Cellular & Molecular Immunology www.nature.com/cmi

REVIEW ARTICLE

Targeting innate sensing in the tumor microenvironment to improve immunotherapy

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The innate immune sensing pathways play critical roles in the defense against pathogen infection, but their roles in cancer immunosurveillance and cancer therapies are less defined. We propose that defective innate immune sensing inside the tumor microenvironment might limit T-cell responses to immunotherapy. A recent mechanistic understanding of conventional therapies revealed that both innate immune sensing and T-cell responses are essential for optimal antitumor efficacy. T-cell-based immunotherapy, particularly immune checkpoint blockade, has achieved great success in reactivating antitumor immune responses to lead to tumor regression, but only in a small fraction of patients. Therefore, incorporating conventional therapy that can increase innate sensing and immunotherapy should lead to promising strategies for cancer patients. Here, we review the innate sensing pathways related to cancer initiation/progression and therapies, summarize the recent key findings in innate immune sensing related to conventional therapies, evaluate current combination strategies, and highlight the potential issues of combinational therapies in terms of antitumor efficacy and toxicities.

Keywords: Innate immune sensing; Conventional therapy; Immunotherapy

Cellular & Molecular Immunology (2020) 17:13–26; https://doi.org/10.1038/s41423-019-0341-y

INTRODUCTION

The innate immune system serves as the front line of host defense against invasion of pathogens by sensing pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs) with pattern-recognition receptors (PRRs).[1,2] Recognition of PAMPs or DAMPs by PRRs initiates the inflammatory response by activating the NF-κB, IRF3/7 or inflammasome signaling pathways and producing proinflammatory cytokines, particularly type I interferons (IFNs), which subsequently leads to the activation of adaptive immune responses that clear the pathogens.[3,4] In addition to their role in infection, accumulating evidence shows that both the innate and adaptive immune systems play critical roles during tumor occurrence and progression. Evidence suggests aberrantly increased cell proliferation and cellular turnover may result in increased cell stress and release of tumor-derived DAMPs.[5] These DAMPs are recognized by PRRs and trigger the innate immune system to eliminate the vast majority of incipient cancer cells. However, the activated adaptive immune system misses the weakly immunogenic variants, which are allowed to grow and form tumors.[6–8] The initial innate immune sensing of tumors results in recruitment, activation, and clonal expansion of tumor-specific $CD8^+$ T cells, which have the potential to kill cognate tumor cells and are associated with better outcomes and improved overall survival in cancer patients treated with conventional therapies or immunotherapies.[9–17] Therefore, increasing the innate immune sensing of tumor cells will be a very potent strategy for improving cancer therapy.

Surgery, radiotherapy, chemotherapy, and targeted therapy are considered the major conventional anticancer therapies.[18] The major antitumor effects of all these conventional therapies have been thought to reduce tumor burden though direct killing of tumor cells.[19–22] Intriguingly, numerous studies in the past decade have demonstrated that conventional therapies also activate host innate immunity and adaptive immunity to cause tumors to regress, especially innate immune sensing and type I IFN production.[21,23–27] However, the induction of immune responses is inconsistent and often suppressed by further prolonged treatment or high doses. A strength of conventional therapies is their high response rates because of their direct cell killing and reduction of tumor burden with potentially enhanced innate immune sensing and antitumor immunity. Their weaknesses are systemic toxicity and a short-term response to treatment because of the development of treatment resistance and/or the acquisition of adaptive immune resistance.[28,29] While emerging immunotherapies, especially immune checkpoint blockade (ICB), have shown long-term responses in cancer patients, these benefits are only seen in a small fraction of patients.[30–32] All these features provide potential rationale for clinically developing combinations of conventional therapy and immunotherapy to achieve a high response rate, long-lasting responses, and improved overall survival in cancer patients.[23,28,33–36] Here, we will summarize the innate immune sensing pathways involved in cancer initiation/progression, conventional cancer therapies, the potential strategies and issues for integrating conventional therapies with immunotherapy.

INNATE IMMUNE SENSING PATHWAYS RELATED TO CANCER

Toll-like receptors (TLRs) and cancer

TLRs are a family of type I integral membrane glycoproteins that play a critical role in host defense against pathogens by

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Received: 29 August 2019 Accepted: 24 November 2019
Published online: 16 December 2019

recognizing a variety of PAMPs or DAMPs.[37] TLRs are mainly expressed on immune cells, such as macrophages, dendritic cells (DCs), monocytes, neutrophils, mast cells, eosinophils, B cells, natural killer (NK) cells, and T cells.[3] These receptors localize to either the cell surface (TLR1, TLR2, TLR4, TLR5, and TLR6) to recognize lipid and protein ligands or endolysosomal compartments (TLR3, TLR7, TLR8, and TLR9) to sense nucleic acids (NAs).[38] Upon ligand engagement, TLRs transmit downstream signals by recruiting adapter proteins, including myeloid differentiation factor 88 (MyD88), TIR domain-containing adapter protein, TIR domain-containing adapter inducing interferon (IFN)-β (TRIF), TRIF-related adapter molecule, and sterile α- and armadillo motif-containing protein (SARM).[39,40] All TLRs, excluding TLR3, signal through MyD88 to activate the canonical NF-κB pathway to produce proinflammatory cytokines, such as interleukin (IL)-1β, tumor necrosis factor-α (TNF-α), and IL-6. In addition, TLR3 and TLR4 signal through TRIF to activate TNFR-associated factor family-member-associated NF-κB activator binding kinase 1 (TBK1)/inducible IκB kinase and IRF3/7 to produce type I IFNs (Fig. 1).[41–43] These signaling pathways are essential for further triggering the host innate and adaptive immune responses against infection.

Emerging evidence has also indicated that TLRs play important roles in cancer initiation/progression and cancer therapy. On the one hand, TLR signaling may drive cancer initiation/progression by provoking proinflammatory cytokines or antiapoptotic, proliferative, and profibrogenic signals in cells that eventually transform into tumor cells.[44] TLR signaling stimulates the production of tumor-promoting inflammatory cytokines through the transcription factor NF-κB, including TNF-α, IL-1β and IL-6, which promote tumorigenesis in the intestine, liver, stomach, and skin.[45] For example, in a model of adoptively transferred tumor cells, systemic lipopolysaccharide (LPS) administration increased the growth of tumor cells in a host TLR4 signaling-dependent manner. Mechanistically, the LPS-enhanced TLR4 signaling increased the systemic level of TNF-α, which in turn led to the upregulation of NF-κB-regulated antiapoptotic factors, such as B-cell lymphoma 2 (BCL-2) and inhibitor of apoptosis (IAP), in tumor cells.[46,47]

On the other hand, TLR signaling may drive antitumor effects by eliciting inflammatory cytokines in the tumor microenvironment (TME) to trigger antitumor immune responses or induce apoptosis and programmed necrosis of tumor cells.[48] Activation of TLR signaling induces the maturation of antigen-presenting cells (APCs), including macrophages and DCs, to produce inflammatory cytokines, especially type I IFNs, and upregulates the costimulatory molecules CD80, CD86, and CD40, which further activate innate immune cells and tumor-specific T-cell responses.[3,49] TLR agonists have been demonstrated to achieve potent antitumor effects in both mice and human studies. Among all the TLR agonists, the TLR7/8 agonist imiquimod is the most successful and has been approved by the Food and Drug Administration (FDA) for the treatment of basal cell carcinoma.[50,51] Bacillus Calmette–Guérin, of which the antitumor effect is due to the stimulation of TLR2 and TLR4, has been approved for the treatment of bladder cancer.[52] Other agonists, such as a CpG-containing TLR9 agonist, flagellin (TLR5 agonist), and poly I:C (TLR3 agonist), are still under investigation in the clinic. In addition, TLR agonists might achieve their antitumor effect through direct killing. Flagellin has been reported to induce HeLa cell death.[53] Poly I:C has been reported to trigger both apoptosis and programmed necrosis in tumor cells.[48,54] Therefore, targeting TLRs with agonists for cancer immunotherapy will not be a straightforward process. Further

