Cancer Letters 452 (2019) 51–58

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Harnessing immune checkpoints in myeloid lineage cells for cancer immunotherapy

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ARTICLE INFO

Keywords:
Macrophages
Dendritic cells
Phagocytosis
Antitumer immunity

ABSTRACT

Myeloid lineage immune cells, such as macrophages and dendritic cells, play important roles in the induction of antitumor immunity during the initial stage of the cancer-immunity cycle, eliciting antitumor adaptive immunity by phagocytosing cancer cells and processing cancer-specific antigens, and then presenting these antigens to T cells. During this process, cancer cell phagocytosis can be prevented by inhibitory signals, and the signaling cascades that elicit immune responses against cancer antigens can be inhibited by immunosuppressive myeloid cells in the tumor microenvironment. A number of therapeutic strategies for enhancing cancer cell phagocytosis and promoting antitumor immunity by targeting myeloid lineage cells have recently been developed. Here, we discuss recent advances in cancer immunotherapy that involve the targeting of myeloid lineage immune cells to induce effective antitumor immunity.

1. Introduction

Multiple genetic alterations are common in most cancers and may provide neoantigens that can elicit cancer-specific immune responses. A series of events, referred to as the cancer-immunity cycle, are prerequisites for the induction of antitumor immunity [1]. First, antigen-presenting cells (APCs) must phagocytose and process cancer cells or cancer-specific antigens, and then dendritic cells (DCs) must present these captured antigens to T cells to elicit activation of effector T cell responses against these cancer cells and antigens. Several molecules, including programmed cell death 1 (PD-1) and cytotoxic T-lymphocyte–associated protein 4 (CTLA-4), have been shown to constitute immune checkpoints that regulate T cell activation [2]. Moreover, immunotherapies targeting T cell immune checkpoints have been shown to elicit endogenous immune responses that can lead to the effective killing of cancer cells and the regression of human tumors [3,4]. However, cancer patients that do not produce tumor-specific T cells do not respond to current immune checkpoint blockades, and their limited therapeutic response serves as a reminder of the importance of the uptake and processing of cancer antigens, a prerequisite for generating tumor-specific T cells.

A new type of immunotherapeutic strategy based on therapeutics that target tumor cell phagocytosis has recently emerged. As is the case for T cells, the phagocytosis of cancer cells or their antigens by APCs can be regulated by stimulatory or inhibitory signals. The processing of engulfed antigens following phagocytosis is also regulated by various signals [5]. APCs of the myeloid lineage, such as macrophages and DCs, are important for the induction of host immune responses and for linking innate immunity and adaptive immunity against cancer cells. In addition, immunosuppressive myeloid cells in the tumor environment, such as tumor-associated macrophages (TAMs) and myeloid-derived stromal cells (MDSCs), modulate immune responses to cancer antigens. This review focuses on recent advances in therapeutic strategies that target immune cells of the myeloid lineage and on promising targets for tumor cell phagocytosis and regulation of antitumor immunity.

2. Phagocytosis of cancer cells and cancer antigens

2.1. Anti-phagocytic signals

Anti-phagocytic signals, also known as “don't eat me signals”, collectively constitute a regulatory mechanism that protects normal cells from phagocytic clearance. To date, several molecules have been proposed to act as signals that prevent tumor cells against phagocytic clearance, including cluster of differentiation 47 (CD47), major histocompatibility complex (MHC)−I, and programmed death ligand 1 (PD-

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https://doi.org/10.1016/j.canlet.2019.03.018
Received 6 December 2018; Received in revised form 20 February 2019; Accepted 1 March 2019

