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# Signal-transducing innate receptors in tumor immunity

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## Abstract

The signal-transducing innate receptors represent classes of pattern recognition receptors (PRRs) that play crucial roles in the first line of the host defense against infections by the recognition of pathogen-derived molecules. Because of their poorly discriminative nature compared with antigen receptors of the adaptive immune system, they also recognize endogenous molecules and evoke immune responses without infection, resulting in the regulation of tumor immunity. Therefore, PRRs may be promising targets for effective cancer immunotherapy, either by activating or inhibiting them. Here, we summarize our current knowledge of signal-transducing PRRs in the regulation of tumor immunity.

**KEYWORDS**
cytokines, inflammation, innate immunity, pattern recognition receptor, tumor microenvironment

## 1 | INTRODUCTION

Rapid progress in cancer immunotherapy has been witnessed over the past few decades. Although this field has had a long history of aspiration and frustration,[1,2] it has become clear that the immune system indeed functions in the fight against cancer.[3-5] In this regard, much attention has been focused on the role of adaptive immune responses in tumor immunity. Numerous studies have also revealed that the innate immune system is a critical regulator of tumor pathogenesis.[6] The production of type I interferons (IFNs) and inflammatory cytokines is a hallmark of innate immune activation and these cytokines affect tumor growth through various mechanisms, either positively or negatively, in a context-dependent manner. For example, type I IFNs directly induce apoptosis of cancer cells and they also promote maturation of DCs and cytotoxic activity of CD8^{+} T cells, thereby augmenting anti-tumor immunity.[7] However, type I IFN can also induce the expression programmed cell death-1 (PD-1) in T cells, promoting their exhaustion and, therefore, impeding anti-tumor immunity.[8] Furthermore, multiple cytokines can skew the differentiation of myeloid cells to suppressive phenotype and also

promote recruitment of those cells in the tumor microenvironment (TME).[9]

The induction of these cytokines is largely dependent on the activation of pattern recognition receptors (PRRs). PRRs are highly expressed in innate immune cells and recognize both pathogen-associated molecular patterns (PAMPs) or endogenous molecules termed damage-associated molecular patterns (DAMPs).[10,11] PRRs are subdivided into:

(i) membrane-associated PRRs, which include Toll-like receptors (TLRs) and C-type lectin receptors (CLRs);

(ii) cytoplasmic PRRs, which include the RNA-sensing retinoic acid-inducible gene-I (RIG-I)-like receptor (RLR) family, DNA-sensing receptors, nod-like receptor (NLR) family and the absent in melanoma 2 (AIM2)-like receptor (ALR) family;

(iii) soluble PRRs, which include complement receptors, collectins, pentraxin proteins, and others.[11-14]

The first 2 PRRs classes, but not the soluble PRRs, can transmit signals to the nucleus upon binding with their cognate ligand(s) to alter gene expression profile of the cell.

Accumulating evidence has been provided to show that anti-tumor immunity is regulated by the activation of PRRs.[15] Engagement of

PRRs by their exogenously administered ligands induces robust anti-tumor immune responses in animal models,[16-18] while exacerbated tumor growth has been observed in some PRR-deficient mice.[19,20] In this way, several adjuvants, such as TLR3 agonist poly(I:C), TLR9 agonist CpG, and stimulator of IFN genes (STING) agonist cGAMP, have been reported to show beneficial effects by improving the immune checkpoint inhibitor therapy for cancer in preclinical models.[21-23]

Conversely, as the activation of PRRs evokes inflammatory responses, this raises the question of whether PRRs may inhibit cancer immunity, as the progression of several types of tumors is often associated with persisted inflammation.[24,25] The chronic activation of PRRs by endogenous ligands such as DAMPs released from tumor cells may promote tumor progression through pro-inflammatory responses, which in turn augments the proliferative, anti-apoptotic, and pro-fibrogenic signals within the TME.[15,24] These seemingly contradictory results indicate that innate immune activation by PRRs confers a dual role, ie, an immune-enhancing role that potentiates anti-tumor immunity and a tumor-promoting role through the induction of persisted inflammation.

In this review, we summarize our current understanding of PRRs that play a critical role in the regulation of oncogenesis. For the broader biology of innate immune cells and PRRs, excellent reviews are available.[10,26-28]

## 2 | MEMBRANE-ASSOCIATED PRRS

### 2.1 | Role of TLRs in the regulation of tumor development

The TLR family is by far the best characterized class of PRRs, which function as sentinels of pathogen infections.[12,13,29] TLRs can recognize microbial PAMPs and signal through the recruitment of cognate adaptor proteins.[30] In general, TLRs commonly utilize the adaptor protein myeloid differentiation primary response gene 88 (MyD88).[30] TLR3 employs the TIR domain-containing adaptor-inducing IFN-β (TRIF, also called TICAM) adaptor protein, although there is evidence that it also utilizes MyD88.[30,31] TLR4 requires both MyD88 and TRIF adaptor proteins for the full-blown activation of its downstream signaling pathway.[30] Upon binding to TLRs, those adaptor proteins engage additional downstream proteins that mediate the activation of transcription factors such as nuclear factor-kappa B (NF-κB), IFN regulatory factors (IRFs), and protein kinases such as mitogen-activated protein kinase (MAPK) to execute transcriptional control of target genes, including those for type I IFNs and inflammatory cytokines.[13,30]

The fact that TLRs are highly expressed in antigen-presenting cells and the activation of some TLRs by their cognate ligands induces anti-tumor mediators such as type I IFNs, has led to efforts to harness TLR agonists for cancer therapies. Indeed, plenty of clinical trials targeting TLRs for cancer therapy are ongoing.[32] However, TLR signaling also induces chronic inflammatory responses, which potentially also favor tumor growth.[33] An emerging notion is that, in addition to PAMPs, TLRs also recognize a wide range of self-derived molecules called DAMPs that are released upon cellular damage and that can alter the tumor microenvironment (TME) by induction of inflammatory responses.[33,34] Therefore, as described later in this review, TLRs apparently show both anti-tumor and pro-tumor functions[33,35] (Tables 1 and 2).

