Macrophages in melanoma: A double-edged sword and targeted therapy strategies (Review)

QIUJUN ZHOU[1], TINGTING FANG[1], SHENYU WEI[2], SHIQIAN CHAI[1], HUIFENG YANG[1], MAOCAN TAO[3] and YI CAO[3]

[1]Department of First Clinical Medical College, Zhejiang Chinese Medical University; [2]Department of Hepato-Pancreato-Biliary Surgery, The Second Affiliated Hospital, Zhejiang University School of Medicine; [3]The First Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou, Zhejiang 310000, P.R. China

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Abstract. Melanoma, which evolves from melanocytes, is the most malignant skin cancer and is highly fatal, although it only accounts for 4% of all skin cancers. Numerous studies have demonstrated that melanoma has a large tumor mutational burden, which means that melanoma has great potential to achieve immune evasion. Tumor-associated macrophages (TAMs) are an important component of both the immune system and tumor microenvironment. Several studies have demonstrated their double-edged sword effects on melanoma. The present review focuses on the role of TAMs in melanoma development, including regulation of proliferation, invasion, metastasis, angiogenesis and chemical resistance of melanoma. Furthermore, the existing mechanisms of action of the TAM-targeting treatments for melanoma are reviewed. More broadly, the weak points of existing research and the direction of future research are finally identified and described.

Contents

Correspondence to: Dr Maocan Tao or Dr Yi Cao, The First Affiliated Hospital of Zhejiang Chinese Medical University, 98 Wushan Road, Shangcheng, Hangzhou, Zhejiang 310000, P.R. China
E-mail: taomaocan@163.com
E-mail: caoyi1965@163.com

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1. Introduction

Melanoma evolves from melanocytes, which are mainly melanin-producing cells, is the most malignant skin cancer and is highly fatal, although it only accounts for 4% of all skin cancers (1). Globally, it affected 324,600 individuals in 2020, resulting in 57,000 deaths (2). A report from 2017 estimated that melanoma would result in 20,000 new cases annually in mainland China (3). According to annual reports on the status of cancer in the United States published in 2020, the incidence of melanoma is increasing continuously regardless of gender (4,5).

Although current therapies, including immune checkpoint treatment, targeted therapies, radiotherapy and chemotherapy, have resulted in a sustained reduction in the death rates of melanoma (6.1% annually) (4), treatments for melanoma still have room for advancement due to drug resistance (6). Numerous studies have demonstrated that melanoma has a large tumor mutational burden, meaning that melanoma has great potential to achieve immune evasion (7-9). Thus, the understanding of the detailed mechanism underlying immunosuppression in melanoma has become increasingly important. It is now widely accepted that the tumor microenvironment (TME), the complex ecosystem in which tumor cells reside and interact with various types of cells (10), has an important impact on tumor progression and drug resistance (11).

Macrophages are an important component of both the immune system and TME (12), and their infiltration into the tumor is associated with poor prognoses in most solid tumors (13-18). A number of studies have reported their double-edged sword effects on melanoma (19-21). As innate immune cells, macrophages can kill tumor cells via different extracellular mechanisms through phagocytosis, antigen presentation and T cell regulation, resulting in early tumor cell elimination (22,23). Along with tumor progression, the M2-type polarization of macrophages is induced by various signaling factors from the tumor and other stromal cells to promote tumor progression and threaten the life of the patient (22,23). The present review discusses the tumor-suppressive roles, tumor-promoting roles and potential clinical applications of macrophages in melanoma.

2. Overview of macrophages in the TME

Inflammation is an outstanding hallmark of cancer and is important for promoting tumor progression (10). For a number of cancer types, inflammation is an enabling characteristic that precedes malignant transformation with a subsequent shift to immunosuppressive TMEs (24).

Macrophages have different origins. The monocyte-macrophage lineage derives from precursor cells in the bone marrow and is driven by granulocyte-macrophage colony-stimulating factor (GM-CSF) (25). Tissue-resident macrophages with the ability to self-maintain originate from the yolk sac or fetal liver precursors during fetal development and show specialized phenotypes depending on the specific organ (26,27). TAMs are considered to be derived from both circulating monocytes and tissue-resident populations (28,29).