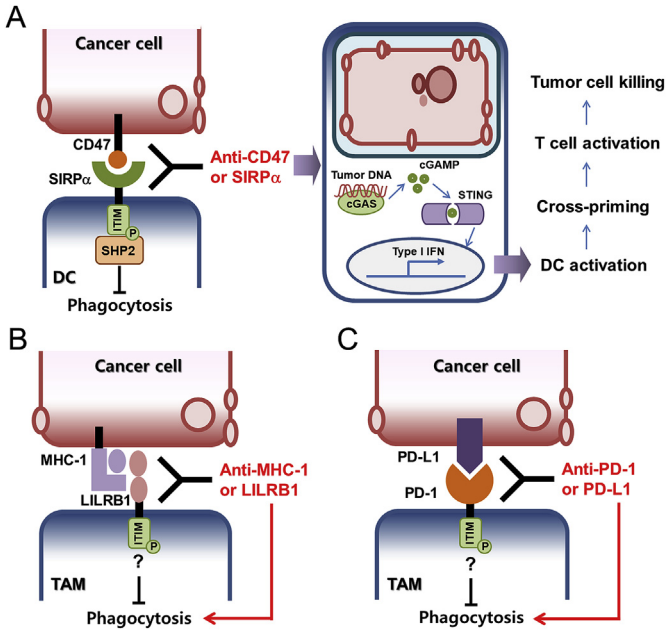

Fig. 1. Working models of anti-phagocytic signal blockade as a cancer therapy strategy. (A) Blockade of the CD47-SIRPα axis enhances cancer cell phagocytosis in DCs; this, in turn, activates the STING pathway for sensing tumor DNA and induces type I IFN production, leading to DC activation and antitumor T cell immunity. (B and C) Blocking the MHC-I–LILRB1 or PD-1–PD-L1 axis in TAMs promotes cancer cell phagocytosis. However, the mechanisms by which inhibition of MHC-I–LILRB1 or PD-1–PD-L1 axes enhances cancer cell phagocytosis remain to be clarified.

L1). CD47 (also known as IAP) is a red blood cell signal that serves to discriminate self and non-self [6]. Upon engagement of CD47 by signal regulatory protein alpha (SIRPα; also known as SHPS-1 or CD172a) on macrophages and DCs, the tyrosine-based inhibitory motif (ITIM) in the cytoplasmic tail of SIRPα activates the inhibitory tyrosine phosphatases SHP-1 and SHP-2, resulting in inhibition of the actin cytoskeleton rearrangement required for phagocytosis (Fig. 1A). CD47 is upregulated on the surface of several cancer cell types, enabling these cells to evade phagocytic removal by immune cells [7,8]. Consistent with this, high CD47 expression is negatively correlated with disease prognosis and survival in many cancers [8–10]. Furthermore, therapeutic reagents that antagonize the CD47-SIRPα axis in macrophages inhibit the growth of several types of tumor, including acute myeloid leukemia and non-Hodgkin lymphoma, by enhancing cellular phagocytosis [11–13]. Interestingly, CD47 blockade has been shown to robustly increase immune responses against tumor antigens by activating DC cross-presentation of tumor antigens [14,15]. Recently, combined therapies of a CD47-SIRPα axis inhibitor and other anticancer therapeutics (e.g., anti-PD-1 antibodies) have been investigated in the context of potentiating antitumor immunity [13,16,17]. In addition, the application of nanotechnology to CD47 blockade has been reported to remarkably enhance tumor cell engulfment by macrophages and efficiently suppress tumor growth in *in vivo* tumor models. For example, ferritin nanocage therapeutics carrying both CD47 and doxorubicin induce the release of danger signals, also known as damage-associated molecular patterns (DAMPs), from dying tumor cells, thereby promoting cancer cell phagocytosis and subsequent cross-presentation of cancer antigens by APCs [18]. In addition, exosomes harboring SIRPα variants have been shown to markedly augment cancer cell phagocytosis, and thus induce effective T cell responses to cancer [19]. Currently, 13 clinical trials are underway to evaluate the efficacy of therapeutic reagents targeting the CD47-SIRPα axis (Table 1).

MHC class I component β2-microglobulin (β2M) was recently found to act as another potential anti-phagocytic signal in cancer cells [20]. MHC-I on the cancer cell surface is sensed by leukocyte immunoglobulin-like receptor B1 (LILRB1; also known as LIR1) on TAMs, resulting in negative regulation of cancer cell phagocytosis (Fig. 1B). LILRB1 is an immunoglobulin-like receptor for the gene product of human cytomegalovirus UL18, a homolog of cellular MHC class I antigens [21]. LILRB1 in natural killer (NK) cells inhibits Fc receptor-mediated signaling in monocytes by activating the inhibitory phosphatase SHP-1 [22]. Moreover, ablation of either MHC-I on cancer cells or LILRB1 on macrophages has been shown to promote the phagocytic clearance of tumor cells and inhibit tumor growth in an animal model [20]. However, the molecular mechanism by which MHC-I-LILRB1 signaling inhibits phagocytosis in TAMs remains to be elucidated.

