Dual roles of myeloid-derived suppressor cells induced by Toll-like receptor signaling in cancer (Review)

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Abstract. Myeloid-derived suppressor cells (MDSCs) are one of the major components of the tumor microenvironment (TME), and are the main mediators of tumor-induced immunosuppression. Recent studies have reported that the survival, differentiation and immunosuppressive activity of MDSCs are affected by the Toll-like receptor (TLR) signaling pathway. However, the regulatory effect of TLR signaling on MDSCs remains controversial. TLR-induced MDSC can acquire different immunosuppressive activities to influence the immune response that can be either beneficial or detrimental to cancer immunotherapy. The present review summarizes the effects of TLR signals on the number, phenotype and inhibitory activity of MDSCs, and their role in cancer immunotherapy, which cannot be ignored if effective cancer immunotherapies are to be developed for the immunosuppression of the TME.

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Abbreviations: AP-1, activator protein 1; Arg1, arginase 1; BCG, Bacillus Calmette-Guerin; BLP, bacterial lipoprotein; BM, bone marrow; CCL2, chemokine (C-C motif) ligand 2; DCs, dendritic cells; ERK, extracellular regulated protein kinases; Hsp, heat shock protein; IFNgR1, interferon gamma receptor 1; IFN-γ, interferon-γ; Imiq, imiquimod; iNOS, inducible nitric oxide synthase; IRF, interferon regulatory factor; MAPK, mitogen-activated protein kinase; M-MDSCs, monocyte-myeloid-derived suppressor cells; MM, multiple myeloma; Moto, motolimod; MPL, monophosphoryl lipid A; MyD88, myeloid differentiation primary response 88; NF-κB, nuclear factor kappa-B; NK, natural killer; pDCs, plasmacytoid DCs; RNS, reactive nitrogen species; ROS, reactive oxygen species; TCR, T cell receptor; TLR, Toll-like receptor; TME, tumor microenvironment; TRIF, Toll-IL-1 receptor-domain containing adaptor-inducing interferon-β; VISTA, V-domain immunoglobulin suppressor of T-cell activation

Key words: MDSC, TLR signals, antitumor, protumor

Contents

1. Introduction

Myeloid-derived suppressor cells (MDSCs) are heterogeneous cell populations that are precursors of dendritic cells (DCs), macrophages and/or granulocytes (1,2). Under physiological conditions, MDSCs mature and differentiate into DCs, macrophages and granulocytes, while their differentiation is inhibited in the context of inflammation and tumors, leading to their accumulation in tumors and lymphoid organs as a negative balance mechanism to prevent excessive T cell activation (3). Tumor cells use this mechanism for immune escape, and MDSCs are directly involved in several processes that promote tumor development, damaging the immune response of T cells and natural killer (NK) cells, particularly $CD8^+$ T cell activation and effector function (4). High levels of circulating MDSCs in patients with tumor are associated with a worse prognosis and disease progression (5). Thus, one of the directions of development in cancer immunotherapy is to target either the MDSC populations and/or the signals involved in their recruitment and function (6-8).

MDSCs express several TLR family members, including TLR2 (9), TLR3 (10), TLR4 (11), TLR5 (12) and TLR7/8/9 (13) in mice, and TLR2 (14) and TLR7/8 (15) in humans. MDSCs accumulate in the tumor microenvironment (TME) and are an important target for TLR signaling regulation (16-18). However, the effects of TLR signaling on MDSCs and its effect on tumor growth are not yet fully understood. Previous studies have demonstrated that TLR ligands are inducers of MDSCs, and have emphasized that myeloid differentiation primary response 88 (MyD88) is essential for acquiring the direct suppressive activity of MDSCs and the ability to promote tumor growth, whereas these abilities are inhibited by blocking MyD88-mediated signaling (19-21). The accumulation of MDSCs mediated by MyD88-nuclear factor kappa-B (NF-κB) signaling increases the production of IL-10, which

inhibits the function of DCs in liver cancer (22). In addition, it has been demonstrated that increased expression of interferon regulatory factor (IRF)4, a negative feedback regulator of TLR signaling (23), decreases the MDSC population, particularly the G-MDSC population (24).

Furthermore, immune-checkpoint protein V-domain immunoglobulin suppressor of T-cell activation (VISTA) is a chief myeloid cell-intrinsic immune-checkpoint protein that can control antitumor immunity (25). VISTA modulates the polyubiquitination and protein expression of TNF receptor associated factor 6 to inhibit TLR-mediated activation of the mitogen-activated protein kinase (MAPK)/Activator protein-1 (AP-1) and IKK/NF-κB signaling cascades, which decreases the ability of MDSCs to produce proinflammatory mediators and enhance their T cell-suppressive functions, thereby protumor progression (26).

However, other groups have disputed these findings, reporting that TLR signaling activation can decrease the immunosuppressive activity of MDSCs (13,27,28). Loss of MyD88 results in an increase in prostate intraepithelial tumors and highly differentiated adenocarcinoma areas in TRAMP transgenic mice, accompanied by an increase in the frequency of MDSCs and the production of inducible nitric oxide synthase (iNOS), prostate arginase 1 (Arg1), and cytokine Interleukin (IL)-10 (27). Furthermore, other studies have reported that TLR stimulation decreases the MDSC population and enhances their differentiation into tumoricidal macrophages (13,28). Thus, the regulation of TLR signaling on MDSCs is diverse. The present review summarizes the effects of TLR signals on the number, phenotype and inhibitory activity of MDSCs, and their role in cancer immunotherapy. It remains essential to address this in the present and future cancer immunotherapies for the immunosuppression of the TME.

