Research progress on the role of tumor-associated macrophages in tumor development and their use as molecular targets (Review)

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Abstract. The tumor microenvironment (TME) is a complex system composed mainly of tumor cells, mesenchymal cells and immune cells. Macrophages, also known as tumor-associated macrophages (TAMs), among innate immune cells, are some of the most abundant components of the TME. They may influence tumor growth and metastasis through interactions with other cell populations in the TME and have been associated with poor prognosis in a variety of tumors. Therefore, a better understanding of the role of TAMs in the TME may provide new insight into tumor therapy. In the present review, the origin and classification of TAMs in the TME were outlined and their polarization and dual effects on tumor cells, as well as emerging strategies for cancer therapies targeting TAMs, were discussed.

Contents

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1. Introduction

Malignant tumor is a major health problem worldwide and a leading cause of death, ranking second only to cardiovascular disease. Immunotherapy based on the tumor microenvironment (TME) has become a promising cancer treatment strategy (1). The TME is a complex system composed mainly of tumor cells, mesenchymal cells, innate immune cells [macrophages, monocytes, natural killer (NK) cells, dendritic cells and myeloid derived suppressor cells] and adaptive immune cells (T cells and B cells). Macrophages, also known as tumor-associated macrophages (TAMs), among innate immune cells [macrophages, monocytes, natural killer (NK) cells, dendritic cells and myeloid derived suppressor cells], which are found in almost all tissues and organs, are the first line of defense against exogenous and endogenous injury- or pathogen-associated molecular patterns (2). Macrophages that infiltrate tumor tissue or congregate in the microenvironment of solid tumors are defined as tumor-associated macrophages (TAMs). As an important part of the TME, macrophages have a complex role in tumorigenesis and tumor progression. They can not only inhibit tumor growth by releasing pro-inflammatory cytokines and exerting cytotoxic activities, but also promote tumor progression by affecting the occurrence and growth of tumor cells, participating in tumor angiogenesis and metastasis, and shaping an immunosuppressive microenvironment (3). In the present review, the heterogeneity of the origin of TAMs, the factors affecting the polarization of TAMs and the complex role of TAMs in tumor progression were summarized, and therapeutic approaches targeting TAMs, such as consuming TAMs or re-educating TAMs were discussed in order to provide a reference for colleagues to gain insight for tumor immunotherapy.

2. Origin and classification of TAMs

Origin. It is generally thought that TAMs are mainly derived from monocytes produced by hematopoietic stem cells in bone marrow (4). However, recent evidence suggested that certain TAMs (such as alveolar macrophages, brain macrophages and liver macrophages) originate from pre-natal embryonic precursors (yolk sac or fetal liver) (5). These cells were recruited into the TME under the action of chemokines [such as C-C motif

ligand 2 (CCL2), CCL3, CCL4 and CXCL12], colony-stimulating factor 1 (CSF-1), interleukin-6 (IL-6), IL-1β and vascular endothelial growth factor (VEGF) produced by tumor cells or stromal cells to become TAMs (6,7). As an important part of the TME, these TAMs can affect tumorigenesis and development, tumor angiogenesis and immune regulation through interactions with other cell populations in the TME. Furthermore, TAMs are also associated with poor prognosis of various tumors, such as breast cancer (8), bladder cancer (9), head and neck neoplasm (10), glioma (11), melanoma (12) and prostate cancer (13). However, more recently, it has been found that high macrophage infiltration is associated with better prognosis in colorectal and gastric cancers (14). This opposite effect may be related to the plasticity of macrophages and the resulting heterogeneity in phenotype and function of various cancers.

Classification. Macrophages are highly plastic and their phenotype and function are regulated by the surrounding microenvironment. Macrophages usually exist in two distinct subsets: Classically activated macrophages (M1) and alternately activated macrophages (M2). M1 macrophages, normally recognized and induced by type I T-helper cell (Th1) cytokines [e.g. IFN-γ and tumor necrosis factor (TNF)-α] or bacterial lipopolysaccharide (LPS), secrete pro-inflammatory cytokines such as IL-12 and TNF-α, produce high levels of nitric oxide (NO) and reactive oxygen species (ROS), and have powerful anti-microbial and anti-tumor activities (15). By contrast, M2 macrophages are activated by Th2 cytokines (IL-4 and IL-13), secrete anti-inflammatory cytokines such as IL-10, IL-13 and IL-4, and express abundant arginase-1 (Arg-1), mannose receptor (CD206) and scavenger receptor (CD163), which have the functions of removing debris, promoting angiogenesis, tissue reconstruction, damage repair, as well as promoting tumorigenesis and development (16). However, depending on the different activation stimuli, M2 macrophages can be further divided into four distinct subgroups, including M2a, M2b, M2c and M2d. The M2a subgroup is induced by IL-4 and IL-13 to produce high levels of CD206, decoy receptor IL-1 receptor II and IL-1 receptor antagonist (17,18). The M2b subgroup can be induced by immune complexes combined with IL-1β or LPS, which produces anti-inflammatory and pro-inflammatory cytokines IL-10, IL-1β, IL-6 and TNF-α (17,18). The M2c subgroup is generally induced by glucocorticoids and IL-10, releases large amounts of IL-10 and TGF-β and exhibits strong anti-inflammatory activity against apoptotic cells (17,19). Finally, the M2d subgroup, induced by Toll-like receptor (TLR) agonists via adenosine receptors, inhibits the production of pro-inflammatory cytokines and induces the secretion of anti-inflammatory cytokines and VEGFs (20-22). Overall, M2a and M2b macrophages have an immunomodulatory role and promote helper T-cell responses, while M2c macrophages are associated with immune response suppression and tissue remodeling. M2d macrophages are involved in angiogenesis and tumor progression (17,23) (Fig. 1).