PD-1 (also known as CD279) is one of the best-characterized immune checkpoint targets for cancer immunotherapy, and inhibiting it has proven to be the most successful immunotherapy strategy to date [3,23]. PD-1 is upregulated in activated T cells, leading to immune tolerance [24]. Engagement of PD-1 with its cognate ligands, PD-L1 and PD-L2, on cancer cells causes phosphorylation of ITIM in the PD-1 cytoplasmic tail and recruitment of phosphatase SHP-2, leading to suppression of T-cell receptor activity [25–27]. Recently, Gordon et al. found that PD-1 is expressed in TAMs, but not in circulating monocytes or splenic macrophages, and that it can negatively regulate cancer cell phagocytosis (Fig. 1C) [16]. In addition, the authors found that blockade of PD-1 or PD-L1 enhanced the phagocytic activity of TAMs towards tumor cells *in vivo* and inhibited tumor growth in a macrophage-dependent fashion, suggesting that the PD-1/PD-L1 axis is an immune checkpoint for regulating both innate and adaptive immunity. They further found that PD-1 blockade also changes the nature of TAMs from immunosuppressive M2-like cells to proinflammatory M1-like cells. However, the mechanism responsible for the inhibition of tumor cell phagocytosis following blockade of PD-1 signaling needs further clarification, and the extent to which blockade of the PD-1/PD-L1 axis in TAMs contributes to the induction of antitumor immunity remains to be determined.

2.2. Cytoskeletal regulators

The small GTPase RhoA and its downstream effectors, Rho-associated kinases (ROCKs), play important roles in actin cytoskeleton organization and dynamics and the diverse biological processes to which these processes contribute, including cell adhesion, migration, and phagocytosis; as such, RhoA and ROCKs are proposed as potential targets of cancer therapy [28,29]. Therapeutic strategies that target ROCK activity inhibit tumor cell invasion and metastasis in many cancer types [30–33]. For example, it has been found that ROCK signaling promotes invasive growth of pancreatic ductal adenocarcinoma cells by increasing collagen-remodeling activity [34]. Recently, Campbell et al. showed that Δ133p53, a truncated p53 mutant detected in many cancers, activates the RhoA-ROCK axis, resulting in increased migration and invasion of colorectal cancer cells [35]. However, some studies have reported that inhibition of the RhoA/ROCK axis increases invasion and metastasis in other types of cancer, including nasopharyngeal carcinoma, breast cancer, and BRAF-mutant melanoma [36–38]. Thus, the detailed molecular mechanism by which the RhoA/ROCK axis controls tumor cell migration will require further clarification. On the other hand, RhoA/ROCK and Rac1 signaling have been shown to exert antagonistic regulation of cytoskeletal reorganization during phagocytosis [39]. Nam et al. recently described a therapeutic strategy based on targeting RhoA/ROCK signaling that directly potentiates the phagocytic ability of APCs [40]. These authors found that blocking ROCK increased the ability of macrophages or dendritic cells to engulf cancer cells *in vitro*, as assessed by phagocytosis assays using co-cultures of bone marrow-derived macrophages (BMDMs) or dendritic cells (BMDCs) with tumor cells, and *in vivo*, using phagocytosis assays of tumor-draining lymph nodes and tumor tissues from CT26 tumor-bearing mice. Intriguingly, the enhanced phagocytosis induced by ROCK

Table 1
Clinical trials of CD47-SIRPα axis-targeting therapeutic agents.