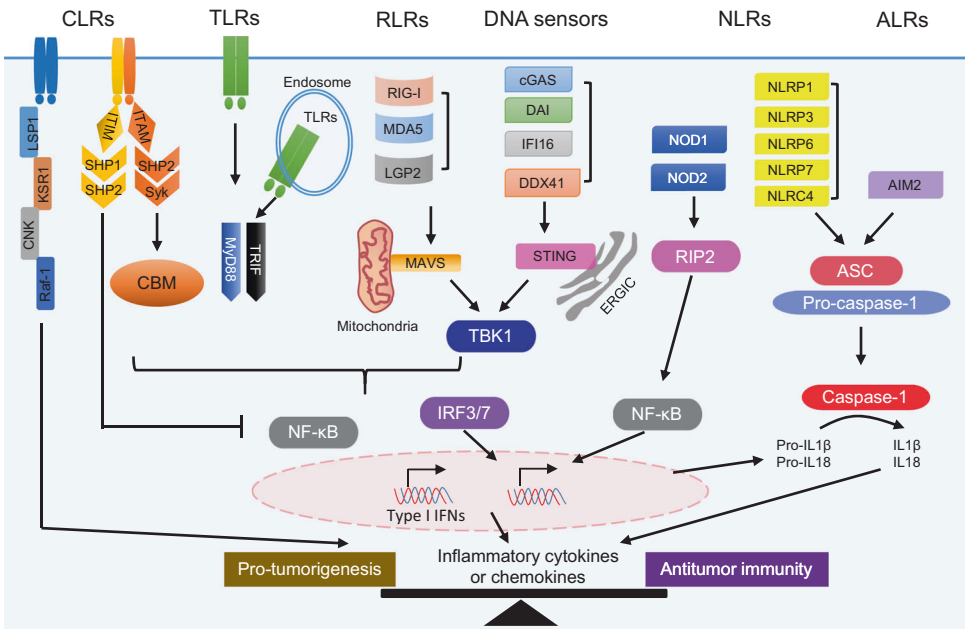

Fig. 1 Innate immune sensing pathways and cancer. Innate immune receptors, including C-type lectin receptors (CLRs), Toll-like receptors (TLRs), RIG-like receptors (RLRs), DNA sensors, NOD-like receptors (NLRs), and AIM2-like receptors (ALRs), are expressed on and/or in various cell types. They cooperate to recognize a variety of danger-associated molecular patterns (DAMPs) from pathogens, damaged cells, or stressed cancer cells and activate downstream signaling for the production of multiple cytokines/chemokines to initiate antipathogen or antitumor immune responses. Here, we list only the key signaling pathways under the receptors upon their ligand binding. For instance, CLRs function through ITAM/Syk/CBM, ITIM/SHP-1/2, or LSP1/KSR1/CNK/Raf-1, TLRs function through MyD88 or TRIF, RLRs function through MAVS, DNA sensors function through STING, and NOD1/2 function through RIP2 to activate cells via the NF-κB and/or IRF3/7 signaling pathways and produce inflammatory cytokines and chemokines. NLRs (such as NLRP1/3/6/7 and NLRC4) and ALRs (AIM2) can form inflammasomes to activate caspase-1, which results in the release of IL-1β and IL-18. The functions of inflammatory cytokines and chemokines in tumorigenesis and antitumor immunity are still controversial, and the balance of these cytokines and chemokines might affect the outcome of activation of the innate sensing pathways in tumorigenesis and antitumor immunity.

understanding the key factors of TLR signaling involved in cancer initiation and progression will guide the clinical application of TLR agonists as cancer therapeutics.

C-type lectin receptors (CLRs) and cancer

CLRs are a large superfamily of receptors that contain at least one carbohydrate-recognition domain, which is important for recognizing a variety of ligands, including galactose, N-acetylgalactosamine, carbohydrate ligands, such as β-glucan, and non-carbohydrate ligands, such as lipids and proteins.[55–57] CLRs work as PRRs and are mostly expressed on myeloid cells and have been traditionally associated with fungal infection.[58] According to the specific motifs in their cytoplasmic domains, CLRs can be divided into activating and inhibitory clusters. Some CLRs transduce their downstream signals through an integral immunoreceptor tyrosine-based activation motif (ITAM)-like/ITAM, which results in cellular activation, and the CLRs that function this way mainly include dectin-1/2/3, Mincle, MCL, BDCA-2, DCAR, DCAR1, DNGR-1, and mannose receptor (MR). CLRs possessing immunoreceptor tyrosine-based inhibition motifs (ITIMs) in their cytoplasmic domain usually suppress cellular activation, and the CLRs that function this way mainly include DCIR, MICL, MAgH, and Ly49Q.[59–61] Upon ligand binding, activating CLRs, such as dectin-1, dectin-2, dectin-3, and mincle, initiate the phosphorylation of ITAM-like/ITAMs and further activate the Syk kinase. Subsequently, the activated CARD9–Bcl10–Malt1 (CBM) complex ultimately activates several transcription factors (such as NFAT, IRF1, IRF5, and NF-κB) to promote the production of both proinflammatory (such as TNF-α, IL-12, IL-6, and IL-1β) and anti-inflammatory cytokines (such as IL-4, TGF-β, and IL-10).[62] Engagement of inhibitory CLRs, such as MICL and DCIR, results in the phosphorylation of ITIMs, which further recruit and activate SHP-1 and SHP-2 and ultimately inhibit cellular activation signaling. Interestingly, these inhibitory CLRs can also enhance cellular activation signaling by inhibiting inhibitory cellular responses.[63] In addition, myeloid CLRs also contain members that do not have ITAM or ITIM domains, mainly including MMR, DEC-205, DC-SIGN, langerin, and MGL. The intracellular domains of these receptors interact with a signalosome composed of LSP1, KSR1, CNK, and the kinase Raf-1, which can modulate cytokine production and endocytic machinery for antigen processing and presentation to T cells (Fig. 1).[64]

Recently, CLRs have attracted increasing attention for their various functions in shaping both innate and adaptive immune responses related to cancer development and therapy. It has been demonstrated that tumor-associated carbohydrate antigens are specifically recognized by certain CLRs. For example, a well-defined tumor-associated antigen (TAA), carcinoembryonic antigen, which is overexpressed on almost all human colorectal, gastric, and pancreatic adenocarcinomas, 70% of non-small-cell lung carcinomas, and 50% of breast carcinomas, is recognized by DC-SIGN.[55,65] DC-SIGN can also recognize mucin 1 with cancer-specific glycosylation changes.[66] Moreover, dectin-1, a CLR for β-glucans, has been reported to recognize N-glycan structures on tumor cells. In addition to recognizing TAAs, dectin-1, DC-SIGN, and MGL have been well studied for their activity in promoting DC maturation and enhancing tumor-specific T-cell responses.[67,68] On the other hand, CLRs expressed on tumor-associated macrophages (TAMs), such as MR, DC-SIGN, MGL, MICL, and DICR, can also cause TAMs to produce immunosuppressive cytokines (such as IL-10 and TGF-β) to impair antitumor T-cell responses upon ligand engagement.[69,70] For instance, a study reported that activation of MR on TAMs either by tumor mucin engagement or an anti-MR antibody promoted the transition of TAMs toward a more immunosuppressive state (increased IL-10, no IL-12, and decreased CCL3).[71] Due to the immunoregulatory effect of CLRs in antitumor immunity, several CLR agonists or antagonists have been considered for cancer therapy. Targeting dectin-1 with β-glucans has shown promising antitumor activity either alone or in combination with chemotherapy in both preclinical and clinical studies.[72,73] Moreover, several CLRs, such as DEC-205, DNGR-1, and DC-SIGN, have been intensively studied for targeted delivery of antigens to APCs in combination with potent adjuvants for vaccine purposes.[74–77]

As discussed above, CLRs, especially MR and DC-SIGN, are involved in inducing both immune tolerance and eradication of tumor cells. The balance and crosstalk between different CLRs and between CLRs and other innate immune signaling pathways in the TME may affect the final outcome of cancer patients. Further understanding of how CLR signaling affects immunological outcomes, immune activation, and immune suppression will facilitate the application of CLR agonists or antagonists in the clinic to benefit patients with cancer- or CLR-related diseases.

Nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) and cancer

Unlike TLRs and CLRs that are commonly located on the membrane, NLRs are a large family of intracellular sensors, the members of which are characterized by sharing a C-terminal leucine-rich repeat domain, a central NOD, and a variable N-terminal effector domain.[43,78] Based on their diverse functions, NLRs can be classified into three clusters: receptors (such as NOD1 and NOD2), adapters (such as NLRP3, NLRC4, and NLRP6), and regulators (such as NLRX1, NLRC3, NLRC5, and NLRP4).[79] NOD1 and NOD2, receptors of PAMPs from microbes, have been well studied. Upon activation by their ligands, g-D-glutamyl-meso-diaminopimelic acid (iE-DAP) for NOD1 and muramyl dipeptide for NOD2, NOD1, and NOD2 self-oligomerize and recruit and interact with the CARD-containing adapter receptor-interacting protein kinase 2 (RIP2 or RIPK2)[80] to form the signaling complex termed "the nodosome," which subsequently leads to the activation of canonical NF-κB pathway- and MAPK pathway-mediated inflammatory responses.[81] Several other NLRs, including NLRP1, NLRP3, NLRP6, NLRP7, and NLRC4, can form different types of inflammasomes.[79,82] The NLRP1 inflammasome was the first complex to be identified, while the NLRP3 inflammasome is the most widely studied. Upon activation, NLRs recruit the inflammatory protease caspase-1 and the apoptosis-associated speck-like protein (ASC) to form large protein complexes termed "inflammasomes," which further promote the secretion of IL-1β and IL-18 and induce a type of inflammatory cell death named pyroptosis (Fig. 1).[83–85]

As we discussed above, NLRs are involved in the regulation of inflammation, which is considered a major hallmark of cancer. Indeed, numerous studies have shown that NLR signaling is very important for cancer development and therapy. For instance, both *Nod1*$^{-/-}$ mice and *Nod2*$^{-/-}$ mice are susceptible to dextran sulfate sodium (DSS) and azoxymethane (AOM)-induced colorectal cancer (CRC).[86,87] In addition, NOD2 polymorphisms are associated with increased risk and the prevalence of gastric, breast, and lung cancers.[80] It has also been reported that increased expression of both NOD1 and NOD2 was observed in head and neck squamous cell carcinoma biopsies compared with healthy nasal biopsies, indicating the role of NOD signaling in enhancing head and neck cancers.[88] Moreover, accumulating evidence suggests that abnormal activation of the inflammasome is closely linked to various types of human cancers. NLRP3 polymorphisms, such as mutations that render NLRP3 constitutively active, are correlated with melanoma susceptibility, CRC prognosis, and overall survival in myeloma.[89] Consistently, NLRP3-deficient mice formed less pulmonary metastasis than control mice in an orthotopic transplant mammary adenocarcinoma mouse model.[90] Mechanistically, NLRP3 activation increased the myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs) in the TME and suppressed NK and T-cell-mediated antitumor activity.[91] Although all of these findings indicate the protumorigenic role of NLRP3, several studies have shown an antitumor role. NLRP3-deficient

mice are more susceptible to AOM–DSS-induced mouse CRC than control mice. Similarly, the NLRP3 expression level was significantly lower in hepatic parenchymal cells in hepatocellular carcinoma biopsies than in the hepatic parenchymal cells in noncancerous samples.[92] In addition to the well-studied NOD signaling, several other NLRs, such as NLRC4, NLRP6, and NLRP12, are also correlated with tumorigenesis. Mice deficient in these NLRs showed increased tumor numbers and burden upon AOM–DSS treatment. In terms of the mechanism, the cellular intrinsic role of NLRC4 in intestinal epithelial cells might be more important for tumor progression, while NLRP6 and NLRP12 mostly achieved their protective roles by regulating the NF-κB signaling pathway and its downstream proinflammatory cytokines and chemokines, such as TNF-α, IL-6, IL-1β, IL-18, CXCL12, and CXCL13.[93–95] These findings highlight the potential roles of NLRs in tumorigenesis, but like the TLRs and CLRs, conflicting evidence still exist. Protumorigenic signaling is balanced by inflammasome-mediated pyroptosis, which enhances antitumor innate and adaptive immunity. Therefore, further studies focusing on understanding the precise effects of NLR signaling in tumorigenesis and discovering novel NLR ligands (agonists or antagonists) might provide potential therapeutic strategies for inflammation-related diseases and cancer.

NA-sensing pathways and cancer

In addition to TLRs, CLRs, and NLRs, cytosolic NA sensors are also important groups of PRRs in the innate immune system that can recognize cytosolic DNA or RNA. The cyclic GMP-AMP synthase (cGAS)–stimulator of IFN genes (STING) axis is the major pathway for cytosolic DNA sensing,[96] while the RIG-I-like receptor (RLR)–MAVS axis is responsible for RNA sensing.[97] Upon the engagement of double-stranded DNA, cGAS catalyzes the synthesis of cyclic-di-GMP-AMP (cGAMP), which in turn binds the adapter protein STING on the endoplasmic reticulum (ER) and promotes TBK1-dependent IRF3 and NF-κB activation for further production of type I IFNs, proinflammatory cytokines, and chemokines to initiate antiviral responses.[98,99] In addition, several other DNA sensors, such as ZBP1, DDX41, DNA-PK, RNA polymerase III, and AIM2-like receptor family members (AIM2 and IFI16), have also been shown to detect cytosolic DNA to activate inflammasome or type I IFN signaling pathways.[96,100] As RNA sensors, RIG-I preferentially recognizes 5′-triphosphate-ending (5′-3p) RNA and short dsRNA, while MDA5 detects long dsRNA. After ligand stimulation, RIG-I or MDA5 interacts with the protein MAVS on the mitochondrial membrane, which activates transcription factors such as IRF3/7 and NF-κB and thus elicits innate/adaptive immunity against viral infection (Fig. 1).[97,101] The NA-sensing pathways described above have been mainly discovered and intensively studied in the field of RNA and DNA virus infection. However, mounting evidence has shown that damaged NAs released from stressed or dying cancer cells can be recognized by the cGAS–STING axis and/or RLRs in DCs to initiate innate immune responses in the TME. Subsequent type I IFN production promotes the activation and maturation of DCs to further cross-prime the tumor-specific T cells for tumor control.[102,103] In addition, several human studies also indicate that NA sensors can serve as tumor suppressors and can be considered prognostic and predictive biomarkers in certain types of cancers. For instance, in human hepatocellular carcinoma, the expression of STING has been negatively correlated with advanced tumor stages and patient survival.[104] Based on the role of NA sensing in antitumor immunity, cGAS-STING and RIG-I/MDA5 agonists have been developed for cancer immunotherapy. Notably, some controversial studies have also shown that inappropriate activation of STING and RIG-I signaling can contribute to the suppressive TME and promote tumor growth and metastasis.[105,106] Further characterization of the outcome of activated NA-sensing pathways in the TME is required to better apply agonists of the involved proteins alone or in combination with other potential therapies to benefit cancer patients.