### 2.2 | TLR 2

TLR2 is expressed on the cell surface.[30] TLR2 forms heterodimers with TLR1 or TLR6 and recognizes a variety of PAMPs including lipoteichoic acid present in Gram-positive bacteria.[30] TLR2 has also been shown to recognize endogenous molecules such as hyaluronic acid, versican, and surfactant protein A.[30]

### Anti-tumor role of TLR2

TLR2-deficient mice showed enhanced tumor growth in a N-nitrosodiethylamine (DEN)/carbon tetrachloride ($CCl_4$) model of hepatocellular carcinoma (HCC).[36] Mechanistically, multiple cytokine levels, including IFN-γ, are decreased in the liver of TLR2-deficient mice. It has been speculated that attenuated cytokine induction fails to induce senescence to tumor cells, thereby promoting tumor growth.[36] Another study also showed that TLR2-deficient mice developed more intestinal tumors in the DEN/$CCl_4$ model of colon cancer.[37] The TME in TLR2-deficient mice is characterized by increased levels of IL-6 and phosphorylation of signal transducer and activator of transcription 3 (STAT3) in the intestinal tumor, both of which are known to promote tumor growth.[37] Furthermore, a recent study on a model of mouse glioma showed that TLR2 on microglia had an anti-tumor role. Mechanistically, microglia in the TME upregulate major histocompatibility complex-I (MHC-I) in a TLR2-dependent manner and function as antigen-presenting cells (APCs). This in turn promotes anti-tumor immune responses by CD8+ T cells.[38]

### Pro-tumor role of TLR2

Conversely, in a mouse gastric cancer model in which cancer cells displayed hyperactivation of STAT3, TLR2 deficiency resulted in a reduced tumor burden.[39] This effect is independent of inflammation and, is dependent on impaired proliferation and increased apoptosis of the cancerous cells by the lack of TLR2.[39] These phenomena are explained by the suppression of multiple kinases such as phosphatidylinositol-3 kinase (PI3K), and NF-κB signaling pathways due to TLR2 deficiency.[39] TLR2-deficient mice also show slower metastatic growth of Lewis lung carcinoma (LLC) cells in the lung compared with wild-type (WT) mice.[40] Enhanced tumor metastasis in WT mice has been attributed to the activation of the TLR2:TLR6 complex by the tumor cell-derived glycoprotein versican, resulting in the secretion

**TABLE 1** Roles of TLRs in tumor immunity: genetic studies

| Receptor | Cancer model | Phenotype | Reference |
|---|---|---|---|
| TLR2 | DEN/CCl4 HCC model | TLR2-deficient mice show enhanced tumor growth | [36] |
| TLR2 | DEN/CCl4 colon cancer model | TLR2-deficient mice show enhanced tumor growth | [37] |
| TLR2 | GL26 glioma model | TLR2-deficient mice show enhanced tumor growth | [38] |
| TLR2 | $gp130^{F/F}$ mice of gastric cancer model | TLR2-deficient mice show reduced tumor burden | [39] |
| TLR2 | LLC lung metastasis model | TLR2-deficient mice show reduced metastasis | [40] |
| TLR3 | TRAMP prostate cancer model | TLR3-deficient mice show enhanced tumor growth | [41] |
| TLR3 | LLC and B16 lung metastasis model | Lung metastasis is suppressed in TLR3-deficient mice; | [50] |
| TLR4 | Apc$^{min}$ colon tumor model | Mice harboring constitutively active TLR4 show reduced tumor burden | [51] |
| TLR4 | DEN HCC model | TLR4-deficient mice exhibit enhanced tumor burden | [52] |
| TLR4 | DMBA skin cancer model | TLR4-deficient mice exhibit enhanced tumor burden | [53] |
| TLR4 | DMBA mammary cancer model | TLR4-deficient mice exhibit enhanced tumor burden | [54] |
| TLR4 | 4T1 lung metastasis model | TLR4-deficient mice exhibit enhanced lung metastasis | [55] |
| TLR4 | AOM/DSS colon cancer model | TLR4-deficient mice show reduced tumor burden | [56,57] |
| TLR4 | AOM/DSS colon cancer model | Mice harboring constitutively active TLR4 show enhanced tumor burden | [58] |
| TLR4 | DEN/CCl4 HCC model | TLR4-deficient mice show reduced tumor burden | [59] |
| TLR4 | DEN HCC model | TLR4-deficient mice show reduced tumor burden | [60] |
| TLR4 | DMBA/croton oil skin cancer model | TLR4-deficient mice show resistance to carcinogenesis | [61] |
| TLR4 | HGF-CDK4 (R24C) melanoma model | TLR4-deficient mice show reduced lung metastasis | [62] |
| TLR4 | p48Cre;Kras$^{G12D}$ pancreatic cancer model | TLR4-deficient mice show reduced pancreatic intraepithelial neoplasia | [63] |
| TLR5 | p53;Kras$^{G12D}$ sarcoma model | TLR5-deficient mice show ameliorated tumor growth | [64] |
| TLR7 | p48Cre;Kras$^{G12D}$ pancreatic cancer model | TLR7-deficiency in hematopoietic compartment ameliorated tumor growth | [69] |
| TLR7 | LLC lung metastasis model | TLR4-deficient mice show reduced lung metastasis | [70] |
| TLR9 | p48Cre;Kras$^{G12D}$ pancreatic cancer model | TLR9-deficient mice show ameliorated tumor growth | [79] |

Abbreviations: AOM, azoxymethane; DEN, *N*-nitrosodiethylamine; DMBA, 7,12-dimethylbenz[a]anthracene; DSS, dextran sodium sulfate; HCC, hepatocellular carcinoma.

of tumor necrosis factor-α (TNF-α) by myeloid cells, which in turn promotes metastatic tumor growth.[40]

## 2.3 | TLR3

TLR3 is localized within endosomes where it recognizes endocytosed double-stranded RNA (dsRNA), typically derived from viruses,[30] as well as self-derived messenger RNA released from dead cells.[30]