2. General aspects of MDSCs and TLR signaling

MDSCs. MDSCs can be divided into two categories, including polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs or G-MDSCs) that are phenotypically and morphologically similar to neutrophils, and monocyte-myeloid-derived suppressor cells (M-MDSCs), which are similar to monocytes. In mice, the phenotype of G-MDSCs is $CD11b^{+}Ly6G^{+}Ly6C^{lo}$, and M-MDSCs is $CD11b^{+}Ly6G^{-}Ly6C^{hi}$ (29). In humans, the phenotype of G-MDSCs is $CD11b^{+}CD14^{-}CD66b^{+}$ or $CD11b^{+}CD14^{-}CD15^{+}$, and the phenotype of M-MDSCs is $CD11b^{+}CD14^{+}HLA^{-}DR^{-/lo}CD15^{-}$ (29). Increasing evidence suggests that MDSCs promote tumor progression and metastasis through their immunosuppressive activity, the mechanism of which can be summarized as i) Arg1 and iNOS produced by MDSCs consume arginine and cysteine, which are nutrients required by lymphocytes, leading to the downregulation of the ζ chain in the T cell receptor (TCR) complex and inhibiting the proliferation of antigen-activated T cells (30); ii) reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated by MDSCs induce the formation of oxidative stress, leading to the loss of T cell ζ chain expression and interfering with IL-2 receptor signaling cascades (31,32); iii) MDSCs interfere with the transportation and survival of lymphocytes, affecting the migration of $CD8^{+}$ T cells to the TME and restricting T cell recycling to the lymph nodes (33,34) and iv) MDSCs improve naïve $CD4^{+}$ T cell differentiation into regulatory T cells, thereby inhibiting T cell function (35). Thus, it remains essential to develop cancer immunotherapies that aim to decrease the negative impact of MDSCs on effector immune cells.

Previous studies have demonstrated that targeting the MDSC population and the signals involved in their function can delay tumor progression (36,37). Thus, understanding the regulators and signaling pathways involved in the survival, development, differentiation and activation of MDSCs is essential for cancer immunotherapy targeting MDSCs.

Currently, two-signal models are used to describe the differentiation of MDSCs. The model includes two stages: The first stage is the expansion of immature bone marrow cells and the inhibition of terminal differentiation; the second stage is the activation stage, which transforms immature bone marrow cells into MDSCs (38). Notably, the TLR signaling pathways are involved in both stages of MDSC differentiation, and TLR receptors are expressed positively on MDSCs. TLR signals are considered important regulators of the differentiation and acquisition of the immunosuppressive function of MDSCs (39).

TLR signaling. TLRs are type I transmembrane proteins, with 10 existing in humans (TLR1-10) and 12 in mice (TLR1-9, TLR11-13) (40). TLR1, TLR2, TLR4, TLR5 and TLR6 are located on the cell surface and are mainly involved in the detection of extracellular bacterial products, while TLR3, TLR7, TLR8 and TLR9 are located in intracellular compartments that are involved in the detection of nucleic acids from viral and bacterial sources (40). These TLRs recognize pathogen-associated molecular patterns to initiate the appropriate host immune response. Binding of the TLR to the ligand results in the activation of two major signaling pathways, the MyD88-dependent and Toll-IL-1 receptor-domain containing adaptor-inducing interferon-β (TRIF)-dependent signaling pathways. Excluding TLR3, all TLRs activate MyD88-dependent signaling pathways, and both TLR3 and TLR4 activate TRIF-dependent signaling pathways. The MyD88-dependent pathway activates nuclear factor NF-κB and the MAPK pathway, and induces the development of inflammatory responses. However, the TRIF-dependent pathway activates the interferon IRF pathway and induces antiviral type 1 interferon, which is involved in the antiviral response (40).

Currently, Bacillus Calmette-Guerin (BCG, TLR2/TLR4 agonist), Monophosphoryl lipid A (MPL, TLR4 agonist), and Imiquimod (Imiq) (TLR7 agonist) have been approved by the Food and Drug Administration (FDA) for clinical treatment of patients with cancer (41). Furthermore, TLR agonists are extensively used as adjuvants for cancer vaccines to enhance their antitumor effects. The antitumor effect of TLR agonists is attributable to the activation of TLR signals that enhance antigen-specific humoral and cellular immune responses. However, due to the existence of the immune tolerant microenvironment, TLR agonists alone or as cancer vaccine adjuvants can only produce moderate clinical benefits in cancer treatment (42,43). Thus, it remains critical to identify and develop therapeutic approaches to overcome the immunosuppressive TME and to enhance the efficiency of current tumor

immunotherapies. Understanding the effect of TLR signaling on MDSCs will help optimize the role of TLR agonists in anti-tumor therapy and provide novel insight into the development of cancer immunotherapies.

3. Pro-tumorigenic effects of MDSCs induced by TLR signaling

Increasing evidence suggests that the accumulation and activation of MDSCs is associated with tumor progression, recurrence and a negative clinical outcome (44). Recently, a number of studies have demonstrated that TLR signaling induces MDSC accumulation and enhances the ability to inhibit tumor-specific T cell responses, resulting in tumor progression (45,46). Related studies and potential TLR signaling pathways are summarized in Table I and Fig. 1.