Reagent	Composition	Treatment	Start	Phase	ID	Patients
Hu5F9-G4	Humanized monoclonal anti-human CD47 antibody	Monotherapy	Aug 2014	1	NCT02216409	Solid tumors
		Monotherapy	Nov 2015	1	NCT02678338	AML Myelodysplastic syndrome
		+ cetuximab	Nov 2016	1b/2	NCT02953782	Solid tumors Advanced colorectal cancers
		+ rituximab	Nov 2016	1b/2	NCT02953509	NHL DLBCL Indolent lymphoma
		Monotherapy + azacitidine	Sep 2017	1b	NCT03248479	AML Myelodysplastic syndrome
		+ avelumab	May 2018	1b	NCT03558139	Solid tumors Ovarian cancer
CC-90002	Monoclonal anti-human CD47 antibody	Monotherapy + rituximab	Mar 2015	1	NCT02367196	Hematologic neoplasms
		Monotherapy	Mar 2016	1	NCT02641002 (Terminated)	AML Myelodysplastic syndrome
TTI-621	Recombinant SIRPα-Fc protein (Human IgG1 Fc)	Monotherapy + rituximab + nivolumab	Jan 2016	1a/1b	NCT02663518	Hematologic malignancies Solid tumors
		Monotherapy + PD-1/PD-L1 inhibitors + pegylated IFN-α2a + T-Vec + radiation	Sep 2016	1	NCT02890368	Solid tumors Mycosis Fungoides Melanoma Merkel-cell carcinoma Squamous cell carcinoma Breast cancer HPV-related malignant neoplasm Soft tissue sarcoma
ALX148	High affinity SIRPa fusion protein with inactivated Fc	Monotherapy + pembrolizumab + trastuzumab + rituximab	Feb 2017	1	NCT03013218	Metastatic cancer Solid tumors Advanced cancer NHL
SRF231	Monoclonal anti-human CD47 antibody	Monotherapy	Mar 2018	1/1b	NCT03512340	Advanced solid cancers Hematologic cancer
TTI-622	Recombinant SIRPα-Fc protein (Human IgG4 Fc)	Monotherapy + rituximab + RD-1/PD-L1 inhibitors + proteasome-inhibitor regimen	May 2018	1a/1b	NCT03530683	Lymphoma Myeloma
IBI188	Monoclonal anti-human CD47 antibody	Monotherapy + rituximab	Nov 2018	1	NCT03717103	Advanced malignancies

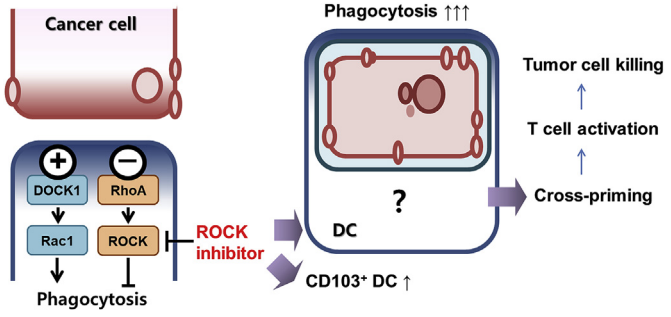

Fig. 2. Working model of the mechanism underlying the anticancer effect of ROCK blockade. Rac1 and RhoA antagonistically regulate cytoskeletal reorganization during phagocytosis: Rac1 facilitates macrophages phagocytic activity, whereas RhoA inhibits it. Inhibition of ROCK, a downstream effector of RhoA, promotes phagocytosis of cancer cells by DCs, which in turn increases T cell priming and results in antitumor T cell immunity. ROCK blockade also increases the population of $CD103^+$ DCs.

blockade was shown to induce T cell immune responses against tumor cells by increasing DC cross-presentation and T-cell priming (Fig. 2). It was further shown that depletion of $CD8^+$ T cells abolishes the antitumor effect of ROCK blockade in a syngeneic tumor model [40,41]. Interestingly, ROCK inhibition was shown to enhance tumor cell phagocytosis in macrophages and DCs, but the resulting antitumor immunity was dependent on $CD103^+$ DCs [40]. Nam et al. additionally proposed a therapeutic strategy combining ROCK blockade and an immunogenic cell death inducer that is known to facilitate the release of danger signals and induce the expression of calreticulin, a phagocytic signal, on dying tumor cells [42,43]. These authors found that this combination therapy significantly increased $CD8^+$ T cell infiltration into tumor tissues and markedly reduced tumor growth in syngeneic and genetically engineered tumor models. This study provided an important strategy for inducing antitumor immunity by enhancing phagocytosis and immunogenic cell death [40]. However, the molecular mechanism by which ROCK blockade induces antitumor T cell immunity in DCs is not completely understood.

2.3. A possible link between tumor cell phagocytosis and antitumor immunity

In some cases, therapeutics targeting phagocytosis increase T-cell antitumor immunity. However, cell death is a common event in solid tumors during tumor progression, and apoptotic cell clearance (also known as “efferocytosis”) in the tumor microenvironment is canonically immunosuppressive [44]. Indeed, blockade of efferocytosis using annexin V or a monoclonal antibody that blocks engagement of phosphatidylserine (a representative phagocytic signal) with phagocytic receptors on macrophages markedly reduces tumor progression and metastasis [45,46]. If this is the case, how can promoting cancer cell phagocytosis increase immune responses against cancer cells in the tumor microenvironment? A recent study revealed that the mechanism