### Anti-tumor role of TLR3

Accumulating evidence suggests that TLR3 functions to promote anti-tumor immune responses. In an implanted model of prostate cancer, TLR3-deficient mice exhibited increased tumor growth.[41] In this model, the TLR3-type I IFN axis enhanced the activation of NK cells for their anti-tumor responses.[41] Consistent with this finding, several studies have revealed the therapeutic effects of

polyinosinic-polycytidylic acid (poly(I:C)), a synthetic TLR3 ligand, in the treatment of cancers. The proposed mechanisms of the poly(I:C)-TLR3 axis are: (i) induction of IRF3-dependent NK-activating molecule (INAM) on DCs;[42] (ii) skewing the differentiation of tumor-infiltrating macrophages toward M1 macrophages, which promote anti-tumor adaptive immune responses;[43] and (iii) activation of DCs, including the production of type I IFNs, to enhance effective cyto-toxic T cell responses.[44]

Poly(I:C) has been considered a promising adjuvant for cancer immunotherapy for several decades. Although effective, this ther-apy has been shown to cause life-threatening side effects, such as cytokinemia.[45-47] In this context, a recent study reported a new type of synthetic RNA, called ARNAX, that has been designed to selectively activate the TRIF pathway, thereby effectively activat-ing NK cells and cytotoxic T cells without inducing a severe cy-tokine storm induced by other types of dsRNA.[48,49] Interestingly, ARNAX treatment in combination with tumor antigen induced the activation of tumor-specific CD8$^+$ T cells and overcame anti-PD-1 resistance.[21]

**TABLE 2** Representative agonists of PRRs with anti-tumor activity

| Receptor | Agonist | Cancer model | Mechanism of action | Reference |
|---|---|---|---|---|
| TLR3 | Poly(I:C) | B16 melanoma model | Induction of INAM on DCs | [42] |
| TLR3 | Poly(I:C) | LLC lung cancer model | M1 macrophage polarization | [43] |
| TLR3 | Poly(I:C) | B16 melanoma lung metastasis model | Activation of DCs | [44] |
| TLR3 | ARNAX | B16 melanoma model | Selective activation of TRIF pathway | [21,48,49] |
| TLR7 | Imiquimod | MC26 colon carcinoma model | Induction of IFN-α | [65,66] |
| TLR7 | 1V270 | B16cOVA melanoma and SCC7 head and neck cancer model | M1 macrophage polarization | [67,68] |
| TLR9 | CpG-ODN | C3 cervical cancer model | Increased infiltration of CD8$^+$ T cells in the tumor | [76] |
| TLR9 | CpG-ODN | C26 colon cancer model | Peritumoral injection of CpG-ODN provokes long-term immunological memory response | [77] |
| TLR9 | SD-101 | CT26 colon cancer model | Expansion of tumor-specific CD8$^+$ T cells | [22] |
| RIG-I | 3p-siRNA | B16 melanoma model | Activates dendritic cells and induced apoptosis of tumor cells | [95] |
| RIG-I | 5-AZA-CdR | LIM1215 colon cancer model | Induction of a type I IFN signaling and apoptosis in cancer cells | [96] |
| STING | cGAMP | Colon 26 colon cancer model | Promotes DC maturation | [101] |
| STING | cGAMP | B16 melanoma model | Activation of CD8$^+$ T cells | [102] |

Abbreviation: cGAMP, cyclic GMP-AMP.

## Pro-tumor role of TLR3

One report has suggested a tumor-promoting role for TLR3. Lung metastasis of subcutaneously injected LLC lung cancer and B16F10 melanoma cells was suppressed in TLR3-deficient mice.[50] It has been suggested that RNA(s) derived from tumor exosomes can activate TLR3 expressed in lung epithelial cells and induce cytokine expression, leading to neutrophil recruitment and the development of a pre-metastatic niche, which favors tumor metastasis.[50]

## 2.4 | TLR4

TLR4 is expressed on the cell surface and recognizes lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria.[30] TLR4 may also recognize various endogenous ligands such as high-mobility group box protein 1 (HMGB1), heat shock proteins (HSPs), biglycan, and oxidized low-density lipoprotein.[30]

## Anti-tumor role of TLR4

Mice harboring constitutively active TLR4 in the intestinal epithelial cells showed a decreased tumor burden in the APC$^{min/+}$ mouse model of colon tumors.[51] It was revealed that tumor cells isolated from the intestine of these mice showed elevated expression levels of IFN-β and caspase-3 activation, with increased apoptosis of tumor cells.[51] Another study showed that TLR4-deficient mice exhibited an enhanced tumor burden in a DEN-induced HCC model.[52] Mechanistically, TLR4 deficiency results in impaired DNA repair and subsequent accumulation of oxidative stress, which can promote HCC carcinogenesis.[52]

In a skin cancer model, induced by 7,12-dimethylbenz[a]anthracene (DMBA), TLR4-deficient mice exhibited a higher tumor burden. This was accompanied by elevated levels of serum IL-17 and decreased levels of IFN-γ, suggesting the development of impaired Th1-type anti-tumor responses.[53] Furthermore, TLR4 has been shown to be protective for both a DMBA-induced

mammary cancer model[54] and a 4T1-inoculated lung metastasis model.[55]

### Pro-tumor role of TLR4

In a mouse colon cancer model of azoxymethane (AOM)/dextran sodium sulfate (DSS), TLR4-deficient mice showed a diminished tumor burden.[56,57] It has been suggested that TLR4 signaling induces changes in the TME characterized by induction of prostaglandin $E_2$, a well known tumor-promoting lipid mediator, and amphiregulin, which activates epidermal growth factor receptor (EGFR) signaling. Consistently, mice carrying a constitutively active TLR4 protein in intestinal epithelial cells are more susceptible to tumor development in the same model.[58]