3. Polarization of macrophages

The factors influencing TAM polarization are diverse and regulated by a variety of signals in tumor cells and stromal cells in the TME, mainly including the following factors, immunogenic signals, hypoxia (tumor-derived metabolic signals) and extracellular matrix (ECM) components.

Immunogenic signals. Immunogenic signaling mainly refers to the cytokines, chemokines and growth factors released by tumor cells, stromal cells and other infiltrating cells in the microenvironment, which are key determinants of TAM polarization. Among these factors, CCL2 and CSF-1 are the most well-studied stimulators. Studies have shown that tumor-derived chemokine CCL2 binds to C-C chemokine receptor 2 (CCR2) expressed on the surface of macrophages to polarize macrophages towards the pro-tumor phenotype. Blocking the CCL2-CCR2 interaction by gene ablation or antibodies was observed to significantly inhibit tumor metastasis, prolong the survival of tumor-bearing mice and reduce the expression of pro-tumor cytokines (24-26). Later, it was proved that, in addition to the influence of CCL2 derived from tumor cells on the polarization of macrophages, the chemotactic signal of CCL2 expressed by tumor-associated fibroblasts could also recruit macrophages to the tumor site and drive the polarization of macrophages towards the pro-tumor phenotype to increase the aggressiveness of cancer cells and the occurrence of breast tumors (27). CSF-1, another important factor affecting the polarization of macrophages, is generally widely overexpressed at the invasive margins of various tumors and is associated with tumor progression (15). A study on follicular lymphoma found that CSF-1 derived from tumor cells promoted the polarization of macrophages toward pro-tumor M2-like phenotypes and the use of CSF-1R inhibitor (PLX3397) resulted in the repolarization of macrophages toward the M1-type, which showed synergistic anti-tumor effects when combined with anti-CD20 rituximab (28). In addition to the above factors, there are other factors involved in the induction of macrophage polarization, such as VEGF-A, epidermal growth factor (EGF) and prostaglandin E2 (29-31).

Hypoxia. Hypoxia, as one of the key drivers of macrophage recruitment and polarization in the TME, is caused by tumor cells with vigorous metabolism and rapid growth but poor vascular organization, which is a common feature of most solid tumors (32). Hypoxia can regulate the phenotype of TAMs through various factors, particularly through lactic acid produced by glycolysis affecting macrophage polarization (33). For instance, a study on gastric cancer showed that lactic acid produced by glycolysis can induce changes in the macrophage phenotype through monocarboxylate channel transporter-hypoxia-inducible factor 1α signaling, polarizing macrophages towards an M2-like state (34). In addition, the hypoxic TME can stimulate the secretion of tumor-derived exosomes, regulate the macrophage phenotype and promote tumor development. For instance, exosomes of hypoxic tumor cells are enriched with immunomodulatory proteins and chemokines, including CSF-1, CCL2, ferritin heavy chain, ferritin light chain and TGF-β, which influence macrophage recruitment and promote macrophage polarization to the M2 phenotype (35). Therefore, the hypoxic microenvironment can shape a specific macrophage phenotype, which promotes immune escape and metastasis of tumor cells.