underlying the antitumor effect of CD47 blockade is largely dependent on the sensing of mitochondrial DNA (mtDNA) in DCs [14]. Unlike macrophages, DCs can maintain an alkaline phagosomal lumen, which delays DNA degradation [47] and facilitates effective sensing of phagocytosed cancer DNA. It is therefore likely that enhanced phagocytosis must be combined with effective sensing of tumor DNA to induce antitumor immunity. On the other hand, TAMs are a major component of myeloid lineage immune cells in tumors. The induction of antitumor immunity through TAM-mediated phagocytosis is an important topic. However, the TAM population consists primarily of M2-like macrophages, which contribute to immunosuppression in the tumor environment. Thus, it may be necessary to target pathways that drive anti-inflammatory and tolerogenic signals in response to tumor cell engulfment or to reprogram M2-like TAMs into M1-like TAMs. Therapeutic strategies related to this are discussed in the following section.

3. Regulation of antitumor immunity

3.1. Tolerogenic signals

It has been suggested that tolerogenic signals generated following phagocytosis of dying tumor cells are immunosuppressive mediators of immune responses against engulfed antigens [48]. This might explain why TAMs play a minor role in eliciting antitumor immunity despite the superior ability of these cells to engulf tumor cells in the tumor microenvironment. Several signaling pathways have been proposed to contribute to immune tolerance in response to the engulfment of dying tumor cells in myeloid compartments (Table 2).

Microtubule-associated protein 1A/1B-light chain 3 (LC3)-associated phagocytosis (LAP), which is a form of non-canonical autophagy, is required for the clearance of dying cells, the processing of engulfed cells, and the regulation of immune responses in macrophages [49,50]. LAP is activated in myeloid lineage immune cells, such as macrophages and conventional and plasmacytoid DCs, through the engagement of Toll-like receptors, Fc receptors, or Tim-4 (T cell immunoglobulin and mucin domain containing 4) [51]. Recently, Cunha et al. showed that LAP in myeloid cells suppresses T cell function in the tumor microenvironment, and thereby promotes tumor growth [52]. These authors further found that impairment of LAP increases type I interferon (IFN) responses in TAMs through activation of the STING (stimulator of interferon genes) pathway [52], which is essential for the sensing of tumor DNA in DCs [53,54].

In DCs, antibody-dependent cellular phagocytosis (ADCP) is known to trigger Fc gamma receptor (FcγR) signaling, which in turn activates the STING pathway and induces the production of type I IFN, an important component for bridging innate and adaptive immunity against cancer antigens [15]. However, Su et al. recently reported the somewhat unexpected finding that ADCP can convert macrophages into an immunosuppressive phenotype and inhibit NK cell-mediated antibody-dependent cellular cytotoxicity (ADCC) and T cell-mediated cytotoxicity [55]. Specifically, they found that FcγR signaling induced by ADCP activates Aim2 (absent in melanoma 2), a cytosolic sensor of double-stranded DNA, and subsequently upregulates PD-L1 and indoleamine 2,3-dioxygenase (IDO) to cause the observed immunosuppression. Furthermore, blockade of PD-L1 or IDO has been shown to inhibit the immunosuppressive effect of TAMs following ADCP in a tumor model [55], suggesting that an ADCP-induced therapeutic antibody might need to be combined with immune checkpoint blockades. However, how tumor DNA engulfed by ADCP triggers different sensor pathways in TAMs and DCs remains to be investigated.

Tryo3/Axl/MerTK family tyrosine-kinase receptors are important for signal transduction in response to apoptotic cell engulfment, and their activation leads to homeostatic regulation of immune responses [56]. Mer tyrosine kinase (MerTK) signaling has been shown to play a crucial role in inhibiting processing of apoptotic cell-associated antigens by DCs [57], suggesting that activation of MerTK in TAMs can lead

Table 2
Therapeutic targets that inhibit tolerogenic signals or promote TAM repolarization.

