on Langerhans cell as well as dermal DC [59]. In the skin-draining lymph nodes and spleen, Langerin is also expressed by the resident $CD8^+$ DC at lower levels [60]. The proportion of $CD8^+Langerin^+$ DC in lymphoid tissues varies among inbred mouse strains and is more prominent in Balb/c mice [61]. With genetically engineered antibody targeting extracellular domain of Langerin, Idoyaga et al. [62] showed that OVA targeting Langerin results in efficient presentation to OVA-specific $CD4^+$ and $CD8^+$ transgenic T cells. Intravenous administration of horse cyt c was previously shown to specifically deplete cells capable of shuttling Ag through the cytosol, thereby removing cells capable of cross-presentation [63]. Interestingly, only the $Langerin^+$ compartment of the $CD8\alpha^+$ splenic DC population showed a dramatic dose-dependent reduction in response to horse cyt c, and they are critically involved in the cross-presentation of systemic soluble antigen [64]. Among five distinguishable skin DC subsets, the $CD207^+CD103^+$ dermal DC subset is endowed with the unique capability of cross-presenting antigens expressed by keratinocytes [65].

Splenic marginal metallophilic macrophages (MMM) could efficiently capture and transfer antigen exclusively to splenic $CD8^+$ DC for cross-priming cytotoxic T lymphocytes; thus, tumor antigen targeting to MMM is very effective as an antitumor immunotherapy [66]. After irradiation or chemotherapy, $CD11b^+$ tumor stromal cells (containing immature myeloid cells, macrophages, bone marrow-derived endothelial precursors, and other pro-angiogenic cells such as pericytes) [67] could acquire and cross-present tumor antigen, thus facilitating direct killing of parental tumor cells as well as bystander elimination of antigen loss variants. This process requires the cooperation of $CD4^+$ and $CD8^+$ T cells and can lead to the complete destruction of well-established solid tumors [68–70]. Besides the myeloid lineage, cells of the lymphoid lineage could also act as antigen-presenting cells. Freshly isolated human peripheral blood gammadelta T cells can phagocytose via Ab opsonization and CD16 (FcgammaRIII) [71] and upregulate expression of co-stimulatory MHC class I and II molecules, leading to Ag processing and presentation [72].

Possible mechanisms for the transfer of tumor antigen to DC for cross-presentation include phagocytosis of cell-associated antigens, pinocytosis/endocytosis of soluble antigen, capture of soluble antigen bound to HSP, capture of exosomes, "nibbling" of live tumor cell membranes, and "cross-dressing" whereby DC acquire peptide MHC complexes from contact with dying tumor cells [73]. Interestingly, DC could also receive preprocessed antigenic peptides from tumor directly through gap junction. Infecting both human and murine melanoma cells with *Salmonella* can induce connexin 43 (Cx43) upregulation and facilitate gap junction formation between DC and tumor cells. The Cx43-dependent cross-presentation pathway provides a novel strategy for DC loading [74].

4 Antitumor therapy and tumor microenvironment

4.1 Immune effectors in tumor microenvironment

The tumor microenvironment contains innate immune cells ($\gamma\delta$ T, NK cells, neutrophils, macrophages, mast cells, myeloid-derived suppressor cells (MDSC), and DC) and adaptive immune cells (T and B lymphocytes) in addition to tumor cells and their surrounding stroma (fibroblasts, endothelial cells, pericytes, and mesenchymal cells) [75].

Natural cytotoxicity receptors and DNAX accessory molecule-1 (DNAM-1) are critical for NK cell-mediated innate immunity to melanoma cells [76, 77]. Reduced DNAM-1 expression on bone marrow NK cells is associated with impaired killing of $CD34^+$ blasts in myelodysplastic syndrome [78]. DNA-damaging agents can induce the expression of NKG2D ligands on tumor cells [79]. Myeloma cells treated with low doses of common therapeutic agents, such as doxorubicin, melphalan, and bortezomib, upregulate DNAM-1 and NKG2D ligands [80]. Novel therapeutic drugs such as histone deacetylase inhibitors could induce MICA and MICB [81], poliovirus receptor (CD155), and Nectin-2 (CD112) [82] expression on tumor cells, leading to better NK-mediated killing via NKG2D and DNAM-1. Chemotherapy-induced genotoxic stress could promote MHC-independent NKR-P1B:Clr-b missing self-axis in leukemia cells and enhance cytotoxicity mediated by NKR-P1B(+) NK cells [83]. Immunomodulatory drugs lenalidomide could induce $\gamma\delta$ T cell expansion, improvement of IFN-γ secretion, and enhancement of cytotoxicity as well as inducing expression of CD1c on tumor cells. Proteasome inhibitors bortezomib, the first proteasome inhibitor to be used in the treatment of multiple myeloma, can also sensitize tumor cells to chemotherapeutic drugs or radiation through the improvement of TRAIL-mediated lysis or NK-mediated killing by downregulation of HLA class I molecules and upregulation of DNAM-1 and NKG2D ligands [84]. Azacytidine enhances tumor antigenicity by upregulating MHC class I and tumor antigen expression, increasing the release of pro-inflammatory cytokines and danger signals, and promoting antigen uptake by DC and killing by NK cells.