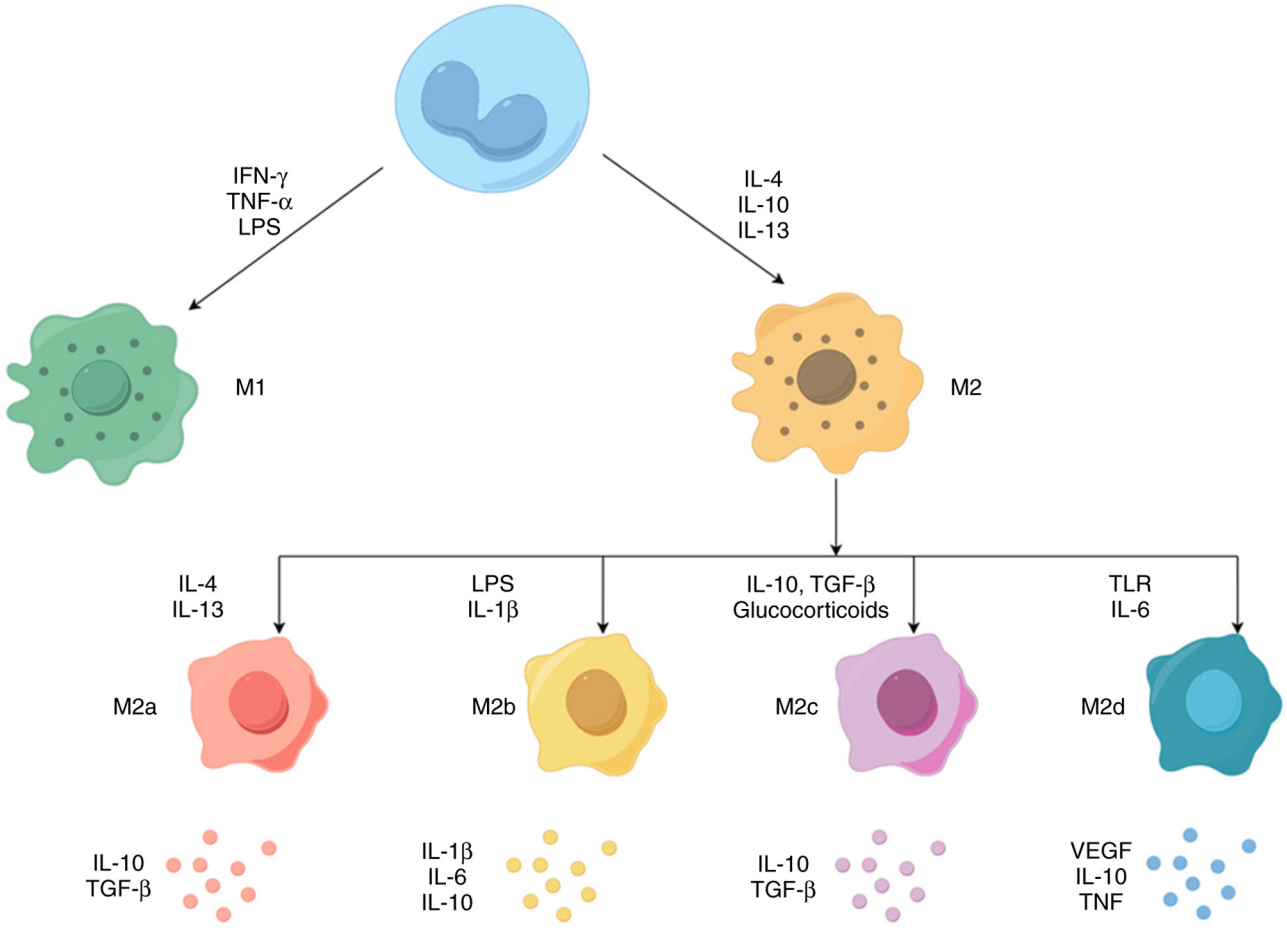

Figure 1. Polarization of macrophages in the tumor microenvironment. LPS, lipopolysaccharide.

ECM components. Last but not least, the ECM component of the TME also has a regulatory effect on macrophage polarization. The ECM is a highly dynamic and complex macromolecular network consisting of a variety of fibrins, proteoglycans and stromal cell-associated proteins (36). The ECM molecule, elastin microfibril interfacer 2 (EMILIN-2), which belongs to the EDEN protein family, exerts a tumor-suppressive effect by affecting the polarization state of macrophages in a variety of tumors (37,38). In a study on colorectal cancer, EMILIN-2 was found to promote M1 polarization through activation of the TLR-4/myeloid differentiation factor-88/NF-κB signaling pathway. EMILIN-2 deficiency is associated with increased M2 macrophage infiltration (39). This suggests that certain components of the ECM are key regulators of the tumor-associated inflammatory environment and may represent promising prognostic biomarkers for tumor patients. In summary, the polarization of TAMs is regulated by complex biological networks, which is closely related to cancer development. Understanding the mechanisms of macrophage polarization may enable researchers to manipulate the macrophage polarization status to stimulate their anti-tumor potential for therapeutic purposes.

4. Role of TAMs in tumorigenesis and tumor progression

Inhibition of tumor development. TAM is a key player in the interaction between cancer cells and their microenvironment and has a dual potential in tumorigenesis and development. As a tumor suppressor, M1-type macrophages have high cytotoxicity and immunostimulatory effects against tumor cells, and can kill tumor cells through two different mechanisms. One is that M1-type macrophages directly mediate the cytotoxic effect of killing tumor cells, i.e., macrophages directly target infected cells or tumor cells by releasing lysosomal enzymes or cytotoxic molecules (such as ROS and NO), which is a slow process (40). Another way to kill tumors is antibody-dependent cell-mediated cytotoxicity, which usually requires the involvement of anti-tumor antibodies to kill tumor cells in a short period of time (41). Therefore, M1-type macrophages are considered anti-tumor or 'good' macrophages (Fig. 2).

Promotion of tumor development. In contrast, in most formed tumors, macrophages contribute to cancer initiation, progression and metastasis through a variety of mechanisms, including promoting cancer cell survival and proliferation, angiogenesis and suppression of immune responses (Fig. 2).

Effects on tumorigenesis and proliferation. TAMs not only provide structural support within the tumor stroma but also promote tumorigenesis and tumor cell growth by producing growth factors, cytokines and chemokines such as EGF, platelet-derived growth factor, TGF-β, hepatocyte growth factor, basic fibroblast growth factor, IL-10 and other cytokines, e.g. CXCL, CCL and VEGF (42). For example, M2-type macrophages have a promoting role in the proliferation and