In an HCC model, ie, the DEN/CCl4 model, decreased tumor development was observed in TLR4-deficient mice.[59] Mechanistically, microbial PAMPs from the intestine stimulate TLR4 on liver-resident cells, prevent apoptosis, and increase proliferation of tumor cells partly by expression of epiregulin, a hepatomitogen.[59] Similarly, a reduction of the development of HCC was observed in TLR4-deficient mice in the DEN-induced HCC model.[60] In a skin cancer model, induced by the combination of DMBA and croton oil, TLR4-deficient mice showed resistance to carcinogenesis.[61] Mechanistically, it is proposed that HMGB1, released from dying keratinocytes, activates TLR4, and enhances inflammation, thereby promoting tumor development.[61] Furthermore, in a genetically engineered mouse model of melanoma, TLR4 deficiency ameliorates UV-induced enhancement of lung metastasis.[62] TLR4 was activated by extracellular HMGB1 released from UV-damaged keratinocytes and promoted the recruitment of neutrophils that induced angiogenesis and migration of melanoma cells toward endothelial cells, facilitating the dissemination of tumor cells.[62]

Additionally, in the pancreas, mice lacking TLR4 in the hematopoietic cell compartment showed a reduced burden of pancreatic intraepithelial neoplasia in a p48Cre;Kras[G12D] pancreatic cancer model.[63]

## 2.5 | TLR5

TLR5 is expressed on the cell surface[30] where it recognizes flagellin, a component of bacterial flagella.[30]

### TLR5 in tumor immunity

Reports on the role of TLR5 in tumor immunity are rather limited. One report demonstrated that TLR5 can promote tumor growth. Here, TLR5-deficient mice showed ameliorated tumor growth in a genetically engineered mouse model of sarcoma.[64] TLR5 deficiency is associated with a decreased level of IL-6, leading to impaired recruitment of myeloid-derived suppressor cells (MDSCs), which strongly suppress anti-tumor immune responses by CD8^{+} T cells and NK cells. Importantly, TLR5-dependent acceleration of tumor growth depends on commensal microbiota.[64]

## 2.6 | TLR7/8

TLR7 recognizes single-stranded RNA (ssRNA), typically derived from RNA viruses, within endosomes.[30] It is highly expressed on plasmacytoid DCs (pDCs) and crucial for the massive release of type I IFNs against RNA viruses.[30] Human TLR8 also recognizes viral ssRNA. However, TLR8-deficient mouse cells showed no defects in cytokine production against viral ssRNA.[30] TLR7 can also recognize self-derived ssRNA bound to autoantibodies.[30]

### Anti-tumor role of TLR7/8

Small molecule agonists of TLR7/8 have long been proposed as anti-tumor drugs. Imiquimod has been known to activate the TLR7-MyD88-type I IFN axis to exert an anti-tumor effect.[65,66] Another study revealed that intratumoral injection of 1V270, a low-molecular-weight agonist of TLR7, potently suppressed tumor growth in a B16 mouse melanoma model.[67] This compound is designed to circumvent an unwanted systemic cytokinemia and, when combined with anti-PD-1 antibody, enhanced the efficacy of the immune checkpoint inhibitor.[68] Mechanistically, it is proposed that this agonist promotes differentiation of TAMs into M1 phenotype and enhances the infiltration of tumor-specific CD8^{+} T cells.[68]

### Pro-tumor role of TLR7/8

Some genetic studies have shown a pro-tumorigenic role for TLR7. One study showed that TLR7 deficiency in hematopoietic cells abrogated tumor development in a genetically engineered mouse model of pancreatic cancer.[69] Here, TLR7 expression was upregulated in both epithelial and stromal compartments in human and murine pancreatic cancer. In a mouse pancreatic cancer model, TLR7 stimulation enhanced tumor progression, accompanied by the modulation of several factors, including STAT3 activation, which are involved in tumor development. Therefore, the blockade of TLR7 protected against carcinogenesis. TLR7 ligation may modulate pancreatic cancer by driving stromal inflammation. Another study demonstrated that TLR7-deficient mice are associated with less tumor burden and prolonged survival in LLC lung cancer metastasis model.[70]

It is difficult to clearly explain the seemingly discrepant results between studies utilizing synthetic ligands and genetic studies. One possible explanation is a dual nature of type I IFN's function. In fact, transient, short-term production of type I IFNs promotes the maturation of DCs for priming CD8^{+} T cells,[71,72] thereby exerting an anti-tumor response. In addition, type I IFNs directly act on cancer cells and induce apoptosis.[73] Conversely, persistent production of type I IFNs results in the expression of immunosuppressive molecules such as PD-1 and IL-10 in T cells and other immune cells such as DCs.[8,74,75] Therefore, type I IFNs show pro-tumor activity. A deeper understanding of these dual functions of type I IFN and PRRs may allow the development of efficient PRR-targeting anti-cancer drugs.

It is also desirable to develop a tool to monitor at what point the switch from anti-tumor to pro-tumor response occurs.

## 2.7 | TLR9

TLR9 within endosomes recognizes unmethylated CpG DNA motifs, which are frequently found in viral and bacterial genome.[30] TLR9 can also recognize self DNA bound to autoantibodies in a manner similar to TLR7.[30]

### Anti-tumor role of TLR9

The role for TLR9 signaling in anti-tumor immunity has been underscored by numerous reports investigating the efficacy of synthetic TLR9 ligands in the anti-tumor response. Most notably, the therapeutic effect of CpG-oligodeoxynucleotide (CpG-ODN), a well known TLR9 ligand, on tumor immunity has been extensively studied.[76-78] CpG-ODN treatment induced a significant anti-tumor effect in several mouse models, in which the tumor regressions by this therapy are mediated by CD8^{+} T cells.[76,77] Furthermore, peritumoral injection of CpG-ODN provoked a long-term immunological memory response, as CpG-ODN treated mice are protected against tumor re-challenge.[77] Of note, 1 report showed that CpG-ODN treatment overcame resistance to checkpoint blockade therapy. Intratumoral injection of SD-101, a type of CpG-ODN, induced the expansion of functional tumor-specific CD8^{+} T cells and reverted resistance to PD-1 blockade in the CT26 murine colon carcinoma model.[22]

### Pro-tumor role of TLR9

TLR9 promotes tumor growth in a genetic model. Orthotopically implanted pancreatic cancer cells carrying mutated genes for K-Ras and p53 showed decreased growth in TLR9-deficient mice.[79] Mechanistically, TLR9 activation in pancreatic stellate cells (PSCs) results in chemokine (C-C motif) ligand 11 (CCL11) expression, resulting in tumor cell proliferation via its receptor chemokine (C-C motif) receptor 3 (CCR3). Interestingly, TLR9 can also recruit regulatory T cells (Tregs) and MDSCs to the TME, further exacerbating tumor progression by subverting anti-tumor immunity.[79]