Type I IFN: the bridge between innate immune sensing and adaptive immunity

The generation of adaptive immune responses depends on the activation of the innate immune system. PRRs, such as TLRs, CLRs, NLRs, and NA sensors, are critical in initiating innate immune responses by activating certain key signaling pathways through which several important cytokines are produced to further trigger adaptive immune responses.[43] Among these cytokines, type I IFNs are the best characterized and studied in the field of antiviral immune responses. Type I IFNs consist of a family of class II α-helical cytokines in humans and mice, including IFN-α (with different subtypes), IFN-β, IFN-ε, IFN-κ, and IFN-ω,[107,108] which can be rapidly induced by certain PRRs (such as TLR3, TLR4, TLR7, TLR8, TLR9, NOD1/2, and all NA sensors) upon ligand binding.[109] All type I IFNs share the same receptor, which is a heterodimer of two subunits, IFNAR1 and IFNAR2. The IFN-α receptor is expressed on almost all cells, and type I IFNs can exert direct antiviral effects through it by inhibiting viral replication and inducing apoptosis of infected cells. Type I IFNs can also stimulate the noninfected cells to express genes related to antiviral activity to prevent the virus from spreading.[108,110] On the other hand, type I IFNs can also work on multiple subsets of immune cells, such as macrophages, DCs, NK cells, B cells, and T cells, to regulate host immune responses.[111] This regulatory role places them as the key bridge between innate immune sensing and adaptive immunity. Type I IFNs stimulate the upregulation of both MHC I and MHC II on the cell surface, as well as the costimulatory molecules CD40, CD80, and CD86. In addition, type I IFNs promote antigen retention and cross-presentation by DCs to enhance antigen-specific $CD8^+$ T-cell responses.[112] Furthermore, type I IFNs are also one of the third signals required for human and mouse T-cell activation.[113]

Accumulating evidence has shown that type I IFNs play critical roles during tumorigenesis and cancer therapies. Using a methylcholanthrene (MCA)-induced carcinogenesis model and a transplantable tumor model, it has been demonstrated that endogenous type I IFNs are critical for both immunosurveillance during tumor initiation/progression and the induction of immune responses against transplanted tumors. *Ifnar*$^{-/-}$ mice were unable to reject their tumors.[114,115] Mechanistically, unknown DAMPs (most likely DNA or RNA) released from MCA-treated tissues or transplanted dead tumor cells engage the PRRs to trigger the production of type I IFNs from $CD11c^+$ DCs, which in turn promote the activation and maturation of DCs with captured antigens, especially $CD8\alpha^+$ DCs, to cross-prime T cells against tumor cells.[115,116] Due to the important roles of type I IFNs in enhancing the host innate and adaptive immune responses, type I IFNs could be very potent therapeutics for cancer patients. Indeed, IFN-α has been used in the clinic for several human cancer types in the past few years.[117,118] Several preclinical studies also demonstrated that targeted delivery of type I IFNs into tumor sites could effectively control tumor growth by enhancing the cross-priming capacity of DCs and increasing tumor-specific T-cell responses and further overcoming ICB resistance.[119,120] Furthermore, the antitumor effect of conventional cancer therapies, such as chemotherapy, radiotherapy, and targeted therapies, has also been shown to depend on the type I IFN-enhanced activation of DCs and T-cell responses.[121] All of these studies confirm the essential role of innate immune sensing and type I IFN production in antitumor immunity.

TARGETING INNATE SENSING WITH CONVENTIONAL THERAPIES TO IMPROVE ANTITUMOR IMMUNE RESPONSES

Conventional anticancer therapies, such as radiotherapy, chemotherapy, and targeted therapy, have been historically thought

to act through direct killing of tumor cells. This concept stems from the fact that all these conventional therapies are developed to interfere with key processes of the cell cycle, such as the synthesis of DNA, RNA, and their building blocks, mitotic spindle formation, and specific oncogenic signaling pathways for cancer cells.[21,25,122] However, accumulating evidence indicates that the antitumor activities of such conventional therapies also rely on host innate immunity, especially innate immune sensing, and adaptive immunity (Fig. 2).[121]

Targeting innate sensing with radiotherapy

Radiation therapy is one of the most important components of cancer treatment, with over 50% of patients receiving radiation during their treatment.[123] Canonical radiation treatment for localized tumors consists of small daily doses of ionizing radiation to reduce normal tissue toxicity. Modern advances in imaging and precision targeting have introduced stereotactic body radiation therapy using three-dimensional imaging technology to precisely map the position of the tumor and target higher doses of radiation while better sparing normal tissue.[124] Radiation directly kills cancer cells by inducing DNA damage and increasing reactive oxygen species (ROS) levels, which will restrict the proliferation of irradiated cells and promote cell apoptosis.[125] Intriguingly, our previous study first revealed that the adaptive immune system was required for the optimal antitumor efficacy of radiation. Lee et al. demonstrated that $CD8^+$ T cells were essential for rapid shrinking of irradiated tumor tissue.[126] Similarly, Reits et al. reported that radiation could induce the expression of MHC I on tumor cells, promote antigen presentation, and further facilitate the killing of tumor cells by $CD8^+$ T cells.[127] Furthermore, radiation has also been shown to induce immunogenic cell death (ICD) of tumor cells and promote the release of DAMPs, such as HMGB1, ATP, DNA, and RNA,[26,128–130] which indicates that host innate immune sensing might play a vital role in radiation. Indeed, our previous study observed that radiation significantly increased the production of type I IFNs in the TME and demonstrated that type I IFN signaling was essential for the activation of tumor-infiltrating DCs and the cross-priming of tumor-specific T cells.[131] To further investigate which innate sensing pathway is required for the antitumor effect of radiation, Deng et al. tracked various pathways and observed that radiation induced a therapeutic effect independently of MyD88 and TRIF.[26] They further demonstrated that STING signaling in host cells was required for radiation-induced tumor shrinking. Mechanistically, irradiated tumor-derived dsDNA can be recognized by cGAS in DCs. This facilitates the production of type I IFNs in an autocrine fashion and increases the cytotoxic function of $CD8^+$ T cells via cross-priming.

However, tumor cells themselves fail to produce type I IFN after radiation treatment in vivo. Thus, it is of great interest to induce functional dsDNA innate sensing in tumor cells. Harding and others reported that radiation induces the aggregation of genomic DNA in micronuclei, where cGAS can recognize such DNA fragments and activate the STING-mediated transcription of type I IFNs.[132–134] They further observed that DNA-PK was essential for cGAS to recognize the DNA in micronuclei, as blocking DNA-PK with nu-7741 abolished the production of type I IFNs. Vanpouille-Box et al. found that very high-dose radiation significantly upregulated the expression of three prime repair exonuclease 1(TREX1), which degraded cytosolic DNA and restricted activation of the cGAS/STING pathway in TSA tumor

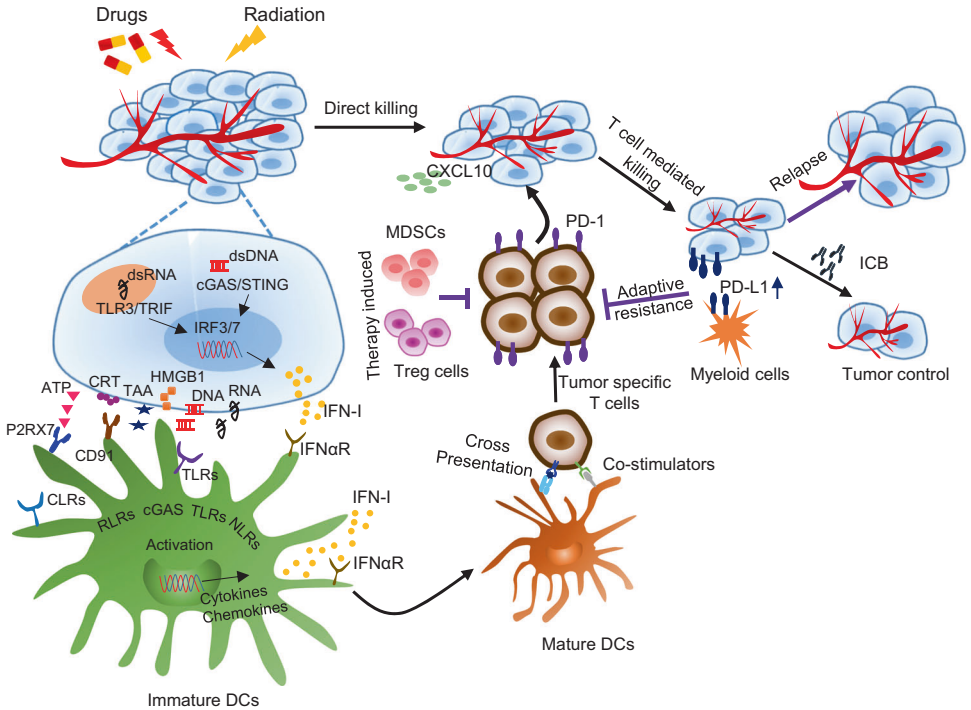

Fig. 2 Immune-based mechanisms of conventional therapies and the rationale of combinational therapy. In addition to direct killing, conventional therapies, such as radiotherapy, chemotherapy, and targeted therapy, can active antitumor immune responses through different innate immune sensing pathways. Conventional therapies can promote the release of tumor-associated antigens (TAAs) and various types of DAMPs, including HMGB1, ATP, DNA, and RNA, and the exposure of CRT. All of these factors can activate DCs (from immature to mature) by triggering innate sensing pathways and the production of type I IFNs (IFN-I) by DCs or tumor cells and promote the cross-priming and recruitment (by increasing CXCL10) of tumor-specific T cells for additional T-cell-mediated tumor cell killing. However, conventional cancer therapies can also increase the immunosuppressive status in the tumor microenvironment (TME), via mechanisms such as upregulation of PD-L1 on both tumor cells and myeloid cells and accumulation of MDSCs and Treg cells in the TME, to cause adaptive immune resistance and result in tumor relapse. Additional immunotherapy, particularly immune checkpoint blockade, will synergize with conventional therapies to further enhance the specific immune response against tumors and achieve better tumor control.