Target	Functions	Treatment	Therapeutic effect	Ref.
Tolerogenic signals following phagocytosis				
LAP	TAM polarization into anti-inflammatory M2 cells	Genetic deletion of Rubcn (a LAP component)	Increased type I IFN responses through STING pathway	[52]
ADCP	Aim2 recruitment and activation; PD-L1 and IDO upregulation	PD-L1 and IDO inhibitors	Inhibition of immunosuppressive effect of TAMs	[55]
Tryo3-Axl-Mer receptors	Inhibition of DC activation for apoptotic cell antigens	Genetic deletion of MerTK	Enhanced expression of pro-inflammatory cytokines; reduction of tumor growth and metastasis; enhancement of antitumor effect of radiation therapy and chemotherapy	[58] [59] [60] [61]
Tim-3	Interference of HMGB-mediated trafficking of nucleic acids into endosome vesicles; upregulation of CXCL9	Anti-Tim-3 antibody and cisplatin	Enhancement of antitumor effect of chemotherapy; regulation of function of $CD103^+$ DCs	[64] [67]
TAM reprogramming				
HCK	Promotion of TAM polarization into M2-like cells	HCK inhibitor RK20449	Suppression of M2-like polarization of TAMs	[75]
PI3Kγ	Promotion of TAM polarization into M2-like cells	PI3Kγ inhibitor	Upregulation of IFN-γ; promotion of antitumor immunity	[76]
CSF-R1	TAM recruitment, differentiation, and survival	CSFR1 inhibitor PLX3397; CSFR1 antibody	Promotion of antigen presentation and antitumor T cell responses	[77] [78]
Let-7	Inhibition of IFN-γ–induced M1-like TAM activation	Genetic deletion of Dicer	Reprogramming of TAMs toward M1-like cells	[79]
miR125b	Promotion of TAM polarization into M1-like cells	Delivery of miR125b using nanovesicles	TAM repolarization toward M1 phenotype	[80]
Microvesicles	Transfer of biomolecules derived from their parent cells into recipient cells	Nanovesicles from M1 macrophages	Repolarization of M2-like TAMs to M1 macrophages; Promotion of antitumor effect of anti-PD-L1	[81]

to the generation of an immunosuppressive environment that favors tumor progression. Loss of Tryo3-Axl-MerTK receptors in APCs results in an increase in the production of type I IFN [58], which play a crucial role in the induction of tumoricidal adaptive immunity. MerTK deletion markedly reduces in tumor growth and metastasis in syngeneic tumor models [59], and increases the antitumor efficacy of radiation therapy in an immunogenic tumor model [60]. Du et al. recently showed that sitravatinib, a tyrosine kinase inhibitor that suppresses Tyro3/Axl/MerTK activity, blunts tumor progression and enhances the efficacy of PD-1 blockade by modulating immunosuppressive tumor microenvironments [61]. Thus, MerTK may be a promising target for therapeutics designed to regulate immune responses following tumor cell phagocytosis.

Tim-3, initially identified as a negative regulator of immune responses of type 1 helper T cells through a galectin-9–dependent mechanism [62,63], is expressed in myeloid cells of the tumor environment, such as TAMs and DCs [64,65]. Chiba et al. found that Tim-3 negatively regulates innate immune response to tumor-derived nucleic acids in tumor-infiltrating dendritic cells (TIDCs) [66]. Tim-3 interacts with high mobility group box 1 (HMGB1) protein, which plays an important role in immune responses through sensing of DNA-containing immune complexes [67], and interferes with HMGB1-mediated trafficking of nucleic acids into endosome vesicles, thereby inhibiting TIDC activation [66]. Recently, de Mingo et al. showed that Tim-3 regulates the function of $CD103^+$ DCs in breast cancers [64]. Intratumoral $CD103^+$ DCs have the ability to cross-present tumor-associated antigens in a manner that depends on the basic leucine zipper transcription factor, BATF3 [68]. Blockade of Tim-3 was shown to increase the expression of chemokine (C-X-C motif) ligand 9 (CXCL9) in $CD103^+$ DCs and thereby promote $CD8^+$ T cell-mediated adaptive immunity, resulting in improved efficacy of paclitaxel chemotherapy in breast cancer models [64].