## 2.8 | Role of CLRs in the regulation of tumors

CLR family members are characterized by their recognition of carbohydrates on bacteria, fungi and viruses,[80] whereas some CLRs can also detect oxidized lipids and other DAMP molecules exposed by damaged cells.[81,82] CLR activation leads to immunoreceptor tyrosine-based activation motif (ITAM)/immunoreceptor tyrosine-based inhibition motif (ITIM)-dependent or -independent signaling to induce host immune responses.[82] Some ITAM-based CLRs, such as Dectin-1, possess hemITAM motif and recruit spleen tyrosine kinase (Syk) to activate NF-κB via caspase activation and recruitment domain 9 (CARD9) adaptor protein.[82] Syk further activates MAPK and nuclear factor of activated T cells (NFAT) pathways and induces reactive oxygen species (ROS) production that leads to NACHT, LRR, and PYD domains-containing protein 3 (NALP3) inflammasome activation.[82] Other ITAM-based CLRs, represented by Dectin-2 and Mincle, associate with ITAM-containing adaptor proteins such as Fc receptor γ (FcRγ) chain, leading to Syk-dependent signal transduction.[82] ITIM-containing CLRs suppress the NF-κB pathway and activation of STAT5, as well as the ITAM-based signaling pathway through Src-homology 2 domain-containing phosphatase-1 (SHP-1) and SHP-2.[82] In addition to the aforementioned signaling pathways, CLRs also drive the phagocytotic activity of myeloid cells to enhance the uptake of invading pathogens and self-derived molecules.[83] Through these mechanisms, CLRs play critical roles in the control of innate and adaptive immune systems. The role of CLRs in the regulation of tumor is summarized in Table 3.

**TABLE 3** Roles of CLRs in tumor immunity: genetic studies

| Receptor | Cancer model | Phenotype | Reference |
|---|---|---|---|
| Dectin-1 | B16 lung metastasis model | Dectin-1-deficient mice show enhanced lung metastasis | [19] |
| Dectin-1 | B16 lung metastasis model | Dectin-1-deficient mice show enhanced lung metastasis | [84] |
| Dectin-1 | p48Cre;Kras[G12D] pancreatic cancer model | Dectin-1-deficient mice show delayed development of dysplasia | [85] |
| Dectin-2 | SL4 liver metastasis model | Dectin-2-deficient mice show enhanced liver metastasis | [20] |
| MCL | SL4 liver metastasis model | MCL-deficient mice show enhanced liver metastasis | [20] |
| Mincle | p48Cre;Kras[G12D] pancreatic cancer model | Dectin-1-deficient mice show ameliorated tumor growth | [87] |

## 2.9 | Dectin-1

Dectin-1 recognizes β-glucan structures and restricts bacterial and fungal infections.[80] Activation of Dectin-1 induces the Syk-dependent signaling pathway through its hemITAM motif.[82]

### 2.9.1 | Anti-tumor role of Dectin-1

In the regulation of subcutaneous tumor growth and lung metastasis, Dectin-1 augmented anti-tumor responses through the enhancement of NK cell cytotoxicity.[19] This anti-tumor response was triggered by Dectin-1 recognition of N-glycan structures on tumor cells. Upon recognition of N-glycan structures, Dectin-1 signaling in myeloid cells was activated and promoted the anti-tumor killing of NK cells in a cell-to-cell contact-dependent manner.[19] Consistent with this action, expression of INAM in DCs, a membrane protein that drives NK cell activation, was upregulated by Dectin-1 when co-cultured with cancer cells.[19] Another study showed that membrane spanning 4 domains A4A (MS4A4A), a tetraspan molecule, on macrophages cooperated with Dectin-1 in lipid rafts and was required for full-blown activation of its downstream signaling.[84] Consequently, MS4A4A is essential for Dectin-1-mediated activation of macrophages and the subsequent NK cell-mediated tumor metastasis control.[84]

### 2.9.2 | Pro-tumor role of Dectin-1

In a genetically engineered mouse model of pancreatic carcinoma, Dectin-1-deficient mice showed delayed development of dysplasia and extended survival.[85] Mechanistically, Dectin-1 expression on tumor associated macrophages (TAMs) promoted their reprogramming to a tolerogenic phenotype and the suppression of anti-tumor immunity.[85] Interestingly, this study also suggested that galectin 9, a member of the β-galactoside-binding family of lectins, expressed on tumor cells was a ligand for Dectin-1. Accordingly, blockade of galectin 9 by a monoclonal antibody, extended the survival of mice harboring pancreatic tumors.[85]

## 2.10 | Dectin-2

Dectin-2 recognizes mannose-rich carbohydrates to activate Syk-dependent signaling.[80] Unlike Dectin-1, Dectin-2 does not possess an ITAM motif. Instead, it associates with the FcRγ chain, which possesses ITAM motif to transduce downstream signaling.[82] Evidence is scarce regarding the Dectin-2 pro-tumor role. Unlike Dectin-1, Dectin-2 was not involved in the control of subcutaneous tumor growth and lung metastasis.[20] Notably, however, Dectin-2 suppressed liver metastasis. This underlying mechanism is unique, in that Dectin-2 augmented phagocytosis of cancer cells by Kupffer cells, liver-residing macrophages, against cancer cells in vitro.[20]

Consistent with this finding, massive liver metastases were observed in Dectin-2-deficient mice. This Dectin-2-mediated phagocytosis of cancer cells appears to be mediated by coupling of Dectin-2 with its family member MCL.[20]

## 2.11 | Mincle

Macrophage-inducible C-type lectin (Mincle) recognizes mannose and trehalose-6,6′-dimycolate (TDM), a mycobacterial glycolipid.[82] Mincle also binds to endogenous molecule ribonucleoprotein spliceosome associated protein 130 (SAP-130), which is released from dying cells.[86] Stimulation of Mincle with its ligands induced Syk-dependent signaling pathway through the ITAM-possessing FcRγ chain.[82]