cells.[135] Relatively low-dose radiation treatment significantly increased the aggregation of dsDNA in the cytosol and induced the transcription of type I IFNs. Thus, they further optimized the radiation dose and observed that a hypofractionated 3×8 Gy schedule could synergize with anti-CTLA4 to induce a stronger abscopal effect in a tumor-derived cGAS-dependent manner. We also observed that 1×15 or 20 Gy was still better than 4×5 Gy in controlling primary tumor growth in the B16 tumor model.[126] We hypothesize that the conventional 30×2 Gy schedule might not generate enough innate sensing and may even kill reactivated T cells. Thus, innate sensing may be dose dependent but may also depend on the tumor type. Which dose and schedules might kill more tumor cells, induce greater innate immune sensing, and generate stronger T-cell immune responses remain to be determined in the clinic. Furthermore, although radiation can induce DNA aggregation in micronuclei and enhance dsDNA innate sensing in tumor cells, only slightly increased type I IFN level was observed, which indicates that the major barrier of dsDNA sensing in tumor cells still needs to be further investigated.

In addition to the STING pathway, the inflammasome is another critical platform for recognizing cytosolic foreign or self dsDNA. AIM2 can recognize cytosolic dsDNA and assemble the AIM2 inflammasome to activate caspase-1.[136] Cytosolic mtDNA and radiation-induced ROS can also activate the NLRP3 inflammasome.[137,138] Previous studies have reported that AIM2 is vital for the radiation-induced death of intestinal epithelial cells and bone marrow cells,[139] while radiation also induces the activation of the NLRP3 inflammasome in macrophages.[140] Interestingly, there is complicated mutual regulation between STING and inflammasome sensing.[141,142] As IFN-stimulated genes, AIM2 and caspase-1 are upregulated by type I IFNs,[143–145] while type I IFNs also regulate the activation or suppression of the inflammasome during different treatments.[142,146] Moreover, cGAS/STING can also induce K^+ efflux to facilitate the activation of the NLRP3 inflammasome in a type I IFN-independent fashion.[147] In contrast, the inflammasome can restrict activation of the cGAS/STING pathway in the following two ways: (1) activated caspase-1 can directly cleave cGAS;[148] and (2) inflammasome-activated gasdermin D promotes pyroptosis and K^+ efflux through membrane pores, which restrict the cGAS-mediated production of type I IFNs.[149] These results indicate that blocking the inflammasome might increase cGAS-mediated production of type I IFNs after radiation. However, the role of the inflammasome in the therapeutic effect of radiation in cancer treatment is still largely unknown. In addition, previous studies have reported that radiation can enhance the transcription of small endogenous noncoding RNA or endogenous retrovirus elements.[130,150] The induced RNA can be recognized by RIG-1 or MDA5 to facilitate type I IFN production through MAVS. However, whether and how MAVS-mediated RNA innate sensing regulates adaptive immunity after radiation treatment are still unclear.

In addition to promoting the tumor cell apoptosis, necroptosis and pyroptosis described above, radiation has been reported to induce tumor cell ferroptosis. Ferroptosis is another regulated form of cell death that results from the iron-dependent accumulation of lipid peroxide.[151,152] Recently, Lang et al. revealed that radiation can trigger tumor cells to undergo ferroptosis by inducing oxidative damage and the accumulation of toxic lipid peroxidation, and also sensitize tumor cells to ferroptosis agonists.[153] Moreover, they demonstrated that $CD8^+$ T cells can regulate tumor cell ferroptosis during immunotherapy.[154] Furthermore, they implicated ferroptosis as a direct link through which immunotherapy and radiotherapy cooperate to improve tumor control. Mechanistically, IFNγ derived from immunotherapy-activated $CD8^+$ T cells synergizes with the radiation-activated ataxia telangiectasia mutated gene to suppress solute carrier family 7 member 11 (SLC7A11), resulting in enhanced tumor lipid oxidation and ferroptosis.[153] However, whether ferroptosis has a negative or positive impact on radiation-induced innate sensing or immunotherapy-induced tumor-specific immune responses is still largely unknown and needs to be further investigated.

Targeting innate sensing with chemotherapy

Beyond the direct killing effect of tumor cells, numerous clinical and preclinical studies have demonstrated that chemotherapeutics can also work directly or indirectly on the immune system, which has been well reviewed by other researchers.[155–157] On the one hand, some chemotherapeutics can induce tumor cell ICD, which will alert the innate immune system, followed by the activation of the host adaptive immune system. However, certain chemotherapeutics can directly affect immune cell populations via mechanisms such as inducing transient lymphodepletion or specifically reducing MDSCs or Tregs to subvert the immunosuppressive microenvironment.[21] Here, we will focus on key findings related to only the innate sensing pathways activated by certain chemotherapeutics. The antitumor activity of numerous chemotherapeutic agents, such as anthracyclines and oxaliplatin, was decreased in TLR4- and MyD88-deficient mice. Mechanistically, HMGB1, which is released by chemotherapy-induced cell death, can promote activation and maturation of DCs by binding to TLR4 and activating its downstream MyD88 signaling pathway to induce antitumor T-cell responses.[27,158] Similarly, loss-of-function polymorphisms affecting TLR4 are associated with decreased time to metastasis among patients with anthracycline-treated breast carcinoma.[27] In addition, it has also been demonstrated that anthracyclines can stimulate the production of type I IFNs by cancer cells through the activation of TLR3, which further promoted the DC cross-priming and CXCL10 production needed for recruiting T cells against the tumor.[159] Moreover, another study revealed that ATP released from dying tumor cells as a result of chemotherapy can act on P2X7 purinergic receptors (P2RX7) on DCs and trigger the NLRP3-dependent inflammasome, which is responsible for the secretion of IL-1β and enhanced tumor-specific T-cell immunity. The priming of tumor-specific T cells fails in the absence of a functional IL-1 receptor 1 and in Nlpr3-deficient or caspase-1-deficient mice unless exogenous IL-1β is provided. Accordingly, anticancer chemotherapy was ineffective against tumors established in purinergic receptor P2rx7-, NLRP3-, or caspase-1-deficient hosts. Consistently, anthracycline-treated individuals with breast cancer carrying a loss-of-function allele of P2RX7 developed metastatic disease more rapidly than individuals bearing the normal allele.[90] Intriguingly, the same group also reported that another two chemotherapeutic agents, gemcitabine and 5-fluorouracil (5-FU), activated NLRP3-mediated inflammasome formation in MDSCs, followed by IL-1β production that can induce IL-17 secretion from $CD4^+$ T cells and further impair the anticancer efficacy of chemotherapeutic drugs. Accordingly, gemcitabine and 5-FU exert increased antitumor effects in NLRP3- or caspase-1-deficient mice, and NLRP3 activation by chemotherapeutic drugs is considered a positive regulator to promote tumor growth.[160] All these results indicate the pivotal and conflicting roles of innate sensing pathways in chemotherapy-induced antitumor immunity. Further investigations are needed to better understand the effects of chemotherapeutics on the immune system, which will help modulate the existing chemotherapy strategies to best benefit cancer patients.