3.2. Tumor-associated macrophages

Macrophages are a major population of myeloid lineage cells in the tumor microenvironment. However, the majority of TAMs are M2-like macrophages, which are known to contribute to an immunosuppressive microenvironment, and thus tumor progression [69]. TAMs can suppress antitumor adaptive immunity through direct cell-to-cell contact with immune effector cells (e.g., T cells, NK cells, and NKT cells) or by secreting anti-inflammatory cytokines such as interleukin (IL)-10 and transforming growth factor (TGF)-β [70]. Numerous studies have shown that extensive TAM infiltration into tumor tissues is linked to poor prognosis in many types of human tumors [71,72]. Tolerogenic signaling in response to the engulfment of dying tumor cells is associated with macrophage polarization toward an M2-like immunosuppressive phenotype [73,74]. Accordingly, considerable effort has been devoted to reprogramming TAMs from the M2 type to the proinflammatory M1 type by targeting signaling pathways involved in their polarization into the M2-like phenotype (Table 2). For instance, blockade of hematopoietic cell kinase (HCK), a key regulator of gene expression in M2-type macrophages, suppresses the M2-like polarization of TAMs and reduces tumor growth in mouse models [75]. Kaneda et al. reported that blockade of phosphoinositide 3-kinase (PI3K)-γ switches TAM activity from immune suppression to immune stimulation [76]. Specifically, they showed that inhibition of PI3K-γ activity leads to upregulation of immune-stimulatory factors, such as IFN-γ, and promotion of antitumor T cell immunity, ultimately reducing tumor burden in mouse models of cancer. It was further found that blocking colony-stimulating factor 1 (CSF1) signaling, which is important for TAM recruitment, differentiation and survival, not only has a TAM-depleting effect, but also reprograms macrophage responses so as to increase antigen presentation and antitumor T cell responses [77]. Hoves et al. recently showed that co-treatment with a CSF1 receptor inhibitor and a CD40 agonist effectively facilitates TAM reprogramming toward a proinflammatory phenotype [78]. Moreover, several studies have sought to reprogram TAMs through epigenetic regulation by targeting microRNAs (miRNAs). For example, Dicer/Let-7 activity inhibits IFN-γ–induced M1-like TAM activation, whereas blockade of Let-7 miRNA by genetic deletion of the riboendonuclease, Dicer, leads to M1-like TAM reprogramming [79]. Parayath et al. repolarized TAMs in a genetically engineered non-small-cell lung cancer model using hyaluronic acid-based nanoparticles encapsulating miR-125b [80], and Choo et al. found that nanovesicles derived from M1 macrophages cause M2-like TAMs to switch to the M1 phenotype, and thus potentiate the antitumor efficacy of an anti-PD-L1 antibody [81].

3.3. Myeloid-derived suppressor cells

MDSCs are a heterogeneous group of myeloid cells with potent immune regulatory activity. MDSCs possess functional properties that exert potent immunosuppressive ability toward immune cells, including secretion of reactive oxygen species (ROS) and nitric oxide (NO), overexpression of PD1, consumption of cysteine and arginine, and secretion of anti-inflammatory cytokines [82]. Notably, it has been shown that the immunosuppressive properties of MDSCs are positively correlated with disease stage and poor outcome in various cancers [83–86]. Therapeutic strategies targeting MDSCs inhibit tumor growth and increase antitumor immunity. Hossain et al. showed that selective silencing of STAT3 using CpG-siRNA conjugates inhibits the immunosuppressive effects of TLR9-positive MDSCs from prostate cancer patients [87]. Treatment with the phosphodesterase-5 (PDE5) inhibitor, tadalafil reduces the number of MDSCs and regulatory T cells in patients with head and neck carcinoma, thereby promoting antitumor T-cell immunity [88]. Furthermore, recent studies have shown that inhibition of MDSCs improves the efficacy of cancer immunotherapy. Entinostat, a selective inhibitor of class I histone deacetylase inhibitor, reduces the immunosuppressive function of MDSCs and enhances the anti-tumor effect of PD-1 blockade in syngeneic mouse models of lung and renal cell carcinoma [89]. In addition, treatment of MDSCs with IPI-145, a selective inhibitor of PI3K-δ and -γ, has been shown to enhance the efficacy of anti-PD-L1 antibodies in T-cell inflamed tumor models [90].

4. Chimeric antigen receptors in myeloid cells

Chimeric antigen receptors (CARs) are engineered receptors designed to enable T cells to recognize cancer-specific antigens [91]. The positive outcomes of CAR-T cell therapies highlight the potential of programmed immunity and suggest that CAR-based strategies can be applied to other immune cell lineages, such as macrophages and DCs. Morrissey et al. recently engineered CARs for phagocytosis (CAR-P) consisting of an extracellular single-chain antibody variable fragment (scFv) and an intracellular domain from Megf10 or FcγR containing immunoreceptor tyrosine-based activation motifs (ITAMs) (Fig. 3A) [92]. They found that CAR-P–expressing macrophages efficiently engulfed synthetic particles or cancer cells bearing a specific antigen. However, the efficacy of CAR-P cell therapy remains to be established in *in vivo* tumor models, and the induction of antitumor immunity by CAR-P cells following cancer cell phagocytosis has yet to be demonstrated.