### 2.11.1 | Pro-tumor role of Mincle

In a pancreatic cancer model of *p48Cre;Kras^G12D* mice, Mincle created an immunosuppressive TME and promoted tumor development.[87] Mincle signaling enhanced the production of IL-10 from T cells and promoted the infiltration of MDSCs and M2-like macrophages into tumors.[87] This process was associated with necroptotic cell death and the induction of SAP-130 expression in the pancreas.[87] SAP-130 administration into the pancreas aggravated tumor growth, indicating that ligation of Mincle with dead cell-derived SAP-130 promoted this oncogenic process.[87]

## 3 | CYTOPLASMIC PRRS

### 3.1 | Role of cytosolic nucleic acid sensors in tumor development

Cytosolic nucleic acid-sensing PRRs are expressed in almost all cell types and detect RNA and DNA or their mimetics to provoke innate immune responses.[88] The induction of type I IFN genes is the hallmark of the activation of these cytosolic PRRs, and this induction is critical for effective anti-viral responses.[88] As the anti-tumor activity of type I IFNs has been well documented, the role of these PRRs in anti-tumor immunity has been the focus of attention.[89] Therefore, multiple agonists for this class of PRRs are under investigation in clinical trials.[90]

RIG-I (also known as DDX58), melanoma differentiation-associated 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2) of the RLR family comprise cytosolic RNA sensors. Multiple DNA sensors have been identified. These include cyclic GMP-AMP (cGAMP) synthase (cGAS), DNA-dependent activator of IRFs (DAI), human IFN-γ-inducible protein 16 (IFI16) and its mouse ortholog p206, DDX41, DNA-dependent protein kinase (DNA-PK), and meiotic recombination 11 (MRE11).[88] Among the above nucleic acid sensors, RIG-I and cGAS have been extensively studied in the context

of tumor immunology, whereas evidence for other sensors is rather limited (Tables 2 and 4.).

## 3.2 | RIG-I

RIG-I (also known as DDX58) senses dsRNA, a replication intermediate for RNA viruses.[91] It is also activated by RNAs bearing 5-triphosphates or 5-diphosphates.[92] Upon ligand recognition, they are recruited by the adaptor MAVS (also known as IPS-1, CARDIF, or VISA) to the outer membrane of the mitochondria, leading to the activation of several transcription factors including IRF3, IRF7 and NF-κB and subsequent production of type I IFNs and inflammatory cytokines.[88]

In humans, low RIG-I expression in HCC tissue samples is associated with a poorer prognosis and a higher resistance to IFN-α therapy.[93] The anti-tumor role of RIG-I has been validated in RIG-I-deficient mice in a model of HCC.[93] It has also been reported that RIG-I activation induced the secretion of extracellular vesicles (EVs) from melanoma cells, which exhibit expression of the NKp30-ligands on their surface, therefore triggering NK cell-mediated elimination of melanoma cells.[94]

Given its ability to induce high amounts of type I IFN, RIG-I has been regarded as a promising target for cancer therapy. One study revealed that short interfering RNA (siRNA) with 5′-triphosphate ends (3p-siRNA) against Bcl-2 exhibited a potent anti-tumor effect for melanoma cells. Recognition of 5′-triphosphate by RIG-I activated dendritic cells and directly induced the expression of type I IFNs and apoptosis in tumor cells.[95] Another report showed that a DNA-demethylating agent, 5-AZA-CdR, triggered cytosolic sensing of dsRNA derived from endogenous retroviral elements. This led to the induction of type I IFN signaling and apoptosis in cancer cells.[96]

## 3.3 | cGAS

Among DNA-sensing PRRs reported so far, cyclic GMP-AMP (cGAMP) synthase (cGAS) is one of the best characterized molecules for its role in anti-viral immunity. Viral DNA released into the cytosol is catalyzed by cGAS and converted to cGAMP, which in turn binds to STING to activate its downstream signaling pathways, including IRF3 and NF-κB, type I IFNs and inflammatory cytokines, respectively.[97]

**TABLE 4** Roles of cytosolic nucleic acid sensors, NLRs and ALRs in tumor immunity: genetic studies

| Receptor | Cancer model | Phenotype | Reference |
|---|---|---|---|
| RIG-I | DEN HCC model | RIG-I-deficient mice show enhanced tumor growth | 93 |
| STING | 1969 cell sarcoma model | STING-deficient mice show enhanced tumor growth | 98 |
| STING | MC38 colon cancer model | STING-deficient mice show impaired efficacy of irradiation | 99 |
| STING | AOM/DSS colon cancer model | STING-deficient mice show increased tumor burden | 100 |
| STING | DMBA skin cancer model | STING-deficient mice show reduced tumor burden | 103 |
| STING | LLC lung cancer model | STING-deficient mice show reduced tumor growth | 105 |
| NLRP3 | AOM/DSS colon cancer model | NLRP3-deficient mice show enhanced tumor burden | 114 |
| NLRP3 | MC38 liver metastasis model | NLRP3-deficient mice are more susceptible to metastatic tumor growth | 115 |
| NLRP3 | DMBA/TPA skin papilloma model | NLRP3-deficient mice develop higher tumor burden | 116 |
| NLRP3 | EL4 lymphoma model | NLRP3-deficient mice show impaired efficacy of cytotoxic chemotherapy | 117 |
| NLRP3 | MCA-induced fibrosarcoma model | NLRP3-deficient mice show decreased tumor burden | 118 |
| NLRP3 | EL4 lymphoma model | NLRP3-deficient mice show enhanced efficacy of cytotoxic chemotherapy | 119 |
| AIM2 | AOM/DSS colon cancer model | AIM2-deficient mice are more susceptible to tumor development | 120 |
| AIM2 | Apc$^{min}$ colon tumor model | AIM2-deficient mice are more susceptible to tumor development | 121 |

Abbreviations: AOM, azoxymethane; DEN, *N*-nitrosodiethylamine; DMBA, 7,12-dimethylbenz[a]anthracene; DSS, dextran sodium sulfate; HCC, hepatocellular carcinoma; MCA, 3-methylcholanthrenerbol-13-acetate; TPA, 12-*O*-tetradecanoylphorbol-13-acetate.