The accumulation and survival of MDSCs induced by TLR signaling

TLR2 and MDSCs. In EG7 tumor-bearing mice, it has been demonstrated that Pam2CSK4, a TLR2 agonist, induces the accumulation of MDSCs and prolongs the survival of MDSCs, leading to the suppression of the antitumor immune response (9,45). In addition, signal transducer and activator of transcription (STAT3) is an important transcription factor for MDSC expansion, which is attributed to the abnormal and continuous activation of STAT3 in myeloid progenitor cells that prevents them from differentiating into mature myeloid cells (4). Recently, some studies have confirmed this effect (46-50). It has been demonstrated that heat shock protein (Hsp72/Hsp70) in tumor cell-derived exosomes and telomeric repeat-binding factor 2 promote the recruitment and expansion of MDSCs through the activation of STAT3, which is induced in a TLR2/MyD88/IL-6-dependent manner (46-50). Furthermore, serum amyloid A3 can activate STAT3 by TLR2/MyD88/tumor necrosis factor (TNF)α signaling, leading to the enhanced survival of MDSCs (51). Notably, STAT3 also regulates the expression of the inflammatory factors S100A8 and S100A9, which act as TLR4 ligands to activate the immunosuppressive activity of MDSCs (52-54).

TLR4 and MDSCs. The anticonvulsant drug valproic acid, which decreases the frequency of MDSCs, is accompanied by the downregulation of TLR4 mRNA expression (55). In addition, both tumor volume and pulmonary recruitment of MDSCs decrease with a TLR4/MD-2 complex antagonist (56). Previous studies have suggested that the TLR4 signaling pathway may also be involved in the accumulation and survival of MDSCs (55,56). Recently, MPL, a TLR2 and TLR4 agonist, has been confirmed to have this effect (57). MPL induces the accumulation of MDSCs both *in vitro* and *in vivo* by inhibiting DC development from myeloid cells (57). In addition, in a melanoma mouse model, soluble calreticulin (sCRT39-272) was demonstrated to promote the migration and survival of tumor-derived MDSCs via interactions with TLR4 (52). Notably, Li *et al* (58) demonstrated that exogenous S100A4 upregulates TLR4 receptor expression on MSC2 cells and protects MDSCs from apoptosis via the TLR4/extracellular regulated protein kinases (ERK)1/2 signaling axis, both *in vitro* and *in vivo*.

TLR7/9 and MDSCs. Notably, some intracellular TLR7/9 signaling pathways also promote accumulation of MDSCs (59,60). CpG ODN (CpG, TLR9 agonist) administration induces the accumulation of tumor-infiltrating MDSCs in pancreatic ductal adenocarcinoma (59). It has also been demonstrated that CL264 (TLR7 agonist) directly interacts with the TLR7 receptor on murine lung adenocarcinoma LLC-Luc cells, which promotes the accumulation of G-MDSCs by increasing the secretion of granulocyte/macrophage CSF and chemokine (C-C motif) ligand 2 (CCL2) in the TME, resulting in an increased number of lung metastases and the promotion of tumor progression (60,61). However, some studies have reported that TLR7 and TLR9 signals weaken MDSC immune activity (16,17).

Differentiation and activation of MDSCs induced by TLR signaling

TLR2 and MDSCs. It has been demonstrated that Pam2CSK4 inhibits TCR-stimulated syngeneic T cell proliferation by inducing M-MDSCs to differentiate into the M2-like ($25F9^+/CD200R^+$) phenotype, and produce IL-6 and IL-10 (14). Another study revealed a novel mechanism of inducing the immunosuppressive activity of M-MDSCs: Pam2CSK4 promotes the differentiation of M-MDSCs into $CD11b^+F4/80^+$ macrophages, which inhibits DC-induced T cell proliferation through nitric oxide (NO) produced by iNOS (45). In addition, TLR2 signaling activates MDSCs by increasing the expression levels of NOS2, Arg1, iNOS, IL-10 and transforming growth factor (TGF)-β, thereby triggering NK and T cell suppression (46).

TLR agonists induce $CD8^+$ T cells to produce interferon-γ (IFN-γ), which is beneficial for killing tumor cells (62). However, studies have demonstrated that the IFN-γ-STAT1-IRF1 axis is essential for the inhibitory activity obtained by M-MDSCs, and may upregulate the expression levels of iNOS and Arg-1 (39,63). This phenomenon has also been confirmed by Shime *et al* (45), who demonstrated that the TLR agonist, Pam2CSK4, induces IFN-γ production by $CD8^+$ T cells, accompanied by interferon gamma receptor 1 (IFNgR1) expression on M-MDSCs. Thus, IFN-γ interacts with IFNgR1 on M-MDSCs, induces iNOS expression and inhibits the proliferation of T cells. These results suggest the rationality of targeting the IFN-γ-STAT1-IRF1 axis in MDSCs while targeting MDSC inhibition.

TLR4 and MDSCs. SA100A8/A9 are important pro-inflammatory cytokines that increase MSC accumulation and immunosuppressive activity in the TME (64). De Veirman *et al* (54) demonstrated that S100A9 acts as a chemokine for multiple myeloma (MM) cells and induces MDSCs to express and secrete inflammatory and promyeloma cytokines, including TNF-α, IL-6 and IL-10. In addition, He *et al* (52) demonstrated that TLR4 signaling inhibits MDSC differentiation into DCs and promotes their functional maturation, and the chemotactic migration of MDSCs by initiating the expression of S100A8 and S100A9. Recent studies on colorectal cancer (53) and MM (54) have demonstrated that S100A9 promotes the expression levels of Arg1, iNOS and IL-10, and ROS production in MDSCs via TLR4-NF-κB signaling cascades, thereby inhibiting $CD8^+$ T cell activity and promoting tumor progression (53). It has also been

Table I. Pro-tumorigenic effects of MDSCs induced by TLR signaling in cancer.