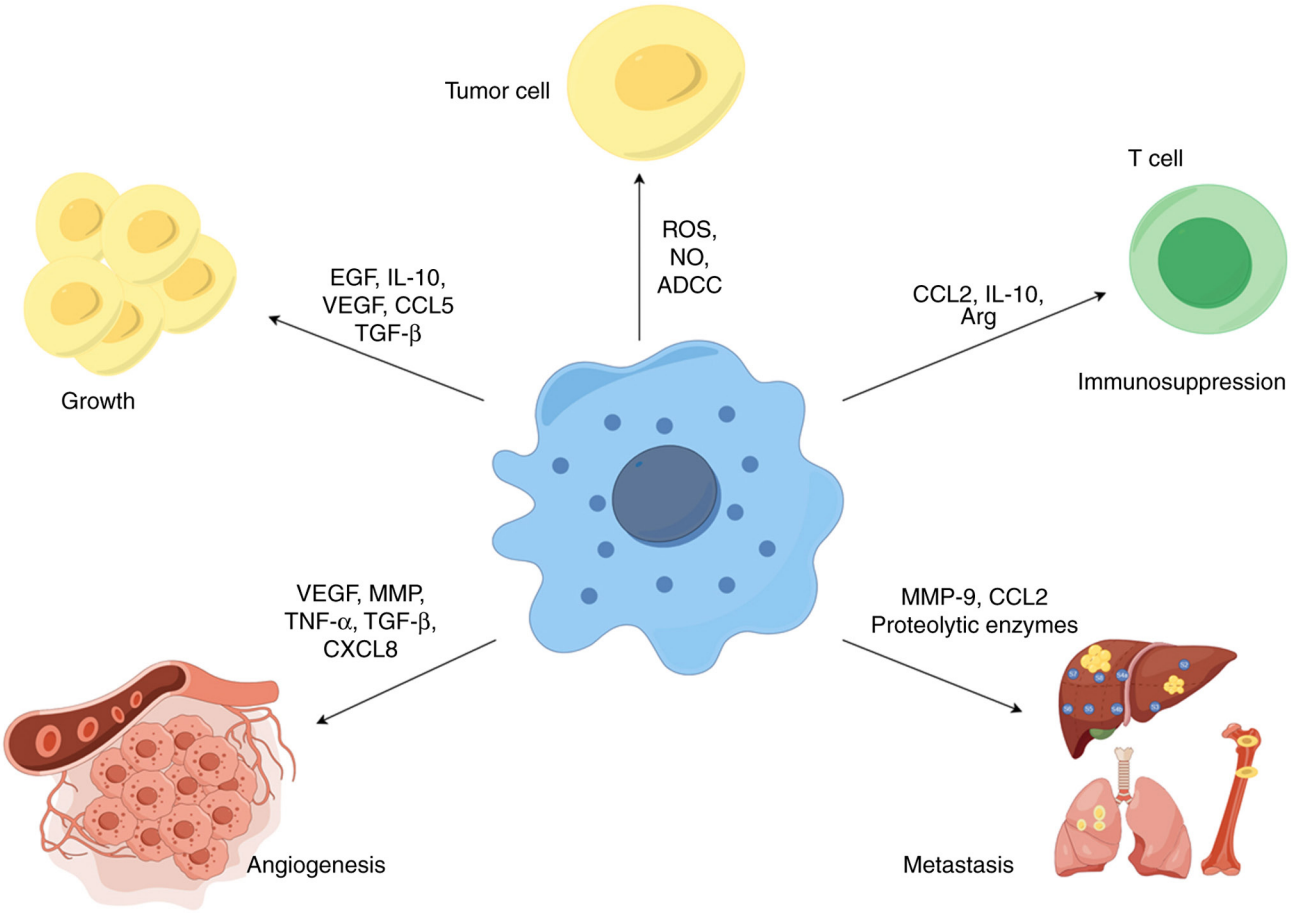

Figure 2. Anti- and pro-tumor functions of macrophages in the tumor microenvironment. On the one hand, tumor-associated macrophages inhibit tumor development by releasing ROS and NO and activating ADCC pathways; on the other hand, tumor-associated macrophages promote tumor development by stimulating tumor growth, angiogenesis and metastasis and shaping an immunosuppressive microenvironment. ADCC, antibody-dependent cell-mediated cytotoxicity; Arg, arginase; CXCL8, C-X-C motif chemokine ligand 8; CCL2, C-C motif ligand 2; NO, nitric oxide; ROS, reactive oxygen species.

invasion of oral squamous cell carcinoma through the production of EGF and the number of $CD206^+$ TAMs is positively correlated with a poorer clinical prognosis in oral squamous cell carcinoma (43). Furthermore, in a study on clear cell renal cell carcinoma (ccRCC), it was found that TAM-derived chemokine CCL5 can promote tumor cell proliferation and the formation of an immunosuppressive TME, which is closely related to the poor prognosis for patients with ccRCC (44). In addition to supporting tumor cell growth, TAM has also been found to have a role in supporting the growth of cancer stem cells (CSCs). In a recent study on breast cancer, TAM-derived IL-6 was found to regulate the enrichment of CSCs in breast cancer through the STAT-3 pathway and lead to tumor growth (45) (Fig. 2).

Effects on tumor angiogenesis. Angiogenesis is essential for tumor growth and metastasis, which is considered a 'hallmark' of cancer. An increasing amount of evidence indicates that TAMs are closely related to angiogenesis in tumors (46). On the one hand, TAMs participate in angiogenesis by secreting pro-angiogenic factors, including VEGF-A, matrix metalloproteinase (MMP), EGF, TGF-β, TNF-α, CCL2, CXCL8 and CXCL12 (47). For example, in a study on bladder cancer, TAM-derived CXCL8 was found to be highly associated with tumor migration, invasion and angiogenesis (48). In addition, the release of thymine phosphorylase (TP) and urine-stimulated plasminogen activator by TAMs can stimulate the migration of endothelial cells, increase ECM degradation and indirectly promote tumor angiogenesis (49). Studies have found that macrophage-derived TP is significantly associated with tumor angiogenesis and poor prognosis in gastric cancer (50). In addition, TAMs also promote tumor angiogenesis by secreting inflammatory mediators such as IL-1 and IL-6. A study on breast cancer found that TAM-derived IL-6 affected breast cancer cell migration and angiogenesis and induced CSC populations, leading to tumor growth (51,52). In summary, these studies suggest that TAMs promote tumor vascularization in different ways and are closely related to tumor progression (Fig. 2).