Targeting innate sensing with targeted therapy

Cancer targeted therapies, including targeted monoclonal antibodies (mAbs) and small molecules, were previously thought to prevent tumor growth by directly blocking oncogenic signaling and inducing tumor cell apoptosis. Accumulating evidence suggests that the antitumor efficacy of these tumor-targeted therapeutics also relies on host innate and adaptive immunity. Here, we focus on summarizing the important discoveries related to targeted therapy and innate immune sensing.

Antibody-based targeted therapy

Targeted antibodies, such as trastuzumab (target ERBB2/HER2), cetuximab (target EGFR), and rituximab (target CD20), have been approved for use in patients with oncogene mutations or overexpression.[161,162] In addition to blocking tumor cell growth signaling and triggering apoptosis, these mAbs engage several innate immune effector processes, including complement-dependent cytotoxicity, antibody-dependent cell cytotoxicity, and antibody-dependent cellular phagocytosis mediated by myeloid cells and NK cells.[121] Previous studies with xenograft models revealed that direct growth signaling blockade or innate immune cell-mediated killing by these mAbs was sufficient to control tumors, but the antitumor efficacy was relatively limited.[163–165] Whether the adaptive immune system was involved could not be determined in these xenograft models. However, later studies demonstrated that these mAbs that triggered adaptive antitumor immune responses were more efficient in controlling tumor growth and provided long-lasting protection.[24,166,167] In the clinic, patients who have been treated with trastuzumab exhibit a substantial increase in $CD8^+$ T cells and NK cells, which is correlated with improved clinical outcomes.[168] Although much clinical evidence has shown this kind of correlation, the mechanisms by which the adaptive immune responses are enhanced were still largely unknown until recent studies with syngeneic tumor models in immunocompetent mice. Using a mouse mammary tumor isolated from HER2/neu transgenic mice, our previous study first demonstrated that the antitumor effect of anti-HER2/neu depended on both host innate sensing and adaptive immunity,[24,169] which has been further confirmed by others.[170] Mechanistically, the HER2/neu antibody induces release of the stress protein HMGB1 from treated tumor cells, which can initiate the MyD88/type I IFN innate sensing pathway and further enhance tumor-specific T-cell responses.[24] A similar phenotype was also observed with EGFR (cetuximab) and CD20 (rituximab) antibodies. The EGFR antibody was shown to promote the phagocytosis of an EGFR-expressing human colon cancer cell line by DCs and to increase cross-priming of T cells through MyD88-mediated innate sensing.[171] In addition, it has been reported that cetuximab, in combination with chemotherapy, fostered ICD in CRC cells via the ER stress response and an increase in phagocytosis by DCs. The authors also further confirmed the enhanced immunogenicity elicited by cetuximab in a mouse model of human EGFR-expressing CRC.[172] In a preclinical B-cell lymphoma model, an anti-CD20 antibody was reported to trigger macrophages to produce type I IFNs, which in turn promoted the activation and maturation of DCs and further enhanced $CD8^+$ T-cell responses.[166] Unlike the targeted mAbs discussed above, the anti-CD47 mAb, which blocks the "don't eat-me" molecule CD47 that is broadly expressed on multiple cancer types, is a novel and potential target agent for cancer patients.[173] The antitumor effect of CD47-blocking mAbs has been considered to largely depend on the enhanced antitumor phagocytosis by macrophages in studies using xenograft tumor models in immunodeficient mice.[174] Later, studies using syngeneic murine models in immunocompetent mice revealed that the therapeutic effects of the anti-CD47 mAb mainly depend on DC (but not macrophage)-mediated cross-priming of T-cell responses. The authors further demonstrated that CD47 blockade with antibody activated the innate sensing pathway related to the cytosolic DNA sensor STING in DCs, but not macrophages, and promoted the production of type I IFNs for enhanced cross-priming of tumor-specific T-cell responses.[121,175]

Small molecule-based targeted therapy

Unlike targeted mAbs, which were developed to target the extracellular domain of surface receptors, small molecules were always designed to target intracellular tyrosine kinase domains or other molecules essential for maintaining the malignancy of cancer cells.[176] EGFR tyrosine kinase inhibitors (TKIs) are considered one of the most successful targeted therapies used for cancer patients with EGFR/HER2-driven mutations, such as patients with lung cancer, breast cancer, and colon cancer.[177,178] It has been shown that EGFR TKIs show greater potency than targeted antibody therapies or existing chemotherapies.[179–182] Due to a lack of syngeneic murine tumor models, studies investigating the antitumor effect of EGFR TKIs have been performed either in vitro or using xenograft tumor models in immunodeficient mice, and these studies have demonstrated that prolonged treatment can potently suppress tumor growth.[183–185] These results suggest that the therapeutic effect of EGFR TKIs likely occurs through direct blockade of oncogenic signaling and induction of tumor cell apoptosis. However, recent studies have shown that EGFR TKIs might modulate tumor plasticity and enhance the tumor recognition or tumor lysis by innate NK cells and antigen-specific T cells.[167,186] However, the mechanisms by which EGFR TKIs influence the host immune system are still poorly defined. Interestingly, by using EGFR TKI-sensitive mouse syngeneic tumor models, we recently observed that hypofractionated EGFR TKI treatment (a high dose with a low-frequency treatment), but not standard hyperfractionated EGFR TKI treatment (a low dose with daily treatment), could trigger great innate sensing and type I IFN and CXCL10 production through the MyD88 signaling pathway. This innate activation further enhanced tumor-specific T-cell infiltration and reactivation to prevent and limit tumor relapse. Mechanistically, we further observed that a high dose of EGFR TKI treatment rapidly induced cellular stress and apoptosis and then increased the release of DNA and RNA from tumor cells, which may promote innate sensing and type I IFN production. However, which kinds of DAMPs and TLRs are upstream of the Myd88 signaling pathway remain to be determined.[23]

Cabozantinib is a receptor TKI with potent activity against multiple targets, including c-MET, VEGFR2, RET, KIT, AXL, and FLT3, all of which have been associated with tumor growth and survival.[187] Cabozantinib has shown striking responses across several cancer types in the clinic. A clinical study reported that cabozantinib could significantly reduce the myeloid immunosuppressive cell subsets (MDSC and $TIM3^+$ myeloid cells) in pretreated metastatic renal cell carcinoma patients.[188] Another preclinical study demonstrated that cabozantinib reduced intratumoral PMN-MDSCs by suppressing the MDSC-promoting cytokines secreted by cancer cells and enhanced the therapeutic effect of immunotherapy in a metastatic castration-resistant prostate cancer model.[189] However, a recent study from Patnaik et al. reported that cabozantinib could rapidly eradicate spontaneous prostate cancer in PTEN/p53-deficient mice with increased infiltration of neutrophils. HMGB1 released from stressed tumor cells under cabozantinib treatment triggered innate sensing, and CXCL12 production resulted in robust infiltration of neutrophils into the TME for tumor clearance. Accordingly, cabozantinib-induced tumor clearance in mice was abolished by antibody-mediated granulocyte depletion, HMGB1 neutralization, or blockade of neutrophil chemotaxis with the CXCR4 inhibitor plerixafor.[190]

Poly (ADP-ribose) polymerase (PARP), which is responsible for DNA repair, has been demonstrated to be a promising target in cancer patients with BRCA1 or BRCA2 mutation.[191,192] PARP inhibitors have shown promising clinical activities in cancer patients with BRCA mutations based on the concept of synthetic lethality between PARP inhibition and BRCA1 or BRCA2 mutation.[193] The therapeutic mechanisms of PARP inhibition were recently discovered. Using a syngeneic Brca1-deficient ovarian mouse tumor model, the authors found that olaparib, a PARP inhibitor, elicited both $CD4^+$ and $CD8^+$ T cells against tumors. They further revealed that DCs could sense tumor-derived dsDNA fragments and/or cGAMP upon PARP inhibition

and drive STING-dependent type I IFN signaling and T-cell responses.[194] Another study showed that the antitumor efficacy of PARP inhibition mainly depended on tumor-specific T-cell responses in a Brac1-deficient triple-negative breast cancer model. Intriguingly, they demonstrated crosstalk between PARP inhibition and the TME related to cGAS/STING/TBK1/IRF3 pathway activation in cancer cells that governed $CD8^+$ T-cell recruitment, activation, and antitumor efficacy.[195] Therefore, these two studies revealed that in addition to synthetic lethality, the therapeutic effect of PARP inhibition in vivo is mainly caused by triggering innate sensing and eliciting host adaptive immune responses.