TLR	Stimulus	Species	Cancer	Number and phenotype	Function and mediator(s)	(Refs.)
TLR2	Pam2CSK	M	Lymphoma	Accumulation in tumor sites supported survival	-	(9)
		H	Colon, prostate, pancreatic, liver cancer	M2-like ($25F9^+/CD200R^+$)	Inhibited T cell proliferation. Mediator: IL-6, IL-10	(14)
		M	Lung cancer and lymphoma	Prolonged survival	iNOS, NO	(45)
	Hsp72/Hsp70	M, H	Breast cancer melanoma lymphoma and RCC	Expansion	Arg1, iNOS	(47-50)
	TRF2	M	OSCC	Accumulation and activation	Triggered NK and T cell suppression. Mediator: Arg1, IL-10, TGF-β,	(46)
	SAA3	M	Breast and CRC	Prolonged survival inhibited MDSCs differentiation into M1	NOS2, Arg1, Nox2,	(51)
TLR4	MPL	M	-	Accumulation	Suppressed T cell proliferation. Mediator: IL-10, NO	(57)
	S100A9	M	CRC	-	Inhibited $CD8^+$T cell activity. Mediator: Arg1 and iNOS	(53)
		M	MM	-	TNF-α, IL-6, and IL-10	(54)
	S100A4	M	Melanoma lung cancer	Prolonged survival	-	(58)
	PUAF	M, H	Pancreatic cancer		Arg1, NO, and ROS	(65)
	HMGB1	M	-		Suppressed T cell proliferation	(66)
	sCRT/39-272	M	Melanoma	Prolonged survival inhibited MDSCs differentiation into DC	S100A8 and S100A9	(52)
	EV	M, H	Melanoma		Upregulated PD-L1 expression	(69)
TLR9	CpG	M	Pancreatic carcinoma	Accumulation in tumor sites		(59)
TLR7	CL264	M	Lung adenocarcinoma	Accumulation in tumor site		(60)

M-MDSCs, monocyte-myeloid-derived suppressor cells; TLRs, Toll-like receptors; M, mouse; H, human; PMN, polymorphonuclear; OSCC, oral squamous cell cancer; RCC, renal cell carcinoma; CRC, colorectal cancer; MM, multiple myeloma; TNFα, tumor necrosis factor-α; NO, nitric oxide; DC, dendritic cell; iNOS, inducible nitric oxide synthase; Arg1, arginase 1; IFN-γ, interferon-γ; IL, interleukin; TGF-β, transforming growth factor-β.

demonstrated that the TLR4/ERK/AP-1 and TLR4-IRF axis signaling pathways enhance the immunosuppressive function of MDSCs (65,66). Furthermore, TLR4 signaling can increase the production of IL-10 and attenuate the production of IL-12 in MDSCs, thereby enhancing the interaction between MDSCs and macrophages, and promoting a shift from the tumoricidal Th1 response to the pro-tumorigenic Th2 response (67,68).

Notably, Fleming *et al* (69) demonstrated that ret mouse melanoma cell-derived extracellular vesicles can induce the upregulation of PD-L1 on bone marrow (BM)-derived murine immature myeloid cells, the immortalized myeloid suppressor cell line, MSC-2, and normal human $CD14^+$ monocytes in a TLR4-MyD88/TRIF-NF-κB signaling-dependent manner, thereby strongly suppressing $CD8^+$ T cell activation through PD-1/PD-L1 signaling cascades (70,71). Similarly, Ki-67 expression in MDSCs is upregulated by the TLR4 mAb, accompanied by increased PD-L1 and iNOS expression on MDSCs, particularly on M-MDSCs (11). These results suggest the possibility of MDSC inhibition and PD-1/PD-L1 signal inhibition, synergistically breaking the immune tolerance microenvironment.

4. Antitumor effects of MDSCs induced by TLR signaling

Recent studies have demonstrated that activation of TLR signaling decreases the ability of MDSCs to inhibit T cell proliferation, thereby inhibiting tumor growth. This effect is mainly manifested in the decreased number of MDSCs, differentiation of MDSCs into antigen-presenting cells and decreased production of inhibitory mediators (72,73). Related studies and potential TLR signaling pathways are summarized in Table II and Fig. 2.