### 3.3.1 | Anti-tumor role of cGAS

The role of the cGAS-STING pathway in the anti-tumor immune response has been the particular focus of attention.[97] In mice inoculated with tumor cells expressing an immunogenic peptide, cGAS-STING appeared to contribute to the anti-tumor response to these cells, in which tumor-derived DNAs, which are taken up by APCs in the TME, stimulate the cGAS-STING pathway and induce IFN-β production for activation of CD8^{+} T cells.[98] Similarly, cGAS-STING-dependent IFN-β production and CD8^{+} T cell activation were triggered in an irradiation-treated tumor and was required for an anti-tumor effect.[99] Furthermore, STING promoted IL-18 and IL-22BP expression in tumor tissues and suppressed AOM/DSS colon carcinogenesis.[100]

In general, targeting the cGAS-STING pathway for its activation is beneficial in many mouse models for the treatment of cancer. Administration of cGAMP ameliorated tumor growth of colon 26 cells in association with DC maturation.[101] Moreover, cGAMP also retarded B16 melanoma cell growth with activation of CD8^{+} T cells in the TME through type I IFNs signaling. This anti-tumor effect was further augmented when combined with antibodies against PD-1 and CTLA-4 antibodies.[102]

### 3.3.2 | Pro-tumor role of cGAS

There have been reports showing a pro-tumor role of the cGAS-STING pathway. DNA released into the cytosol in carcinogen-damaged cells stimulated the cGAS-STING pathway to induce inflammatory cytokine expression and promote DMBA-induced skin carcinogenesis.[103] Similarly, chromosomal instability in cancer cells released genomic DNA into the cytosol, which in turn activated both the cGAS-STING pathway and downstream noncanonical NF-κB signaling to promote cancer metastasis.[104] It has also been reported that subcutaneous growth and lung metastasis of LLC tumor cells were enhanced by STING by induction of indoleamine 2,3-dioxygenase (IDO), which suppresses anti-tumor immune responses.[105]

In addition, nuclear cGAS may exert tumor-promoting activity by inducing genomic instability. When DNA damage occurs, cGAS is transported into the nucleus in an importin-α-dependent manner. Then, cGAS is recruited to the double-strand breaks (DSBs) and interacts with poly [ADP-ribose] polymerase 1 (PARP1). This cGAS-PARP1 interaction inhibited the formation of the PARP-Timeless complex and subsequent homologous recombination (HR).[106] Another study also showed that cGAS inhibited HR by its self-oligomerization, causing DNA compaction at the binding site and suppressing RAD51-mediated DNA strand invasion.[107] Consistently, knockdown of cGAS inhibited tumor growth.[106]

### 3.4 | Role of NLRs and ALRs in the regulation of tumor development

NLRs are cytosolic sensors for various PAMPs and DAMPs and consist of 4 subfamilies based on their structures of N-terminal regions.[108] Some members of the NLRs constitute the inflammasome, a multiprotein complex comprised of NLRs, the adaptor protein apoptosis-associated speck-like protein containing a CARD (ASC), and caspase-1.[108] Some members of the ALR family also constitute the inflammasome upon recognition of cytosolic DNA.[108] These PRRs recognize various PAMPs and DAMPs after pathogen infection or cellular damage, respectively, to recruit ASC and trigger caspase-1 activation.[108] Activated caspase-1 subsequently cleaves pro-IL-1β and pro-IL-18 into their mature forms and induces strong inflammatory responses.[108] Accordingly, inflammasomes are involved in the host defense against pathogens, as well as in the pathogenesis of auto-inflammatory, neurodegenerative, and metabolic diseases.[109-111] Not surprisingly, there is evidence to suggest the involvement of inflammasomes in the development of cancer.[112] The role of NLRs and ALRs in the regulation of tumor is summarized in Table 4.

### 3.5 | NLRP3

NLRP3 binds to various molecules including bacterial DNA:RNA hybrids, bee venom, ATP, uric acid crystals, aluminum hydroxide, and asbestos.[108]

### Anti-tumor role of NLRP3

In the AOM/DSS colon cancer model, NLRP3-deficient mice showed an enhanced tumor burden.[113] In this model, reduction of IL-18 expression in NLRP3-deficient mice led to the impaired production of IFN-γ and an insufficient anti-tumor immune response.[114] Similarly, in a liver metastasis model of colon cancer cell line MC38, NLRP3-deficient mice were more susceptible to metastatic tumor growth.[115] This was attributed to impaired NK cell cytotoxicity due to impaired production of IL-18[115] Another report describing a DMBA/TPA skin papilloma model, NLRP3-deficient mice developed a higher tumor burden.[116]

NLRP3 has also been cited as a key molecule, determining the efficacy of anti-cancer chemotherapy. One report revealed that chemotherapy with oxaliplatin was inefficient against the EL4 murine lymphoma when established in NLRP3-deficient mice.[117] Mechanistically, it was indicated that dying tumor cells release ATP, activate NLRP3 via purinergic receptors for ATP on DCs, and prime CD8^{+} T cells.[117]

### Pro-tumor role of NLRP3

One report showed the tumor-promoting role of NLRP3. NLRP3-deficient mice in a MCA-induced fibrosarcoma model showed decreased tumor burden.[118] Reduction of tumor burden was associated with an increased frequency of NK cells; it was suggested that NLRP3 on CD11b^{+}Gr-1^{int} myeloid cells was

responsible for the impaired recruitment of NK cells.[118] In the context of anti-cancer chemotherapy, activation of NLRP3 on MDSCs led to the release of IL-1β, and abrogating anti-cancer effect of chemotherapy on EL4 lymphoma cells.[119] As a result, NLRP3-deficient mice showed an enhanced response to anti-cancer chemotherapy.[119]

## 3.6 | AIM2

AIM2 recognizes double-stranded DNA (dsDNA) by its positively charged HIN-200 domain and recruits ASC for caspase-1 activation via its PYD domain.[108]

AIM2-deficient mice in an AOM/DSS colon cancer model were more susceptible to tumor development.[120] Mechanistically, AIM2 deficiency causes proliferation of tumor-initiating stem cells via aberrant activation of Wnt signaling. Furthermore, it was suggested that dysbiosis of gut microbiota in AIM2-deficient mice also contributed to enhanced tumorigenesis.[120] Another report also revealed an anti-tumor role of AIM2 in the AOM/DSS and APC$^{min}$ mouse colon cancer models.[121] Mechanistically, AIM2 interacts with DNA-PKcs and inhibits activation of Akt pathway.[121] Of note, both studies argued that tumor-suppressive activity of AIM2 was independent of the activity of inflammasome.