Effects on tumor metastasis. Macrophages have an important role in every step of the metastasis process, including preparation for pre-metastatic niche formation, intravasation, survival of circulating tumor cells, extravasation and invasion. In terms of forming a pre-metastatic niche, macrophages are recruited to the pre-metastatic site in response to various factors secreted by tumor cells, which provide a roadmap for the homing of circulating tumor cells to the pre-metastatic niche through enhanced expression of chemokines and secretion of molecules such as MMPs and integrins (53-55). In terms of intravasation, macrophages can decompose the surrounding matrix by secreting various proteolytic enzymes

to promote the intravasation of tumor cells. For instance, Gocheva *et al* (56) found that IL-4 induces cathepsin activity in TAMs, promoting tumor growth and invasion. In terms of circulating tumor cell survival, macrophages secrete chemokines or cytokines to promote the successful survival of numerous tumor cells at metastatic sites. A study on breast cancer found that macrophages bind to vascular cell adhesion molecule-1 on the surface of tumor cells via α4 integrin, triggering the PI3K/Akt survival signaling pathway in cancer cells and protecting tumor cells from pro-apoptotic cytokines (57). In terms of extravasation, when tumor cells settle in the capillaries of the target organ, they attempt to attach and extrude through the vessel wall with the assistance of macrophages, and the extravasation rate decreases significantly after the loss of macrophages, indicating that macrophages have an important role in promoting the extravasation of circulating tumor cells (58,59). In terms of invasion, TAM contributes to tumor invasion and metastasis mainly through epithelial-mesenchymal transformation (EMT). A recent study found that CCL2, secreted by TAMs, promotes EMT in triple-negative breast cancer (TNBC) cells through activating the AKT/β-catenin signaling pathway, which may provide a new strategy for the diagnosis and treatment of TNBC (60) (Fig. 2).

Effects on tumor immunosuppressive microenvironment. In addition to their tumor-killing role, TAMs can also mediate immunosuppression, reshape the tumor immune microenvironment and promote tumor development. On the one hand, TAMs can express ligands of inhibitory receptors [such as programmed cell death protein-1 (PD-1) ligand 1, CD80/CD86 and death receptor ligands Fas ligand or tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL)], which bind to the immune cell surface receptors PD-1, cytotoxic T-lymphocyte antigen-4, FaS and TRAIL-RI/-RII, thereby inhibiting the anti-tumor effects of immune cells (such as T cells and NK cells) (61,62). On the other hand, TAMs can also form an immunosuppressive microenvironment by producing chemokines, cytokines and enzymes. For instance, a study in ovarian cancer found that the TAM-secreted chemokine CCL22 recruited CCR4 + T-regulatory cells (Tregs) to promote an immunosuppressive microenvironment (63). It has also been observed in a mouse model of colorectal cancer that CCL20, a TAM-derived chemokine, recruited CCR6(+) Treg cells to the tumor mass, creating an immunosuppressive microenvironment that promoted tumor development (64). In addition, Xu *et al* (9) found that TAM-derived cytokine IL-10 was associated with the depletion of CD8+ T cells and dysfunction of NK cells in the tumor immune microenvironment, which led to poor prognosis in patients with bladder cancer. Recently, a study on pancreatic cancer found that TAM-derived Arg-1 drives immunosuppression by depleting arginine and inhibiting T-cell activation (65,66). In conclusion, these findings support the immunomodulatory role of TAM in promoting tumor progression by shaping the immunosuppressive microenvironment (Fig. 2).

5. Therapeutics targeting macrophages

TAMs are abundant in the TME of most cancer types and are commonly associated with poor clinical prognosis for cancer patients. TAMs are becoming a key target for immunotherapy and the different approaches targeting TAMs that have been explored may be broadly divided into three main categories: i) Eliminating TAMs already present in the TME; ii) inhibiting monocyte recruitment; iii) reprogramming of TAMs (67,68). These strategies have been investigated in preclinical models and some of them have been translated into clinical studies as adjuncts to immunotherapy (69). In the present review, some of the current approaches to targeting macrophages and clinical trials were outlined and the potential advantages and disadvantages of these approaches were discussed (Table I).

Consumption of macrophages already present in the TME. Selective elimination of TAMs has been used in cancer treatment. An attractive strategy for depleting TAMs in the TME is to trigger its apoptosis and restore local immune surveillance in the TME, which can effectively inhibit tumor growth. Several compounds have been shown to induce apoptosis in macrophages, mainly including bisphosphonates and trabectedin.