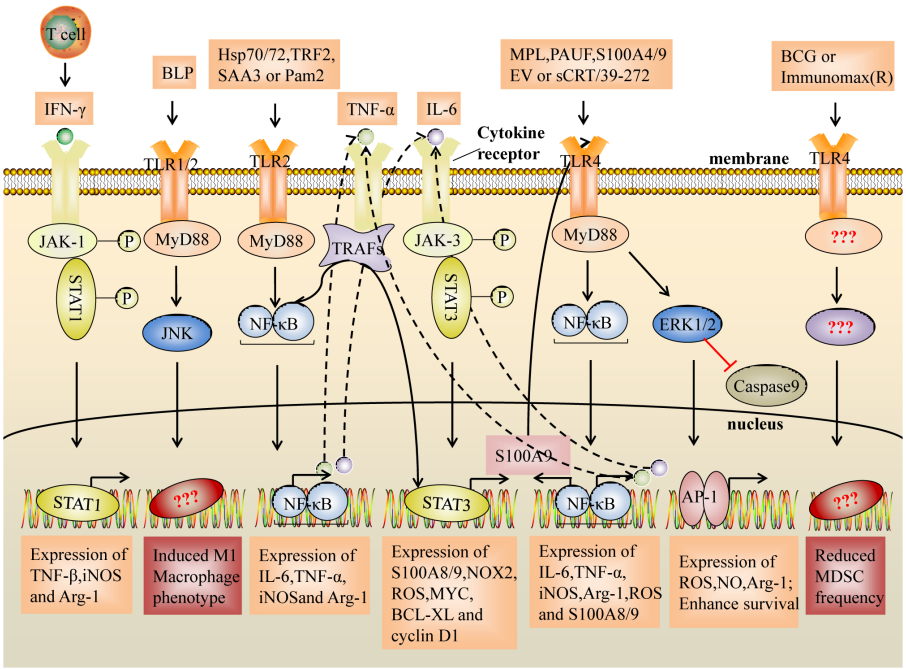

Figure 1. Suppressive activity of MDSCs induced by the TLR2 and TLR4 signaling pathways. The NF-κB pathway activated by TLR2/4 induces the expression of inflammatory factors (IL-6 and TNF-α). In turn, IL-6 and TNF-α activate the STAT3 signaling pathway and the NF-κB signaling pathway. Notably, STAT3 regulates the expression of the inflammatory factors, S100A8 and S100A9, which act as TLR4 ligands to activate the NF-κB pathway, resulting in upregulation of IL-6 and TNF-α expression, and they form a loop that enhances the expansion and activation of MDSCs. In addition, TLR2/JUK signals induce an M1-like macrophage phenotype and decrease the immunosuppressive activity of MDSCs, whereas transcription factors for the differentiation to M1 macrophages or decreasing frequency on MDSC are unclear. TLR, Toll-like receptor; BLP, bacterial lipoprotein; sCRT/39-272, Recombinant CRT fragment 39-272; PAUF, protein pancreatic adenocarcinoma upregulated factor; SAA3, serum amyloid A3; TRF2, telomeric repeat-binding factor 2; EV, extracellular vesicles; Hsp, heat shock protein; MPL, monophosphoryl Lipid A; BCG, bacillus Calmette-Guerin; Pam2, Pam2CSK4; TNFα, tumor necrosis factor-α; IL-6, interleukin-6; IFNγ, interferon-γ; MyD88, myeloid differentiation primary response 88; TRAF, TNF receptor associated factor; ERK, extracellular regulated protein kinases; JNK, c-Jun kinase; JAK, Janus kinase; NF-κB, nuclear factor kappa-B; AP-1, activator protein-1; ROS, reactive oxygen species; iNOS, inducible nitric oxide synthase; Arg1, arginase 1; BCl-XL, B-cell lymphoma XL.

TLR signaling decreases the number of MDSCs. Intracellular TLRs, including TLR3, TLR7, TLR8 and TLR9, are intrinsically capable of detecting nucleic acids, in which TLR7, TLR8 and TLR9 receptors are similar in terms of expressed cells, recognized ligands, localization on cells and activated pathways, and all intracellular TLRs can induce the production of type I IFN (74,75). Some studies have demonstrated that the TLR3/7/8/9 signaling pathway plays a similar role in regulating MDSCs. It has been reported that TLR3/7/8/9 agonists decrease the MDSC frequency by activating TLR3, TLR7, TLR8 and TLR9 signaling pathways *in vivo*, thereby enhancing their antitumor effects (16,17,76) (Table II).

TLR3 and MDSCs. Poly (I:C) treatment decreased the MDSC frequency in BM, blood, spleen and tumors (28,76). In addition, oncolytic reovirus, which activates the TLR3 signaling pathway, mainly decreased the M-MDSC frequency and failed to change the G-MDSC frequency (10). Imiquimod (77,78), SC1 (a novel synthetic agonist with exquisite specificity for TLR7) (18) and ssRNA-Pim-3-shRNA, a synthetic dual-function vector that triggers TLR7 receptors via ssRNA fragments) (79) all decreased MDSCs in the TME, thereby inhibiting the growth of tumors in mice. It has been demonstrated that TLR7 signals decrease the number of MDSCs in a type I IFN-dependent manner (18,79). Similarly, TLR7/TLR9 signal-dependent type I IFN production in plasmacytoid DCs (pDCs) is imperative for decreasing MDSC suppressive activity, as well as promoting antitumor immunity (17,18,79). Thus, it was hypothesized that endosome TLR-induced MDSC inhibitory activity is associated with TLR signaling pathway-induced type I IFN production. However, prospective studies are required to confirm this hypothesis and determine how type I IFN regulates MDSC suppressive activity.

TLR7/8 and MDSCs. Systemic application of R848 (a TLR7/8 agonist) significantly decreases the frequency of M-MDSCs in tumors, blood and spleen instead of bone marrow, as well as the frequency of MDSCs in a mouse subcutaneous CT26 colon cancer model and the mouse 4T1 breast cancer model; however, this decrease is not as obvious as in the CT26 model (16). In addition, motolimod (Moto) treatment significantly increases the cell death of M-MDSCs *in vitro* and in patients with cancer (15). Moto significantly increases the mean fluorescence of FAS on M-MDSCs and upregulates CD69 and FAS-L expression on the T-cell surface; therefore, Moto induces apoptosis of M-MDSCs, in part, through the link between FAS and FAS-L (15).

TLR9 and MDSCs. The effect of TLR9 signals on MDSCs *in vivo* may be associated with the injection methods used to deliver the TLR9 agonist; intratumoral injection of CpG decreases the proportion of M-MDSCs in tumor-bearing mice (13), whereas subcutaneous injection of CpG significantly decreases the amount of G-MDSCs in the spleen of mice

Table II. Antitumor effects of MDSCs induced by TLR signaling in cancer.