## 4 | CONCLUDING REMARKS AND FUTURE PERSPECTIVES

In this review, we focused on the role of pattern recognition receptors in the regulation of tumor immunity. The immune system is intrinsically a double-edged sword in that, while essential to maintaining host homeostasis by eliminating undesirable entities (pathogens, infected cells, and cancerous cells), it also contributes to various harmful events when it is dysregulated. As the activation of innate immune receptors generally evokes the inflammatory response, which may cause either an anti-tumor or pro-tumor response, the role of these receptors in the regulation of tumor development can be variable. In addition, it seems that each oncogenic process (eg, primary tumor growth and metastasis) is modulated by distinct subsets of PRRs.

The complex nature for the functions of PRRs in the regulation of tumor immunity may be attributed to different endogenous tumor ligands among various models. The magnitude and timing of PRR-mediated signaling might also affect the final outcome of tumor control.[7] As the composition of cell types in the TME can be a determinant critical to cancer immunotherapies,[122] different cell composition in the TME may also account for the distinct role of PRRs in each study (Figure 1). Therefore, further clarification of these factors may identify the characteristics of patients who responded to each immunotherapy and dramatically increased the proportion of responders.

FIGURE 1 The general frame of the role of PRRs in the regulation of tumor immunity. Innate receptors on immune cells sense damage-associated molecular patterns (DAMPs) from dead tumor cells or pathogen-associated molecular patterns (PAMPs) from commensal microorganisms. This leads to release of cytokines, interferon, or growth factors. These effecter molecules exert both tumor-promoting and tumor-suppressing functions, depending on their magnitude, chronic or acute production, or composition of the tumor microenvironment. Abbreviations: cGAS, cyclic GMP-AMP synthase; CLR, C-type lectin receptors; RLR, retinoic acid-inducible gene-I-like receptor; STING, stimulator of IFN genes; TLR, Toll-like receptor
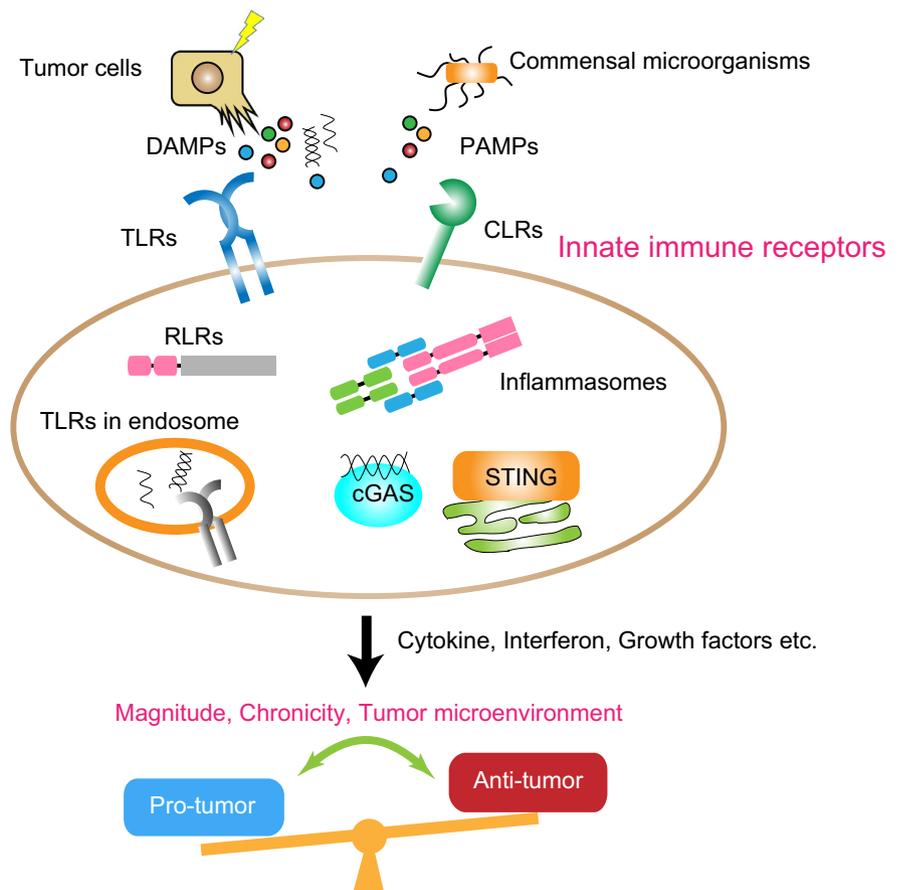
A deeper understanding of the complex nature of regulation of tumor immunity by these receptors warrants further investigation, to seek more effective ways to treat cancers by harnessing these receptors. For example, some CLRs may be exploited to enhance NK cell-mediated tumor killing or phagocytosis of tumor cells. It may also be of particular interest that the type I IFN system, whose anti-tumor activities has been known for many decades, is being "revisited" nowadays.[89] In fact, there is evidence that type I IFNs are involved in the context of rapidly emerging cancer checkpoint therapy fields.[98,123] In addition, the IRF7-IFN-β pathway appears to be critical for optimal anti-tumor activity.[124] Therefore, one possibility may be the development of agonists that selectively activate innate receptors to selectively induce particular pathways, such as those for type I IFN induction. Another possibility may be to combine ligands for activation of more than one receptor, for enhancement of beneficial anti-tumor immune responses.

Clearly, we can expect that further work will cover the basis of improved way(s) to harness the power of innate and adaptive immunity for the treatment of cancer.

## CONFLICT OF INTEREST

The authors have no conflict of interest.

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