Bisphosphonates are a class of anti-bone resorption drugs, which can be divided into two categories according to their structural characteristics: Non-nitrogenous bisphosphonates and nitrogenous bisphosphonates (70). Bisphosphonates exhibit direct or indirect antitumor properties. They can inhibit cancer cell proliferation, induce tumor cell apoptosis, block angiogenesis and interfere with immune surveillance. At the same time, bisphosphonates can also inhibit the proliferation, migration and invasion of macrophages, leading to the apoptosis of macrophages (71,72). For instance, in earlier studies, dichloromethylenediphosphonates (also known as clodronates) from the non-nitrogenous bisphosphonate family were often used to consume macrophages in the liver and spleen when loaded with liposomes (73); Zoledronate, the third generation of nitrogenous bisphosphonates, is selectively cytotoxic to TAMs expressing MMP9 and inhibits the progression of cervical cancer (74). In addition, a study of non-small cell lung cancer found that calcium zoledronate nanoparticles modified with biotin and mannose preferentially targeted biotin-expressing tumor cells and mannose-expressing TAMs, ultimately suppressing tumor growth and survival (75). A study about thyroid cancer found that zoledronic acid inhibits thyroid cancer stemness and metastasis by repressing M2-like TAM polarization and the Wnt/β-catenin pathway, reducing the tumor burden (76). Furthermore, a prospective phase II clinical trial found that zoledronic acid combined with radiotherapy reduced bone pain and improved quality of life in patients with bone metastases from gastrointestinal tumors (77). Trabectedin is a tetrahydroisoquinoline alkaloid that directly kills tumor cells by interfering with multiple transcription factors, DNA-binding proteins and DNA repair pathways (78). Furthermore, it also selectively consumes monocytes and macrophages in the TME by activating caspase 8 through a TNF-related apoptosis-inducing ligand-dependent mechanism (79). In a study on fibrosarcoma, Trabectedin selectively reduced macrophages in the TME and enhanced the anti-tumor response to anti-PD-1 therapy (80). In several clinical trials, Trabectedin was found to show good safety and efficacy in the treatment of soft tissue sarcoma and

Table I. Clinical trials of agents targeting TAMs for cancer treatment.

Mechanism	TAM-targeted agent	Compound	Clinical trial phase	Clinical trial numbers
Elimination	Zoledronate acid	Zoledronate acid	I/II	NCT00588913
				NCT00582790
				NCT00278434
				NCT03664687
	Trabectedin	Trabectedin	II	NCT02194231
				NCT01339754
Recruitment inhibition	CCR2 antagonist	PF04136309	Ib	NCT02732938
				NCT01413022
	CCL2 antibody	CNTO888	Ib	NCT01204996
				NCT00537368
				NCT00992186
		CCX872	Ib	NCT02345408
	CCR2/5 inhibitor	BMS-813160	II	NCT04123379
				NCT03496662
	CSF-1R antibody	RG7155	I/III	NCT01494688
				NCT05417789
		PLX3397	II/III	NCT01217229
				NCT02371369
		Vimseltinib	III	NCT05059262
Reprogramming	CD24	CD24Fc	I/II	NCT04060407
				NCT04552704
	CD47 antibody	Hu5F9-G4	I/II	NCT02216409
				NCT02953509
				NCT03558139
				NCT02953782
		CC90002	I	NCT02641002
				NCT02367196
	SIRP antibody	TTI-621	I/II	NCT02663518
				NCT05507541
		CC-95251	I	NCT03783403
				NCT05168202
	PI3Kγ inhibitor	IPI-549	Ib/II	NCT02637531
				NCT03961698
	TLR agonist	Imiquimod	II	NCT00899574
		Motolimod	II	NCT01836029
		NKTR-262	I/II	NCT03435640
	CD40 agonist	APX005M	I/II	NCT03214250
		RO7009789	I	NCT02665416
		SEA-CD40	I	NCT02376699
		CP-870893	I	NCT01103635

CCL2, C-C motif ligand 2; CCR2, C-C motif receptor 2; TLR, Toll-like receptor; SIRP, signal-regulatory protein; CSF, colony-stimulating factor.

ovarian cancer (81,82). However, disappointing results were obtained in malignant pleural mesothelioma and pancreatic cancer tumors (83,84).

Macrophages have an important regulatory role in maintaining host defenses and tissue homeostasis, and a major problem with the depletion of macrophages is the inability to avoid the side effects that arise from non-selective macrophage depletion. Therefore, the key to minimizing potential toxic side effects is to develop drugs that preferentially target M2-like macrophages. A recent study designed M2-targeting nanoliposomes, which effectively depleted M2-type TAMs, remodeled the TME and effectively inhibited tumor growth (85). Consequently, targeted elimination of M2-like TAMs is a promising approach for cancer immunotherapy.

Inhibition of monocyte recruitment. As mentioned earlier, most TAMs originate from the production of bone marrow monocytes. TAMs are recruited to tumor sites in response to tumor-derived chemokines. Therefore, the application of monoclonal antibodies or small molecule inhibitors to interfere with chemokine signaling may be an effective way to prevent the accumulation of TAMs in the TME.

The expansion of TAMs in tumors is usually mediated by monocyte recruitment on the CCL2-CCR2 axis. Monocytes expressing the CCR-2 receptor are recruited to the tumor site by CCL-2 released by tumor cells, macrophages and stromal cells within the TME, where they further mature into TAMs (86). Therefore, reducing macrophage recruitment and infiltration into the TME by blocking the CCL2/CCR2 axis may be a promising therapeutic anti-tumor strategy. A study on esophageal cancer found that blocking the CCL2-CCR2 axis significantly reduced the tumor incidence by preventing TAM recruitment and also enhanced the anti-tumor effects of CD8+ T cells in the TME (87). Several CCL-2 antibodies are undergoing clinical trials. The two main drugs tested so far are the anti-CCL2 monoclonal antibody Carlumab (CNTO 888) and a targeted small molecule inhibitor (PF04136309), which have shown a certain benefit in tumor control (88,89).