TLR	Stimulus	Species	Cancer	Number and phenotype	Function and mediator(s)	(Refs.)
TLR3	Poly(I:C)	M	Breast cancer	Decreased MDSC frequency, and upregulated MHC II, I-Ad, CD80 and CD86	Decreased ROS production	(76)
		M	CC	Decreased the number of MDSCs	Attenuated the immunosuppressive activity	(28)
		M	Lymphoma		Abrogated the immunosuppressive activity	(88)
	OR	M	Melanoma lymphoma	Decreased M-MDSC frequencies	Abrogated the immunosuppressive activity	(10)
TLR7	Imiq	M	Lung cancer	Decreased the number of MDSCs	-	(77,78)
		M	CC	Phenotype: $Ly6C^{-}F4/80^{+}$ macrophage phenotype	-	(13)
	SC1	M	CC	Decreased the number of G-MDSCs		(18)
	s-P-sh	M	Melanoma	Decreased MDSC proportion	-	(79)
TLR7/8	R848	M	CC	Decreased MDSC frequency, and upregulated CD11c, F4/80, MHC-I and MHC-II	Abrogated the immunosuppressive activity	(16)
		M	CRC	Phenotype: $F4/80^{+}iNOS^{+}$ M1 macrophages	TNF-α and IL-1β	(89)
		H	CC, prostate, pancreatic, liver cancer	Phenotype: M1-like ($25F9^{+}$/CD200R2)	Increased the ability to kill tumor cells and lost immunosuppressive activity. Mediator: IL-6 and IL-12	(14)
TLR8	Resiq	M	Lymphoma	Phenotype: $F4/80^{+}$ macrophages and $CD11c^{+}/I\text{-}A^{d+}$ DCs	Enhanced the proliferation of T cells	(90)
	Moto	H	Melanoma, CC and prostate	Decreased M-MDSC frequency		(15)
TLR9	CpG	M	CC	Decreased M-MDSC frequency, and upregulated F4/80 and downregulated Ly6c and Gr-1	Abrogated immunosuppressive activity. Mediator: IL-6, TNF-α, IL-12	(13)
		M	CC and melanoma	Decreased G-MDSC frequency, and upregulated Sca1(Ly6A/E), F4/80, MHC II and CD11c	Abrogated immunosuppressive activity	(17)
		M	Hepatoma		Attenuated the immunosuppressive activity. Mediator: IFN-γ	(83)
	Ad-CpG/ CpG	M	Melanoma and RCC	Decreased MDSC frequency	-	(80,81)
TLR4	BCG	M	Bladder cancer	Decreased MDSC frequency	-	(84)
	I(R)	M	Metastatic breast cancer	Decreased MDSC frequency	-	(85)
TLR1/2	BLP	M	Lung cancer	Upregulated CD80, CD86, MHCII, F4/80. Phenotype: M1-like macrophage	High levels of NOS2, IL1β, IL-6 and TNF-α, and low levels of Arg1 and CD206	(73)
		M	Glioma	Decreased MDSC frequency in TME	-	(87)
TLR2	pAbM	M	Mammary carcinoma	Upregulated CD86 and MHCII. Phenotype: M1 macrophage	IL-6, IL-12, TNF-α and iNOS	(86)

M-MDSC, monocyte-myeloid-derived suppressor cells; TLR, Toll-like receptor; M, mouse; H, human; PMN, polymorphonuclear; OR, oncolytic reovirus; Imiq, imiquimod; s-P-sh, ssRNA-Pim3-shRNA; Resiq, resiquimod; Moto, motolimod; I(R), Immunomax®; RCC, renal cell carcinoma; TNFα, tumor necrosis factor-α; NO, nitric oxide; DCs, dendritic cells; iNOS, inducible nitric oxide synthase; Arg1, arginase 1; IFN-γ, interferon-γ; IL, interleukin; ROS, reactive oxygen species; TME, tumor microenvironment; CC, colon cancer.