Cyclin-dependent kinases 4 and 6 (CDK4/6), key drivers of the cell cycle, are required for cancer initiation and progression.[196] CDK4/6 inhibitors, such as abemaciclib, palbociclib, and ribociclib, have shown promising activity against several solid tumors.[190] Initially, their primary antitumor effect was considered to be inhibition of the phosphorylation of the retinoblastoma tumor suppressor and induction of G1 cell cycle arrest in tumor cells.[197] Recently, Goel et al. found that CDK4/6 inhibitors not only induced tumor cell cycle arrest but also promoted antitumor immunity. They revealed that the enhanced antitumor immune response had two underpinnings. On the one hand, CDK4/6 inhibitors activate transcription of endogenous retroviral elements in tumor cells and increase intracellular levels of dsRNA. This in turn stimulates the RNA innate sensing pathway and the production of type III IFNs in tumor cells for enhanced tumor antigen presentation. In addition to triggering innate sensing, CDK4/6 inhibitors also markedly suppress the proliferation of Tregs. Ultimately, these events promote tumor-specific cytotoxic T-cell responses for effective tumor clearance.[198]

NAD(P)H: quinone oxidoreductase 1 (NQO1) is a cytosolic two-electron oxidoreductase that is highly expressed in various human cancers.[199,200] While catalase, a hydrogen peroxide (H_2O_2)-scavenging enzyme, has lower expression in tumor tissues than in normal tissue.[201] High NQO1:catalase ratios in tumor cells offer a potential therapeutic target for NQO1 bioactivatable drugs, while low expression ratios protect normal tissues. β-lapachone, an NQO1 bioactivatable drug, can be catalyzed by NQO1 in tumor cells to generate high levels of ROS, which further causes DNA damage and cell death.[202] A recent study demonstrated that β-lapachone triggered tumor-selective innate sensing, leading to T-cell-dependent tumor control. Mechanically, β-lapachone induces HMGB1 release from the oxidation-stressed tumor cells and further activates the host TLR4/MyD88/type I IFN pathway and Batf3 DC-dependent cross-priming to bridge innate and adaptive immune responses against the tumor.[203]

These findings reveal the critical role of innate immune sensing in the therapeutic effect of targeted therapies, showing that they are not as specific as initially designed. However, a lack of proper syngeneic tumor models restricts the characterization of the role of innate sensing in other targeted therapies. Thus, developing syngeneic murine tumor models that are sensitive to certain targeted therapies will allow us to further interrogate the relationship between the immune system and targeted therapeutic effects. This will further promote the clinical development of potential novel combinational strategies for patients with targetable cancers.

INTEGRATING CONVENTIONAL CANCER THERAPIES TO IMPROVE CANCER IMMUNOTHERAPY

Cancer immunotherapy was called the "Breakthrough of the Year" by Science in 2013 due to its great success in the clinic for many forms of cancer.[204,205] There are several types of immunotherapies, such as antibody-based immunotherapies (particularly ICB), adoptive cellular therapy (particularly chimeric antigen receptor (CAR) T cells), cancer vaccines, and cytokine therapy. ICB, including ipilimumab (anti-CTLA-4) and pembrolizumab (anti-PD-1), has been the most attractive form of cancer immunotherapy because of its great success in benefiting various types of cancer patients, such as those with melanoma, CRC, and non-small-cell lung cancer.[206–209] CAR T-cell therapy represents another major immunotherapy because of its good responses and high complete remission rates in patients with hematologic malignancies. However, the application of CAR T-cell therapy in patients with solid tumors remains a significant challenge.[210] Cancer therapeutic vaccines aim to activate the patient's own immune system to fight cancer. Promising results from clinical trials have led to the approval of several cancer vaccines by the U.S. FDA.[211] Based on the potent activity of several proinflammatory cytokines in triggering host immunity and enhancing antitumor efficacy in preclinical murine cancer models, recombinant IFN-α and IL-2 have been approved for several malignancies.[212] However, although immunotherapy can improve T-cell responses against tumors to induce long-lasting responses and significantly improve the overall survival of patients, this only occurs in a small fraction of patients.[213,214] A retrospective analysis of the patients who responded very well to ICB revealed that patients with highly immunogenic tumors (such as a high tumor mutation burden, high PD-L1 levels, and a high frequency of circulating Ki-67^+CD8^+ T cells related to the tumor) and low tumor burden were more likely to respond to ICB or other immunotherapies.[215–217] Accumulating evidence shows that conventional therapies, such as radiotherapy, chemotherapy, and targeted therapies, not only can significantly reduce tumor burden through tumor-intrinsic mechanisms but also can increase tumor immunogenicity by releasing DAMPs and TAAs.[218] DAMPs can trigger innate immune sensing and the production of proinflammatory cytokines and chemokines, which in turn further enhance the cross-priming and recruitment of tumor-specific T cells. Interestingly, ATP, as one of the DAMPs, has been reported to play both positive and negative roles in APC maturation and T-cell proliferation. A low concentration (~250 nM) of ATP can activate the APCs and promote T-cell proliferation, whereas a high concentration (0.1–1 mM) of ATP can induce a disordered maturation of DCs and decrease the cell proliferation ability and IL-2 production of activated T cells, even inducing T-cell death.[219–221] Meanwhile, enhanced innate sensing induces immunosuppressive factors, such as high PD-L1 expression and increased immune-suppressive cell populations (MDSCs and Tregs).[23,222–224] Therefore, the proper combination of immunotherapies based on an understanding of the TME after conventional therapies might achieve the maximum antitumor efficacy (Fig. 2).

Decades ago, most studies focused on the direct killing effect of radiation on tumor cells. However, emerging evidence strongly supported that the adaptive immune responses were essential for the antitumor efficacy of radiotherapy.[225,226] Radiation plays dual roles in antitumor adaptive immunity. On the one hand, radiation significantly induces tumor cell death to reduce tumor burden and enhances antitumor immunity through the cGAS/STING/type I IFN innate sensing pathway.[26] In addition, radiation can also induce the expression of NKG2D ligands on tumor cells, which further promotes NK and $CD8^+$ T-cell-mediated tumor cell killing.[227,228] On the other hand, radiation can also upregulate the expression of PD-L1 on both tumor and immune cells,[229,230] which in turn contributes to the restriction of antitumor immunity. Although more DCs, T cells and NK cells infiltrate into the tumor tissue after radiation treatment,[231,232] radiation also induces a significant increase in the number of MDSCs, Tregs, M2 macrophages, and other immune-suppressive components in the TME.[229] All these immune-suppressive components are responsible for the production of immunosuppressive factors, including TGF-β, IL-10, ARG1, and IDO.[233–237] Combination approaches to overcome these immunosuppressive factors have been studied to achieve optimal therapeutic antitumor immune responses. Several preclinical

studies have shown that a combination of ICB or a cancer therapeutic vaccine with radiation could achieve a better therapeutic effect and generate a stronger systemic antitumor effect than a single treatment alone.[135,222,238–242] Moreover, combining radiation and anti-CTLA-4 or PD-L1/PD-1 blockade has shown synergy in enhancing the abscopal effects in the clinic.[243,244] Recently, clinical studies have shown that radiation can enhance CAR T-cell therapy in patients with B-cell lymphoma without increased toxicities.[245] Whether radiation can increase the antitumor efficacy of CAR T-cell therapy in patients with solid tumors still needs further investigation.[246] Immune stimulatory molecules, such as anti-CD137/CD40 agonists and TLRs/STING agonists, have also been reported to show synergistic antitumor effects with radiation in preclinical studies and also have the potential for clinical application.[26,247–254] However, most of these agonists activate the related pathways in only host immune cells, typically in DCs, macrophages, or T cells, which may result in the activation-induced cell death of immune cells and the upregulation of immunosuppressive factors,[251] all of which eventually limit antitumor immunity. Therefore, to achieve maximal antitumor efficacy in combination treatment with radiation and immunotherapies, several variables of both therapies need to be systematically assessed, including the dose range, schedule, treatment sequence for combination, and target lesion choice.[35]