In addition, BMS-813160, a CCR2/5 inhibitor, has been selected as a clinical candidate for its ability to inhibit the migration of inflammatory monocytes and macrophages. Clinical trials of BMS-813160 are ongoing in non-small cell carcinoma, liver cancer and pancreatic cancer (90) (Table I).

CSF-1 controls the proliferation, differentiation, recruitment, survival and function of mononuclear phagocytes (e.g., macrophages, monocytes) (91). Therefore, targeting the CSF1/CSF1R signaling pathway is considered to be another important and effective strategy for the treatment of malignant tumors. Ries *et al* (92) found that the use of CSF1R monoclonal antibody, RG7155, in patients with advanced diffuse giant cell tumors significantly reduced CSF1R+CD163+ macrophages in the TME. In a study of advanced solid tumors, RG7155 specifically depleted immunosuppressed M2-like macrophages and was used in combination with paclitaxel to enhance anti-tumor responses (93). In addition, in a study on endometrial cancer, it was found to promote TAM recruitment in the TME and tumor cell proliferation, which was significantly diminished by a CSF1R blocker (PLX3397) (94). A phase I clinical trial found PLX3397 to have favorable safety and tolerability in Asian patients with advanced solid tumors (95). In a rare tumor called tendonsynovial giant cell tumor, vimseltinib, a small molecule inhibitor targeting CSF1R, was found to persistently inhibit CSF1R activity *in vitro* and *in vivo*, depleting macrophages and other CSF1R-dependent cells, and inhibiting tumor growth and bone degradation in a mouse model of cancer (96).

Although CSF1/CSF1R and CCL2/CCR2 blockade are the most widely studied axes of TAM depletion, other cytokines have also been shown to have a role in this process. In a mouse model of malignant lobular tumors, monocytes are recruited into tumors through the interaction between CCL5 and CCR5. Blockade of the CCL5-CCR5 axis by CCR5 inhibitors resulted in markedly attenuated monocyte recruitment into tumors and inhibition of tumor growth (97). Therefore, actively exploring the factors that interfere with macrophage recruitment will provide a new idea for targeted macrophage therapy.

TAM reprogramming. As mentioned above, it is widely acknowledged that M2 and M1 macrophages have opposite roles in tumor growth and metastasis. Therefore, therapeutic strategies to re-educate the tumor-promoting M2 phenotype into the tumor-killing M1 phenotype have been proposed. Reprogramming macrophages involves the following two aspects: Restoration of phagocytosis and promotion of the polarization phenotype.

Role of cellular phenotypes in restoring phagocytosis

i) CD24. CD24 is a highly glycosylated glycosylphosphatidylinositol-anchored surface protein that acts as a 'don't eat me' signal. It regulates phagocytosis in macrophages through interaction with sialic-binding IG-10 (Siglec-10), an inhibitory receptor on TAMs (98). CD24 is commonly overexpressed in cancers and its overexpression is associated with poor prognosis in various cancers (99). It has been found in ovarian cancer and TNBC that CD24 expressed by tumor cells can interact with Siglec-10 on TAMs, blocking phagocytosis of macrophages. Blockade of CD24 or Siglec-10 enhanced the phagocytosis of macrophages on tumors and inhibited tumor growth (100). In addition, a recent study in mantle cell lymphoma and follicular lymphoma found that high expression of CD24 was associated with poor prognosis of patients. Treatment with CD24 monoclonal antibody significantly enhanced phagocytosis by macrophages and inhibited tumor progression (101). These studies demonstrate the therapeutic potential of CD24 blockade as a cancer immunotherapy.

ii) CD47. CD47, a transmembrane glycoprotein widely expressed on cancer cells, can block macrophage phagocytosis by binding to the signal-regulatory protein (SIRP)α on the surface of macrophages, enabling cancer cells to escape immune surveillance (102). Based on this characteristic, targeting the CD47-SIRPα axis is an effective modality. In a study of thyroid cancer, it was found that the degree of infiltration of TAMs in xenografted mice treated with anti-CD47 antibody was significantly increased and phagocytosis of tumor cells by macrophages was enhanced, which inhibited tumor growth (103). Furthermore, in small cell lung cancer (SCLC), macrophages showed increased phagocytosis and enhanced anti-tumor effects on GFP-expressing SCLC cells in mice treated with radiotherapy combined with CD47 block, compared with CD47 block alone, a finding that is particularly important for cancer patients suffering from metastatic disease (104). In addition to increasing phagocytosis of tumor cells, anti-CD47 therapy has also been shown to modulate TAM phenotypic changes. For instance, a glioblastoma study found that anti-CD47 treatment not only enhanced macrophage phagocytosis of tumor cells but also shifted the phenotype of macrophages towards the M1 subtype (105). Hence, preclinical studies of CD47-SIRPα blockade suggest its potential for clinical efficacy. To date, clinical studies have indicated that CD47 inhibitors (Hu5F9-G4) in combination with rituximab showed good anti-tumor activity in patients with non-Hodgkin lymphoma, while CD47 inhibitors (CC90002) have provided disappointing results in patients with acute myeloid leukemia (106,107). Clinical trials targeting SIRP molecules are still ongoing (Table I).