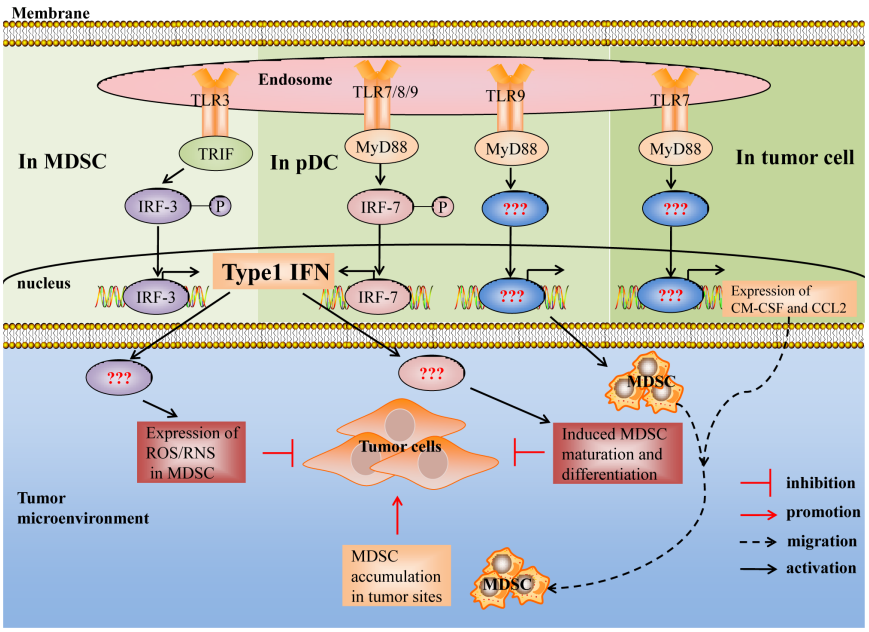

Figure 2. Suppressive activity of MDSCs induced by the TLR3, TLR7, TLR8 and TLR9 signaling pathways. Type I interferon is essential for TLR3/7/8/9 signal induced MDSCs to inhibit the growth of tumors, whereas the mechanism remains unclear. In addition, the downstream signals and transcription factors of TLR7/9 signal induced MDSCs that promote tumor growth remain unclear. TLR, Toll-like receptor; MyD88, myeloid differentiation primary response 88; TRIF, TIR adaptor-inducing interferon-β; IRF3/7, interferon regulatory factor 3/7; ROS, reactive oxygen species; RNS, reactive nitrogen species; GM, granulocyte/macrophage; CCL2, chemokine (C-C motif) ligand 2.

in vivo (17). In addition, Ad5D24 CpG (Ad-CpG), an adenovirus targeting the TLR9 receptor, enhanced the antitumor efficacy in a lung cancer model and significantly decreased the total number and immunosuppressive activation of MDSCs in tumors instead of the spleen (80). Similarly, in a mouse renal cell carcinoma model, CpG treatment decreased the amount and frequency of a large number of MDSCs in tumor-bearing kidney tissues instead of renal blood vessels *in vivo* (81). However, CpG failed to decrease MDSCs in patients with cancer (15,82), which may be due to the negative expression of the TLR9 receptor on human MDSCs (14).

Notably, Lin *et al* (83) demonstrated that CpG significantly increases the M-MDSC frequency in nontumor parts of the liver and suppresses murine hepatic tumor growth. This phenomenon was named 'intrahepatic myeloid aggregation for T cell expansion', which was attributed to CpG promoting the mRNA expression of IFN-γ in both M-MDSCs and G-MDSCs in the TME. Although these CpG-induced MDSCs still express high levels of IL-10 and Arg-1 mRNA, presenting a suppressive phenotype, their suppressive ability is attenuated (83).

TLR4 and MDSCs. The TLR4 signaling pathway induces the activation and accumulation of MDSCs; however, TLR4 signal activated by BCG (84) and Immunomax® (IR) (85) decreases the frequency of MDSCs. Notably, BCG and PD-L1 blockade synergistically inhibit the growth of bladder cancer and decrease the proportion of MDSCs (84). BCG and PD-L1 have been approved for individual use by the FDA for the treatment of cancer. Their combined application synergistically decreases the proportion of MDSCs (84). This suggests the possibility of a combined application of TLR agonists and PD-1/PD-L1 inhibitors to synergistically break the immune tolerance microenvironment.

TLR signaling decreases the immunosuppressive activity of MDSCs

TLR1/2 and MDSCs. TLR2 signaling promotes the accumulation and activation of MDSCs (9,48). However, some studies have demonstrated that TLR2 signaling also weakens the inhibitory activity of MDSCs (73,86).

It has been demonstrated that TLR1/2 agonists decrease the immunosuppressive activity of MDSCs by inducing M1-type macrophage characteristics in MDSCs (73,86). Notably, Deng *et al* (73) reported that TLR1/TLR2/c-Jun kinase signaling promotes M-MDSC differentiation into M1-type macrophages, thereby preventing M-MDSC inhibition. Furthermore, the CCL2-CCR2 signaling pathway was implicated in the attraction of M-MDSCs to the tumor site. The disruption of CCL2-CCR2 signaling notably decreases the monocyte influx into the tumor, decreases the number of TAMs, and generally delays tumor growth (72). Similarly, Zhang *et al* (87) demonstrated that the combination of adoptively transferred antigen-specific T cells and bacterial lipoprotein decreased the MDSC frequency in the TME, which may be associated with low CCL2 expression.

TLR5 and MDSCs. CXCL5 is the main chemokine involved in the migration of MDSCs into tissues, including tumors. It has been demonstrated that intratumoral injection of TLR5 ligand-secreting T cells, engineered tumor-reactive T cells that secrete bacterial flagellin (TLR5 ligand), resulted in a decrease in the number of MDSCs in the spleen and tumor, particularly M-MDSCs, and upregulated the expression levels of CD80, CD86, MHCI and MHCII on MDSCs (12). In addition, the decrease in MDSCs was associated with a striking reduction in CXCL5 levels (12).

However, bacterial flagellin, a TLR5 ligand, failed to influence the ability of MDSCs to inhibit T cell proliferation and slightly affected CD80, MHCI or MHCII expression in MDSCs. It has been suggested that the modification of TLR agonists can change their regulatory effect on MDSCs, which provides new ideas and a theoretical basis for improving the antitumor effect of TLR agonists (12).

TLR3 and MDSCs. In addition to decreasing the number of MDSCs, TLR signals also induce MDSCs to differentiate into antigen-presenting cells and weaken their ability to suppress T cell responses (76). It has been reported that TLR3 signaling activated by PolyI:C decreases the immunosuppressive activity of MDSCs by upregulating MHC II, I-Ad, CD80 and CD86, and decreasing the secretion of ROS in breast cancer models (76). In addition, TLR3 signaling also abrogates the capacity of MDSCs to suppress T cell proliferation in B16 and EL4 tumor models (10). In addition, Shime *et al* (88) demonstrated that G-MDSCs that had been activated with PolyI:C exhibit cytotoxicity and inhibit tumor growth through the production of ROS/RNS in a TLR3/TRIF/type I IFN-dependent manner.