Infiltration of the primary tumor by memory T cells, particularly of the Th1 and cytotoxic types, is the strongest prognostic factor in terms of disease-free and overall survival at all stages of clinical disease [85]. We and others

have shown the critical role of tumor-specific cytotoxic T cells (CTLs) during chemotherapy and radiotherapy [5, 86–88]. Depending on different models, their antitumor effect relies on IFN-γ or cytolytic machinery (perforin, granzyme, TRAIL, TNF-α, etc.). Introduction of tumor-specific Th1-dominant immunity has been reported to be crucial for inducing tumor-specific CTL. A combined therapy of local radiation with Th1 cell could augment the generation of tumor-specific CTL at the tumor site and might also be effective for the treatment of distant metastases [88].

Tumor-infiltrating immune cells could also favor pro-tumor immunity [89] and act as mediators of solid tumor metastasis [90]. MDSCs are $CD11b^{+}Gr1^{+}$ cells which accumulate in peripheral blood of cancer patients as well as in tumors and lymphoid organs [91–93]. It is a phenotypically heterogeneous cell population that includes myeloid progenitor cells as well as immature myeloid cells [94]. The suppressive activity of MDSCs is associated with the intracellular metabolism of L-arginine, which serves as a substrate for inducible nitric oxide synthase (iNOS/NOS2) that generates NO and arginase 1 (ARG1) which converts L-arginine into urea and L-ornithine. Tumor-derived exosome-associated Hsp72 could trigger Stat3 activation in MDSCs and determine their suppressive activity in a TLR2/MyD88-dependent manner [95]. MDSC could suppress the activation of $CD4^{+}$ and $CD8^{+}$ T cells through sequestration of cysteine, and perturb T cell trafficking through down-regulating L-selectin [96]. MDSCs caused dissociation between TCR and CD3zeta molecules, disrupting TCR complexes on T cells [97].

Increasing evidence suggests that regulatory T cells (Treg) accumulate in peripheral blood, ascites, tumors, and tumor-draining lymph nodes in a variety of solid cancers such as lung, head and neck, gastrointestinal, and ovarian. They can be preferentially attracted by the CCR4/CCL22 axis to tumor and lymphoid aggregates and correlate with adverse clinical outcome [98, 99]. More recently, human and murine pancreatic adenocarcinomas have been shown to secrete CCL5, which preferentially attracts Treg through CCR5 [100]. Possible mechanisms of their suppressive activity include cell contact-dependent factors such as membrane TGF-β, CTLA-4, perforin/granzyme, extracellular adenosine, gap junction formation infusing cAMP, as well as soluble immunosuppressive cytokines such as IL-10, TGF-β, and IL-35.

4.2 Role of chemotherapeutic drugs on immune cells

Besides acting on tumor cells, chemotherapeutic drugs also regulate the immune effectors. Paclitaxel, cisplatin, and doxorubicin could upregulate mannose-6-phosphate receptors on tumor cells of mouse and human origin and increase their permeability to granzyme B to facilitate NK and CTL killing [101]. Paclitaxel and cyclophosphamide (CTX) appear to amplify the antigen-specific Th1 response [102] and CTX could reduce Treg in both mice and in end-stage cancer patients [103–105], while tyrosine kinase inhibitors such as imatinib and dasatinib are immunosuppressive, blocking T cell function but sparing $CD4^{+}$ $CD25^{+}$ $FoxP3^{+}$ Treg. Vinblastine, chlorambucil, and docetaxel could inhibit cytotoxic T cell- and NK cell-mediated killing of target cells, while asparaginase, bleomycin, and doxorubicin could enhance it [106]. STAT3 inhibitors and TK inhibitors such as sunitinib could inhibit MDSC maturation, and gemcitabine can block MDSC accumulation [107].

Targeting the tumor microenvironment with more sophisticated and selective tumoricidal drugs could differentially regulate tumor-promoting or tumor-eliminating immune cells and improve the therapeutic outcome [108].

5 Strategies to improve immunogenicity of tumor cell death during cytotoxic therapy

5.1 At the level of tumor

Decreased intracellular drug concentration mediated by multidrug resistance (MDR) gene products, alterations in drug activity, and half-life enhanced DNA repair and defects in cell death pathways could account for chemoresistance, which remains one of the most significant obstacles to the progress of chemotherapy [109]. Possible chemosensitization strategies include cocktail therapy, blocking drug resistance gene expression or activity as well as modulation of the cell death pathways.