In regard to the phenotypic trait, macrophages can be classified into two categories: Classically activated (M1) and alternatively activated (M2) macrophages (Fig. 1). M1 macrophages can activate the adaptive immune system and are characterized by high expression levels of IL-12 and major histocompatibility complex class II, and low expression levels of IL-10 and arginase (30). M2 macrophages highly express the following: Arginase 1, a member of the arginase family; CD206, an important pattern recognition receptor and endocytic receptor in the innate immune system; IL-10, a well-recognized inflammatory and immunosuppressive factor; C-C motif chemokine ligand (CCL) 17; and CCL22, which can attract immune cells to specific locations (31). In the TME, M1 macrophages have antitumor abilities due to pro-inflammatory responses and the ability to produce pro-inflammatory factors such as IL-6, IL-12, C-X-C motif chemokine ligand 10 and tumor necrosis factor (TNF), whereas M2 macrophages have pro-tumor abilities (32,33). Among these, M2 macrophages are similar to TAMs in their phenotypic trait. Studies have demonstrated that the presence of TAMs is associated with poor survival in various tumor types (13,14,34). However, a number of studies have reported that various subtypes of macrophages exist, some of which spread along the spectrum of macrophage phenotypes and have distinct functions (35,36). This reflects the complexity of the TME. During the process of tumor development and aggravation, macrophages, as compartments of intratumor heterogeneity, also evolve under selective pressure, such as low pH, hypoxia, oxidative stress and nutritional deprivation (37).

Macrophages participate in tumor progression by interacting with both tumor and other stromal cells. Tumor cells and other malignant structures reverse the function of macrophages. It will become an adjunct to the tumor. Macrophages can promote tumor proliferation, angiogenesis, immune evasion, invasion and metastasis (38). Thus, an increasing number of studies have been performed to improve the treatment of patients with tumors by restoring tumor-killing abilities, reshaping the plasticity of TAMs from M2 into M1, or depleting M2 macrophages (39,40).

3. Double-edged sword effect of TAMs in melanoma

As previously mentioned, TAMs can be classified into two categories, M1 and M2, which have the opposite effect on tumor development. In this section, the tumor-promoting and tumor-suppressing effects of M2 and M1, respectively, are reviewed with a focus on their role in regulating the proliferation, invasion, metastasis, angiogenesis and chemical resistance of melanoma (Table I).

Regulating tumor proliferation, invasion and metastasis. M1 polarization of macrophages inhibits the proliferation of melanoma (41). By contrast, an increased number of M2 macrophages promotes melanoma growth (42). Furthermore, a study found that macrophages deficient in integrin β3 induced the polarization of M2 macrophages to promote melanoma growth (43). The results of the survival analysis of patients with melanoma treated with isolated hepatic perfusion also indicated that M1 macrophages, rather than M2 macrophages, were associated with longer overall survival, which is due to the inhibition of melanoma growth by M1 macrophages (44). In terms of invasion and migration of melanoma, Kou *et al* (45) reported that increased expression of Connexin 43, a vital gap junction protein in the TME, induced M1 polarization, thereby inhibiting the invasion and migration of melanoma cells *in vitro*. However, another study (46) demonstrated that M2 macrophages lacking tripartite motif 59 (TRIM59), which belongs to the TRIM family of proteins (47), promoted melanoma migration and invasion in a Transwell assay. Further research has demonstrated that M2 macrophages lacking TRIM59 promote the expression of MMP-9 and mucosal vascular addressin cell adhesion molecule 1, which are related to the invasion and migration of melanoma cells (46). *In vivo* models have been widely used to investigate the metastatic ability of melanoma. Park *et al* (48) established a xenogeneic model by planting melanoma cells overexpressing IL-9 in mice and found that the level of lung metastasis of melanoma was lower than that of the wild-type melanoma cells. M1 macrophages in the lungs and spleen were increased. Through *in vitro* experiments, this study also demonstrated that the IL-9-induced cytotoxicity in M1 macrophages was enhanced. However, to the best of our knowledge, the inhibitory effect of M1 macrophages on melanoma has not been directly confirmed *in vivo*, which is a limitation of current research. Eliminating M1 macrophages in mice or using immunodeficient mice may be helpful to further verify these results.

Furthermore, crosstalk between TAMs and other immune cells is also an important mechanism that affects tumor proliferation, invasion and metastasis (49). Nuclear factor of activated T cells (NFAT1) is a transcription factor that can bind to IL-2 and regulate its expression, thereby promoting T cell activation (50). Notably, NFAT1 has been demonstrated to increase the infiltration of M2-TAMs, thereby serving a critical role in enhancing TAM-mediated promotion of growth and metastasis in malignant melanoma (51). However, the activation of natural killer T cells promotes the polarization of M1-TAMs, inhibiting the growth of melanoma (52). Notably, the information exchange between melanoma cells and macrophages also serves an important role in the progression of melanoma (53). Gerloff *et al* (54) reported that melanoma delivered microRNA (miR)-125b-5p into macrophages through exosomes *in vitro*. Subsequently, miR-125b-5p is combined with lysosomal acid lipase A in macrophages, which in turn contributes to M2 macrophage polarization (54). Since exosomes can carry the