TLR7/8 and MDSCs. Increasing evidence suggests that TLR7/8 signaling activated by Imiq (13), R848 (89) and resiquimod (16,90) induces MDSCs to differentiate into tumoricidal M1 macrophages in mice. Furthermore, R848 can induce M-MDSCs to differentiate into an M1-like ($25F9^+$/CD200R2) phenotype in patients with cancer, induce the production of IL-6 and IL-12 in M-MDSCs, increase their ability to kill A549 tumor cells, and lose their ability to inhibit T cell proliferation (14). However, R848 is a topical immune response modifier. When it was administered systemically, undesirable side effects were observed. Thus, novel TLR7/8 (3M-055 and CL-075) agonists were designed and found to be safe when administered to mice (91,92). Studies have demonstrated that each of these agonists duplicates the ability of R848 to induce human M-MDSCs to mature into M1-like macrophages, and that they are safe when administered to mice (14,93). These results are exciting, and they also provide new ideas for the development of TLR agonists that decrease side effects and disrupt the inhibition of MDSCs.

TLR9 and MDSCs. Recently, an increasing number of preclinical and clinical trials have used CpG as a vaccine adjuvant to improve the antitumor effect of cancer vaccines. It has been demonstrated that CpG binding with TLR9 on MDSCs directly induces M-MDSC differentiation into $Ly6C^-F4/80^+$ macrophages and upregulates CD40, CD80 and CD86 expression on MDSCs *in vitro* (13,81). However, another study has reported that CpG indirectly upregulates the expression levels of CD11c, MHCII, CD80 and F4/80 on MDSCs through type I IFN produced by pDCs mediated by CpG (17), which may be attributed to CpG administration. Preclinical trials in our laboratory indicated that the recombinant mucin1-maltose-binding protein vaccine, including recombinant mucin1-maltose-binding protein and CpG 2006, significantly downregulated the ratio of MDSCs in the spleen and tumor microenvironment (94). Taken together, these studies provide a rationality for the application of CpG as a cancer vaccine adjuvant.

TLR-TLR crosstalk and MDSCs. Previous studies have proven that a combination of TLR agonists synergistically enhances the activity of cancer vaccines (95,96). Thus, studying the impact of TLR-TLR crosstalk on MDSCs is essential for understanding the synergistic mechanism of TLR agonists and the rational combined application of TLR agonists.

Notably, TLR7 and TLR9 signals have synergistic effects in regulating MDSCs. 3M-052 and CpG can synergistically decrease the frequency of tumor infiltrating M-MDSCs by nearly 90%, and a synergistic reduction of Arg1 and Nos2 mRNA expression, particularly Nos2 mRNA, resulting in a nearly 90% reduction (97). Furthermore, the combination of CpG plus 3M-052 was more successful against both CT26 colon cancer and B16-F10 melanomas compared with CpG or 3M-052 alone, and cure rates around 80-90% can be achieved via combination therapy (97).

However, Triozzi *et al* (98) demonstrated that Imiq or CpG given individually as an adjuvant both enhance the antitumor effect of tumor vaccines and decrease the MDSC frequency, whereas the combination of Imiq and CpG as adjuvants increases the frequency of MDSCs in the spleen, and the secretion of Arg1 in MDSCs and the production of M2-type macrophages in tumors, accompanied by a reduction in the M1 polarized marker CXCL10, suggesting that TLR7 and TLR9 signals play an antagonistic role in the regulation of MDSCs.

Chang *et al* (99) demonstrated that TLR2 and TLR9 have synergistic effects in regulating MDSCs. It was reported that Rlipo-E7 m, a recombinant lipoprotein that has intrinsic TLR2 agonist activity, significantly decreases MDSC frequency in the circulation and the tumor microenvironment, and this ability to inhibit MDSCs is enhanced when Rlipo-E7 m is combined with CpG ODN.

5. Conclusions and perspectives

The regulation of MDSCs by TLR signals is a double-edged sword in cancer. TLR signaling can activate the immunosuppressive activity of MDSCs to promote tumor progression, and also abrogate the immunosuppressive activity of MDSCs and inhibit tumor growth. Although several compounds have been investigated for the therapeutic targeting of MDSCs, finding TLR agonists that are able to modulate the suppressive function of tumor-expanded MDSCs could be a better choice, which represents a desirable tipping of the balance toward an increase in immunostimulatory activity with the concomitant loss of immunosuppressive MDSCs. In addition, a combination of TLR agonists and immunotherapy targeting MDSC suppression to decrease the activation effect of MDSCs induced by TLR signaling appears to be feasible in cancer treatment. Furthermore, targeted MDSC cancer immunotherapy through modifying TLR ligands may be an attractive direction, enabling enhanced immune activity, accompanying the loss of MDSC immunosuppressive activity and reversing the immunosuppressive microenvironment, which may be expected to cause tumors to regress further. Studies on MDSCs and their subsets (G-MDSC and M-MDSC) regulation by TLR is still relatively limited. G-MDSC and M-MDSC utilize different molecular mechanisms to suppress the immune response in the TME. Thus, understanding the effect of TLR signaling on MDSCs subsets is beneficial to provide new ideas for the development of cancer immunotherapies.

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Authors' contributions

HZ drafted the initial manuscript, edited and critically revised the manuscript. MJ, HY and WN contributed substantially in drafting the manuscript, editing and critically revising the manuscript for intellectual content. GT put forward the concept, critically revised the article for intellectual content, and was responsible for the organization, revision and submission of the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

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Competing interests

The authors declare that they have no competing interests.

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