Direct tumor cell killing is the primary goal of chemotherapy. However, it has become clear that chemotherapeutics can also enact their antitumor activity via host innate and adaptive immunity. Certain chemotherapeutic agents can initiate innate immune sensing and activate tumor-specific adaptive T-cell responses by inducing ICD of tumor cells, as discussed above. In addition, some chemotherapeutics directly affect host innate and adaptive immune cells to enact their antitumor effects. Cyclophosphamide (CTX) is one of the best characterized chemotherapeutic agents that influences host immunity against tumors directly. It was reported that low-dose CTX treatment was associated with enhanced activation of NK cells and switching of M2 macrophages to M1 macrophages, which promoted the expansion and differentiation of DC precursors in the peripheral lymphoid organs and then tumor localization, reducing and suppressing Tregs and increasing MDSCs in both clinical and preclinical studies.[255–257] In addition to CTX, other commonly used chemotherapeutics, such as doxorubicin, cisplatin, 5-FU, gemcitabine, and paclitaxel (PTX), also exert similar impacts on the host immune system.[21,258] All these observations indicate the feasibility of a combination of chemotherapy and immunotherapy. Indeed, combining chemotherapy and ICB (CTLA-4 blockade) has shown promising outcomes in both advanced melanoma and lung cancer patients.[259,260] Previous studies from our group and others have also demonstrated that chemotherapy could significantly enhance the therapeutic effect of cancer vaccines.[261] Notably, intensive chemotherapies are always preferred in the clinic to maximally reduce the tumor mass, which may also impair the host immune responses for long-term protection against tumor recurrence. How to properly use combination therapy to avoid such side effects has become an outstanding issue. Our previous study has shown that the antitumor effect of anti-HER2 treatment largely depends on host T-cell responses. Intriguingly, additional intensive chemotherapeutic drugs after antibody treatment indeed synergistically reduced the tumor burden but also impaired the anti-HER2-generated antitumor T-cell responses and resulted in tumor relapse after tumor rechallenge. Instead, when chemotherapeutic drugs were given before antibody treatment, the antibody-mediated T-cell immunity was significantly diminished, and the synergistic effect in reducing tumor burdening was abolished.[24] Moreover, our group also found that when an anti-CD47 antibody was administered after chemotherapy, but not before chemotherapy, the antitumor effect was enhanced.[175] Therefore, the dosing and timing of each therapy for optimal combination must be carefully considered. How chemotherapy should be combined with immunotherapy in the neoadjuvant or adjuvant setting needs further investigation.

Unlike radiotherapy and chemotherapy, which are widely used in various types of cancer patients, the applications of targeted therapies are restricted to cancer patients with driver mutations or overexpression of specific oncogenic signaling pathways that are essential for cancer cell survival. Together with their strength in reducing tumor burden, mounting evidence has shown the potential activity of targeted therapies in regulating the host immune system. In addition to the targeted therapies described above, other targeted therapies have also been reported to be associated with antitumor immune responses.[122] For instance, BRAF inhibitors (vemurafenib and dabrafenib) and MEK inhibitors (trametinib and cobimetinib) have been demonstrated to increase the expression of MHC I and upregulate the presentation of TAAs on tumor cells, which in turn activate tumor-specific T cells and facilitate T-cell-mediated killing in melanomas with constitutively active BRAF isoforms (mainly the V600E substitution).[35,262,263] Interestingly, MEK inhibitors have been reported to attenuate the terminal differentiation of T cells,[264] while BRAF inhibitors could active the MAPK pathway in T cells.[265] Moreover, BRAF inhibitors can also increase immunosuppressive cells, MDSCs, and Tregs, in the TME, whereas the addition of MEK inhibitors can counteract this suppressive effect.[266] Dasatinib, an oral dual BCR/ABL and Src family TKI, has also been linked to reduced Tregs and MDSCs in the TME in a mouse melanoma model.[267] Several TKIs that inhibit tumor angiogenesis through targeting vascular endothelial growth factor A (VEGFA) have been observed to modulate the TME. Sorafenib (a multiple target TKI) was shown to deplete circulating MDSCs and intratumoral Tregs both in mice and in patients with renal cell carcinoma.[268,269] Sunitinib, another FDA-approved multiple target TKI, was shown to enhance the recruitment of T cells into the TME by upregulating the expression of CXCL10 and CXCL11 on tumor vessels.[270] However, although targeted therapies can initially significantly reduce tumor burden with a high response rate and improve the outcome of patients with immunostimulatory effects, patients will eventually develop drug resistance, and tumors will relapse, resulting in only modest improvements in the overall survival of cancer patients. Given the advantages of immunotherapies, which can induce a long-lasting immune response against tumors and dramatically improve the overall survival of responders, the combination of targeted therapies and immunotherapies is a promising strategy to achieve both a high response rate and significantly improved overall survival in the clinic.[28] Indeed, several clinical trials are ongoing to test certain targeted therapies in conjunction with immunotherapies.[122] Promising synergistic antitumor efficacy has been shown in several clinical studies, whereas substantial toxicity has also increased, and some of the clinical trials have been suspended.[271] Therefore, how to properly combine targeted therapies and immunotherapies to achieve optimal antitumor effects without severe side effects has become an outstanding issue. Our recent study proposed that manipulating the dosing and timing of TKIs and immunotherapy might achieve this. Intriguingly, we demonstrated that anti-PD-L1 should be combined concurrently or early after treatment with a hypofractionated EGFR TKI to obtain the maximal synergistic antitumor effect. If anti-PD-L1 was given when the tumor started to relapse and grow, almost no synergistic antitumor effect was observed. More importantly, we also found that the hypofractionated EGFR TKI/anti-PD-L1 regimen caused much fewer side effects than the hyperfractionated EGFR TKI/anti-PD-L1 regimen.[23] Similarly, we have also demonstrated that an EGFR TKI could synergize with tumor-targeted IL-2 to achieve much better tumor control than either treatment alone.[272] If these observations can be applied to other TKIs or targeted therapies, our findings might open new treatment avenues for targeted therapy and immunotherapy in cancer patients. Another limitation

for completely investigating the immunomodulatory effects of targeted therapies or combinations with immunotherapy is the lack of syngeneic murine tumor models that are sensitive to certain targeted therapies. Developing syngeneic mouse tumor models or xenograft tumor models in humanized mice will be helpful for a deeper understanding of the impact of targeted therapies on the host immune system, which will facilitate the design of combinational therapies in clinical trials.

CONCLUSIVE REMARKS

Innate immune sensing pathways play critical roles in regulating host innate immunity and subsequent adaptive immunity against cancer during cancer initiation/progression and cancer therapy. It is now becoming clear that in addition to their strength in reducing tumor burden directly, all conventional therapies, including radiotherapy, chemotherapy, and targeted therapy, can deeply impact host innate and adaptive immunity by triggering certain innate sensing pathways for the production of type I IFNs. As a bridge between innate and adaptive immunity, type I IFNs enhance the capacity of DCs, especially $CD8\alpha^{+}$ DCs, to cross-prime tumor-specific T cells for tumor control. However, tumors always relapse by developing treatment/drug resistance or acquiring adaptive immune resistance. Recent advances in cancer immunotherapy offer potential combinational strategies of conventional therapies. The combination of conventional therapy and immunotherapy has shown promising clinical activity, whereas the incidence of severe toxicities also increased. A deeper understanding of the impact of conventional therapies on immune cells in terms of treatment regimen (dosing and timing) and the mechanisms of combinational therapies in inducing a synergistic antitumor effect and/or severe toxicities will help in designing potential combinational therapies to achieve maximal antitumor efficacy with minimal toxicities in the clinic.

ACKNOWLEDGEMENTS

We thank Casey Moore for helpful editing and discussions. Y.-X.F. holds the Mary Nell and Ralph B. Rogers Professorship in Immunology. This work was supported in part by Texas CPRIT grants RP180725 and RR150072 (CPRIT scholar in Cancer Research) to Y.-X.F.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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