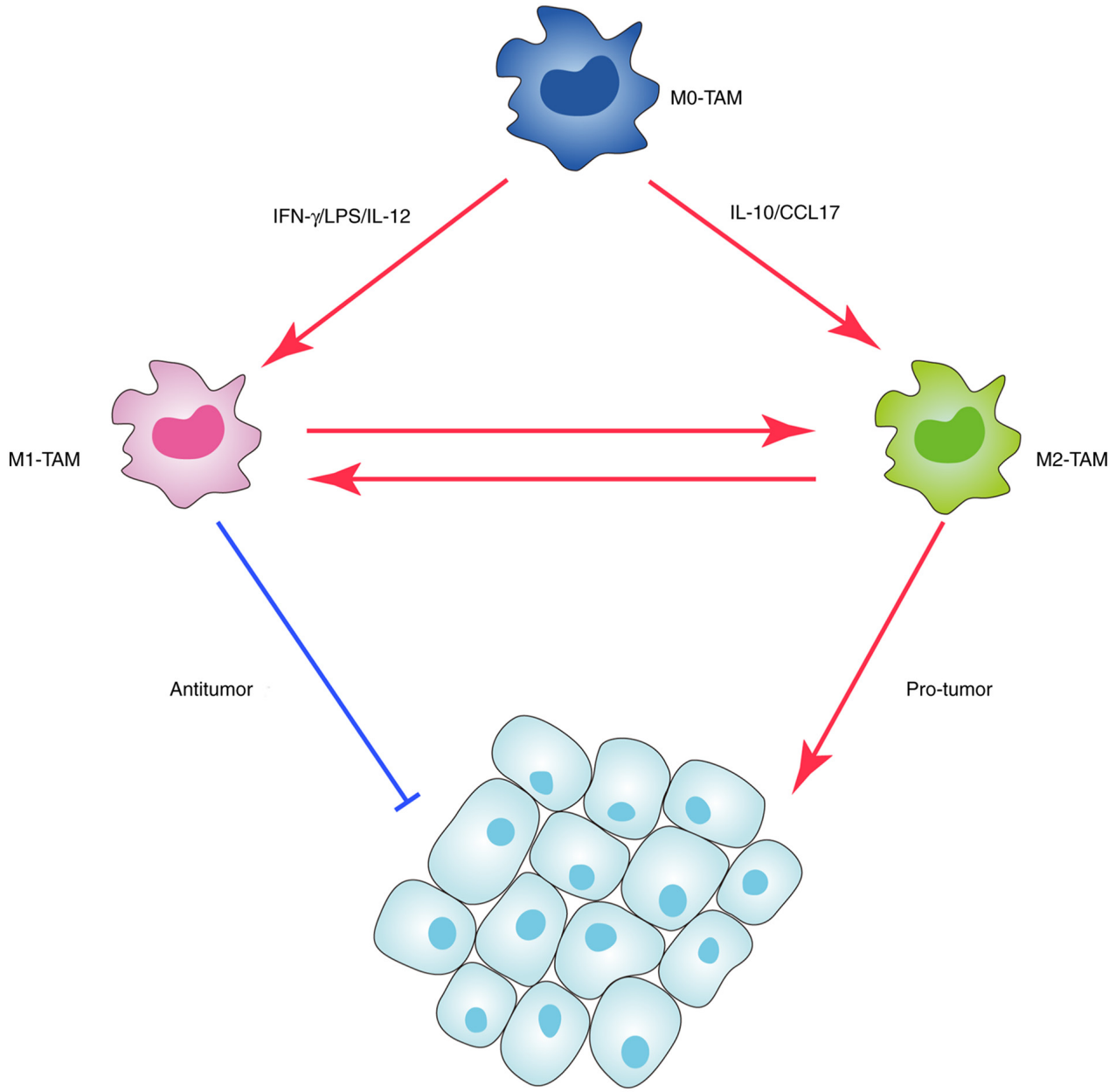

Figure 1. Classification of TAMs and their dual roles in tumors. TAM, tumor-associated macrophage; LPS, lipopolysaccharide; CCL17, C-C motif chemokine ligand 17.

genetic molecules of the source cell, they may also serve an important role in cancer suppression. However, this possibility requires further exploration.

Regulating angiogenesis in melanoma. The present review further explores the role of TAMs in angiogenesis in melanoma. Increased M2 polarization of TAMs has been found to stimulate tumor angiogenesis, leading to tumor progression (55). By contrast, M1-like TAMs trigger immune responses and normalize irregular tumor vascular networks, which sensitize cancer cells to chemotherapy and radiotherapy and further suppress tumor growth (56). This specific mechanism can be attributed to the induction of GM-CSF expression in endothelial cells by melanoma exosomes, thereby enhancing the activity of hypoxia-inducible factor-2α (HIF-2α) in M2-like TAMs. HIF-2α further attenuates VEGF activity by inducing the production of soluble VEGFR-1, promoting improved tissue and vasculature patency, which favors tumor growth (57). However, the results of the studies performed so far are controversial. Jarosz-Biej *et al* (56) analyzed the tissues of 43 patients with melanoma and found that a higher blood vessel density was positively associated with an increased number of M1-like TAMs.