Mutated N-Ras oncogene has recently been implicated in melanoma resistance to cisplatin, both *in vitro* and *in vivo* [110]. Overexpression of Bcl-2, Bcl-xL, and Mcl-1 and mutation of p53 in malignant cells contribute to the anti-apoptotic axis [111]. Therapeutics designed to reboot the pro-apoptotic axis will make chemotherapy-induced death the only option. Secretory clusterin could stabilize Ku70-Bax complexes as a protein chaperone, retenting Bax in the cytosol to prevent cytochrome c release which triggers cell apoptosis [112]. Its expression leads to broad-based resistance to chemotherapeutic agents such as doxorubicin, cisplatin, etoposide, camphothecin, and death-inducing molecules (TNF-α, Fas and TRAIL, or histone deacetylase inhibitors) [113]. Mer (MerTK) and Axl receptor tyrosine kinases are expressed at abnormally high levels in a variety of malignancies. Inhibiting their activity or knockdown of their expression could increase apoptosis and perhaps also reduce the silent clearance of tumor cells by phagocytes [114]. Interestingly, depending on its redox status, reducible HMGB1 induces Beclin 1-

dependent autophagy and promotes tumor resistance to chemotherapeutic agents or ionizing radiation, while oxidized HMGB1 increases the cytotoxicity of these agents and induces apoptosis via the mitochondrial pathway [115]. Galectin-3, a beta-galactoside-binding protein with anti-apoptotic activity, protects papillary thyroid cancer against both TRAIL- and doxorubicin-induced apoptosis, at least partially through he PI3K-Akt axis [116].

Both ER stress and autophagy follow a "yin–yang" principle by which their low to moderate activity is cell protective and supports chemoresistance ("yin"), but where severe conditions will aggravate these mechanisms to the point where they abandon their protective efforts and instead will trigger cell death ("yang") [117].

Some tumor cells survive and grow again after cytotoxic therapy, probably arising from the residual self-renewing tumor stem cells (TSC) which possess MDR properties. Preclinical studies suggested targeting leukemia stem cell surface molecules using antibody to enhance leukemia therapy [118]. Identification of components in the tumor microenvironment required for maintaining self-renewal, differentiation, and quiescence of TSC in the face of cytotoxic therapeutic regimens could also help in targeting TSC niches to prevent ultimate recurrence [119].

5.2 At the level of immune system

5.2.1 Antitumor vaccination

Each of the components of tumor vaccine (antigens, adjuvants, delivery systems) contributes specifically to induction and maintenance of T cell responses. Tumor-specific antigens (MAGE-A, NY-ESO-1, etc.), oncogenic proteins which are overexpressed in tumors (WT1 protein), as well as antigens selectively expressed by tumor-initiating cells or cancer stem cells are ideal targets for vaccine designing [120]. Depending on the delivery vehicles (such as liposomes, virosomes, DC, etc.), antitumor vaccines differ in their ability to induce various immune response as well as the intensity of immune response [121]. The first generation of DC-based tumor vaccine proves that this strategy is feasible to induce, regulate, and maintain T cell immunity [122, 123], and further improvement should be made to generate quantitative and qualitative CTLs and T helper cells as well as to break the immunosuppressive microenvironment. Cytokines (GM-CSF, IL-2, IFN, Flt-3 ligand), saponins, bacterial exotoxins, and, most importantly, TLR/NLR/RLR ligands are commonly used immunoadjuvants for antitumor vaccine [121]. CpG+OVA-liposome administered near the draining lymph node (DLN) of the tumor mass plus radiation-augmented induction of OVA-specific CTLs in DLN of tumor-bearing mice greatly inhibited tumor growth, and approximately 60% of the mice treated were completely cured [124]. Our group developed a combined therapy using vaccination, chemotherapy, and TLR3 agonist ploy(A:U) (VCT) against B16OVA melanoma and GL-26 glioma. Type I IFN and poly(A:U) could induce tumor cells and produce large amounts of CCL5 and CXCL10. Interestingly, VCT therapy relies on CXCR3-expressing CTLs and could be further improved when CCL5 derived from tumor or CCR5 expression on the host is blocked. Combination of chemotherapy and TLR3 agonist could not control tumor growth unless vaccination is given in advance [125]. TLR7