Tumor-derived extracellular vesicles (EVs) are important vehicles for intercellular communication among various types of cells that influence diverse tumor-associated processes, including tumor growth and metastasis, modulation of immune responses, and promotion of angiogenesis [93]. It has been shown that tumor-derived EVs are a source of tumor antigens for eliciting antitumor immunity [94]. Squadrito et al. recently generated an extracellular vesicle-internalizing receptor (EVIR) that selectively induces DCs to engulf cancer cell-derived EVs (Fig. 3B). The extracellular domain of EVIR consists of an IgK signal peptide, an scFv specific for human HER2 protein, and a hinge domain;

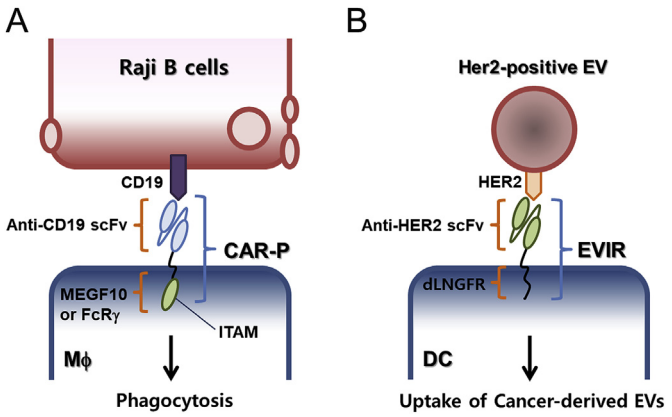

Fig. 3. Working model of CAR-P and EVIR. (A) In CAR-Ps, the extracellular anti-CD19 antibody fragment mediates adhesion of cancerous Raji B cells, and the cytosolic domain of MEGF10 or FcγR triggers pro-phagocytosis intracellular signals. ITAM, immunoreceptor tyrosine-based activation motif. (B) EVIR, containing an extracellular anti-HER2 mediates the uptake of $HER2^+$ EVs by DCs, resulting in enhanced T-cell priming of tumor antigens. dLNGFR, a deleted mutant of low-affinity nerve growth factor receptor.

notably, EVIR-expressing DCs have been shown to enhance the cross-presentation of EV-associated tumor antigens to $CD8^+$ T cells and reduce the growth of HER2-expressing tumors [95].

5. Conclusions and future challenges

Over the past decade, diverse strategies that target myeloid lineage cells have been devised to achieve effective antitumor immunity. Potentiating tumor cell phagocytosis is necessary for the acquisition of tumor antigens needed to trigger antitumor immunity. However, linking innate immunity and adaptive T-cell immunity requires elucidation of the regulatory mechanisms that initiate immunogenic phagocytosis in the tumor microenvironment. This, in turn, requires further understanding of APCs of the myeloid lineage and their phagocytic machineries and signaling pathways in the tumor microenvironment. Various myeloid cells, including TAMs and MDSCs, are present in the tumor microenvironment and provide an immunosuppressive environment that favors tumor progression. Therapeutic strategies targeting immunosuppressive functions of these cells have been found to produce an environment that supports efficient antitumor activity. Application of CARs to myeloid cells has further been shown to promote tumor cell phagocytosis and antitumor immunity. Although recently proposed therapeutics that target tumor cell phagocytosis and immunosuppressive tumor environments represent an intriguing future research evenue, other points in the cancer immunity cycle should be considered as targets in combination with phagocytosis-induced antitumor immunity as a strategy for achieving an effective connection between innate and adaptive immunity. Among other potential targets, we suggest the release of immunogenic DAMPs from dying tumor cells (e.g., produced by radiotherapy or immunogenic chemotherapy) or the activation of effector T cells following DC cross-priming (e.g., T cell immune checkpoint inhibitors). Studies relating to the choice of therapeutic agents and their optimal dosing and scheduling regimen may also be required. Ultimately, understanding the mechanisms that link cancer cell phagocytosis and adaptive antitumor immunity could be key to the development of therapeutic strategies aimed at controlling cancers.

Conflict of interest disclosures

The authors declare no conflicts of interests.

Acknowledgements

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (2017R1A3B1023418), Basic Science Research Program through the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (2017R1A2B4002662), the KU-KIST Graduate School of Converging Science and Technology Program, and the KIST Institutional Program.

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