Use of polarization changes for therapy

i) PI3Kγ. As a member of the class 1B family, PI3Kγ is usually associated with G-protein-coupled receptor signaling and is abundantly expressed on a variety of immune cells, including macrophages and neutrophils, and the PI3Kγ pathway is related to the phenotypic transformation of TAMs as well as immunosuppressive states (108,109). For instance, in a mouse model carrying breast cancer, blocking PI3Kγ was also found to reduce the number of M2-like macrophages and enhance the role of cytotoxic T lymphocytes (CTLs), which significantly prevented tumor progression and prolonged survival (110). In addition, Carnevalli *et al* (111) found that AZD3458, a PI3Kγ inhibitor, did not alter tumor macrophage polarization, but instead promoted antigen-presenting macrophage and cytotoxic macrophage activation, which activated CD8 T cell-mediated antitumor activity of associated immune checkpoint inhibitors. A phase I clinical study found that a highly selective PI3Kγ inhibitor (IPI-549) demonstrated significant anti-tumor activity in patients with advanced solid disease (112). Therefore, the above studies have demonstrated the important role of PI3Kγ in TAMs reprogramming and recruitment of immune cells that inhibit tumor growth, suggesting that PI3Kγ may be a promising target for tumor therapy.

ii) TLRs. TLRs are innate immune pattern recognition receptors that have an important role in the activation of innate immune responses (113). In the reprogramming of macrophages, the phenotype of macrophages can change and they may be polarized in a pro-inflammatory direction. Numerous therapies aim at targeting TLR to repolarize macrophages from an M2-like activated state to an M1-like activated state and to enhance the immune response to cancer cells. For instance, local *in situ* inoculation of melanoma and neck tumor models with the tumor antigen (protein and peptide) adjuvant nanoemulsion loaded with TLR7/8 agonists induced recruitment and activation of innate immune cells, infiltration of lymphocytes and polarization of tumor-associated M2 macrophages, resulting in inhibition of tumor growth and prolonged tumor survival (114). Furthermore, oxaliplatin combined with TLR agonist R848 was found to reverse the polarization of macrophages and enhance the anti-tumor effects of oxaliplatin in colorectal cancer resistant to oxaliplatin (115). In addition to TLR7 and TLR8, it was found that TLR3 and TLR4 agonists also have the effect of altering the macrophage polarization status (116,117). These findings suggest the clinical relevance of TLR signaling and its potential application for cancer treatment by targeting TAMs. To date, several targeting TLRs have been tested in clinical trials with promising results (118-120).

iii) CD40. CD40 is a member of the TNF receptor superfamily and is highly expressed on antigen-presenting cells, such as macrophages. The ligand of CD40 is CD40L, which is mainly expressed by activated T cells, B cells and platelets (121). The CD40-CD40L interaction promotes the polarization of macrophages towards pro-inflammatory macrophages. For instance, in a mouse model of melanoma, it was found that myeloid-derived suppressor cells induce macrophage reprogramming by inhibiting the expression of CD40 on the surface of macrophages, thereby promoting melanoma progression (122). In addition, a study on pancreatic cancer found that CD40 agonists could drive the transformation of the macrophage phenotype towards M1, remodeling the pancreatic cancer microenvironment and inhibiting the development of pancreatic cancer (123). More recently, Frankish *et al* (124) found that HERA-CD40L, a novel molecule targeting CD40-mediated signal transduction, activates the signaling transduction mechanisms in dendritic cells, leading to an increase in intratumoral T cells and manipulating TME phenotypic changes to repolarize M2 macrophages to M1, thereby enhancing tumor control. In addition, a clinical trial in pancreatic ductal adenocarcinoma showed that selicrelumab, an agonist CD40 antibody, induced changes in the TME in patients with resectable pancreatic cancer, a reduction in M2-like TAMs and a significant reduction in tumor burden (125). Based on the above observations, reprogramming TAMs to anti-tumor phenotypes using CD40, rather than targeting and ablating TAMs, may be the preferred therapeutic paradigm for cancer treatment.

6. Conclusion and prospects

TAMs are one of the most important innate immune cell types in TME and have a complex regulatory role in tumor therapy. Therefore, it is crucial to reveal the exact regulatory mechanisms and specific targets of macrophages on tumors in order to optimize the effectiveness of current tumor therapies. In the present review, the heterogeneity of the origin of TAMs, the relevant regulators of recruitment and polarization and the complex roles of TAMs in tumorigenesis, progression and metastasis were discussed. Certain therapeutic approaches targeting TAMs, such as consumption of TAMs or re-education of TAMs were also outlined to provide insight for tumor immunotherapy. Specifically, targeting TAMs is a promising immunotherapy strategy. However, the clinical application of current therapeutic strategies is still very limited. For instance, the efficacy is restricted to certain patients, the anti-tumor spectrum is narrow, the adverse reactions are more frequent and drug resistance may easily occur. These defects limit the clinical application of targeting macrophages in tumor therapy. In addition, numerous questions remain regarding the nature and function of macrophages in the TME; many unknown molecular mechanisms have a crucial role in regulating tumor growth and development, and various potential targets require more research and attention. Therefore, it is necessary to further investigate the dialogue between macrophages and tumor cells. With a greater understanding of macrophage diversity through single-cell sequencing and other techniques, TAM-targeted therapies will be an important complement to cancer immunotherapy.

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Authors' contributions

CL, YL and LM conceived the article. CL wrote the first version of the manuscript with critical input from all authors. XK, HL, HR and XZ performed the literature search. BZ and XN edited the manuscript for important intellectual content. All authors have read and approved the final manuscript. Data authentication is not applicable.

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Competing interests

The authors have no competing interests to declare.

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