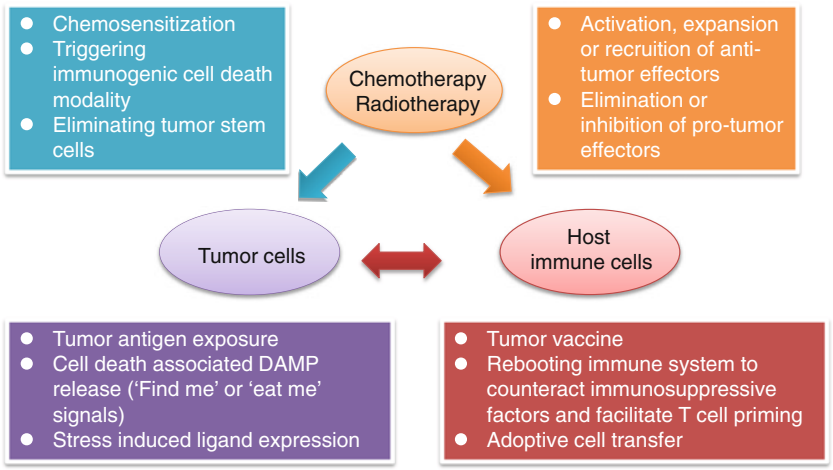

Fig. 1 Strategies to improve the immunogenicity of chemotherapy and radiotherapy. The interplay among chemotherapy and radiotherapy, tumor cells, and host immune system determines the therapeutic outcome. Strategies aiming at triggering sufficient immunogenic cell death and resetting tumor microenvironment at the level of therapy, releasing tumor-derived antigen, danger signals, and stress-induced molecules at the level of tumor are listed. In addition, the efficacy of chemotherapy and radiotherapy could further be improved in combination with tumor vaccine, immunomodulation, and adoptive cell transfer

and TLR8 ligands could also trigger pro-inflammatory cytokines, chemokines, and type I interferon production and upregulate co-stimulatory molecule expression [121].

5.2.2 Blocking immunosuppressive factors

A small population of plasmacytoid DCs in mouse tumor-draining LNs can express immunosuppressive enzyme, indoleamine-pyrrole 2,3-dioxygenase (IDO), which directly activates resting Treg for potent suppressor activity [126]. Inhibiting IDO with 1-methyl-tryptophan could enhance the antitumor efficacy elicited by DC-based vaccine [127]. Stat3 is the key transcription factor mediating immunosuppression. Silencing Stat3 combined with CpG greatly increases killing activity and tumor infiltration of transferred T cells [128]. TGF-β expression in the tumor microenvironment modulates a complex web of intercellular interactions that aggregately promote metastasis and progression. TGF-beta antibodies could reverse this effect [129]. IL-6 is a key molecule involved in malignancies and could activate Stat3 signaling [130]. Targeting IL-6R using antibody could significantly reduce tumor growth and suppress tumor angiogenesis [131, 132].

5.2.3 Enhancing T cell priming

Co-expression of inhibitory molecules LAG-3 and PD-1, Tim-3 and PD-1 on $CD8^+$ T cells is associated with impaired in IFN-γ/TNF-alpha production (T cell anergy or exhaustion) [133–136]. B and T lymphocyte attenuator was identified as a novel inhibitory receptor with structural and functional similarities to CTLA-4 and PD-1 [137]. Antibody targeting these inhibitory receptors could reverse T cell anergy and prolong and sustain T cell activation and proliferation [138–141]. Combination of three agonist antibodies consisting of anti-DR5, anti-CD40, and anti-CD137 could eradicate a large proportion of subcutaneous renal cell carcinoma tumors (75% long-term survival) and orthotopic tumors (55% survival) in combination with IL-2 [142].

5.2.4 Adoptive cellular therapy

Adoptive transfer of autologous tumor-infiltrating T cells expanded *in vitro* leads to potent antitumor responses in patients with refractory metastatic melanoma after lymphodepletion [143]. Without the need for *in vitro* expansion, small numbers of naive tumor-reactive $CD4^+$ T cells transfer into lymphopenic recipients in combination with CTLA-4 blockade and could eradiate poorly immunogenic established B16 melanoma and spontaneous mouse melanoma [144]. After expansion, *in vitro*, polarized tumor-reactive Th17 and Tc17 are capable of rejecting established melanoma [145, 146]. Adoptive transferred haploidentical NK cells can persist and expand *in vivo* and help in the treatment of poor prognosis acute myeloid leukemia [147]. Transfusion of gene-modifying primary mouse NK cells expressing specific receptor for tumor-associated antigen could inhibit tumor progression [148].

6 Conclusions

Effective antitumor therapy should induce sufficient tumor cell death in order to release tumor antigen as well as danger signals attracting phagocytes to uptake and present tumor antigen for specific adaptive immunity. Proper cell death modality should be triggered in both tumor cells, tumor stem cell, and stromal cells. Combining cocktail regimen of chemotherapy and radiotherapy with tumor-specific vaccine using proper immunoadjuvant as well as counteracting the immunosuppressive factors in tumor microenvironment will harness the maximum antitumor response following tumor cell death (Fig. 1).

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