Regulating the resistance to melanoma treatment. Recent research has also indicated that macrophages serve a role in melanoma resistance (58,59). Due to the different phenotypes of macrophages, these can promote resistance in melanoma on one hand and also improve the efficacy of drugs in the treatment of melanoma on the other hand (60). Durable responses in melanoma treatment have been achieved with immunotherapies that target immune checkpoint molecules,

Table I. Double-edged sword effect of TAMs in melanoma.

First author/s, year	TAM Classification	Mechanisms	Effects	(Refs.)
Johansson *et al,* 2020	M1	Increased expression of Cx43 to induce M1 polarization	Inhibiting the invasion and migration	(44)
Kou *et al,* 2017	M2	TRIM59 loss in M2 macrophages	Promoting the invasion and migration	(45)
Tian *et al*, 2019	M1	IL-9-induced cytotoxicity of M1 macrophages	Decreasing metastatic ability	(46)
Shoshan *et al*, 2016	M2	NFAT1 binds to IL-2 and regulates its expression, thereby promoting T cell activation	Increasing metastatic ability	(50)
Liu *et al*, 2018	M1	Activation of NKT cells promotes the polarization of M1-TAMs	Inhibiting the growth of melanoma	(51)
Paul *et al*, 2019	M2	Exosomal miR-125b-5p combines with LIPA in macrophages to induce M2 polarization	Inhibiting the growth of melanoma	(52)
Yamada *et al*, 2016	M1	Unknown	Triggering the immune response and normalizing irregular tumor vascular network	(55)
Jarosz-Biej *et al*, 2018	M2	Melanoma exosomes enhance HIF-2α activity in M2-like TAMs	Promoting vasculature for better reconstruction	(56)
Ribas *et al*, 2016	M2	High IL-34 expression	Inducing melanoma resistance to PD-1 inhibitors	(66)
Han *et al*, 2018	M2	Exosomal PD-L1 induces M2 macrophage polarization	Results in anti-PD-1/PD-L1 therapy resistance	(69)
Liu *et al*, 2021	M1	Blocking the binding of Lgr4 and its ligands R-spondin 1-4 on TAMs to induce the polarization of M1 macrophages	Improving the efficacy of PD-1 immunotherapy	(58)
Heldin *et al*, 2012	M1	Blockade of TGF-βR to induce M1-TAMs	Increasing the efficacy of doxorubicin chemotherapy	(71)

Cx43, connexin 43; HIF-2α, hypoxia-inducible factor 2α; Lgr4, leucine rich repeat containing G protein-coupled receptor 4; LIPA, lysosomal acid lipase; miR, microRNA; NFAT1, nuclear factor of activated T cell transcription factor 1; NKT cells, natural killer T cells; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; TAM, tumor-associated macrophage; TGF-βR, TGF-β receptor; TRIM59; tripartite motif-containing 59.

such as cytotoxic T-lymphocyte antigen 4 (CTLA4) (61-63) and programmed cell death protein 1 (PD-1) (64,65). However, 25% of patients with melanoma who have shown an objective response to PD-1 blockers also develop resistance (66). This finding has prompted scientists to explore the mechanism of melanoma resistance to PD-1 inhibitors (67,68). Melanoma resection specimens, which have been collected from patients with refractory metastatic melanoma who were treated with nivolumab, a PD-1 inhibitor for immunotherapy, exhibit high expression levels of IL-34. Importantly, high expression levels of IL-34 have been found to be positively associated with increased frequencies of M2-polarization TAMs (69). This finding suggests that M2-TAMs may be related to melanoma resistance to PD-1 inhibitors. *In vitro* experiments performed by Liu *et al* (58) further demonstrated that melanoma cell-derived exosomes carrying relatively large amounts of programmed death-ligand 1 (PD-L1) could induce M2 macrophages polarization, eventually resulting in anti-PD-1/PD-L1 therapy resistance. Furthermore, another study has demonstrated that blocking the binding of G protein-coupled receptor 4 on TAM to its ligand R-spondin 1-4 can reduce the polarization of M2 macrophages on the one hand, and promote the polarization of M1 macrophages on the other hand, further improving the efficacy of PD-1 immunotherapy in melanoma treatment (70). Notably, interactions among immune cells may also be involved in melanoma resistance. In particular, myeloid-derived suppressor cells interact with autoimmune macrophages and inhibit the cell surface expression of CD40 and the production of IL-27 (19). Furthermore, low CD40/IL-27 signaling in tumors is associated with high TAM infiltration and immune checkpoint blockade (ICB) therapy resistance in both murine and human melanoma (19). In addition to ICB, macrophages have also been found to serve a role in the resistance of

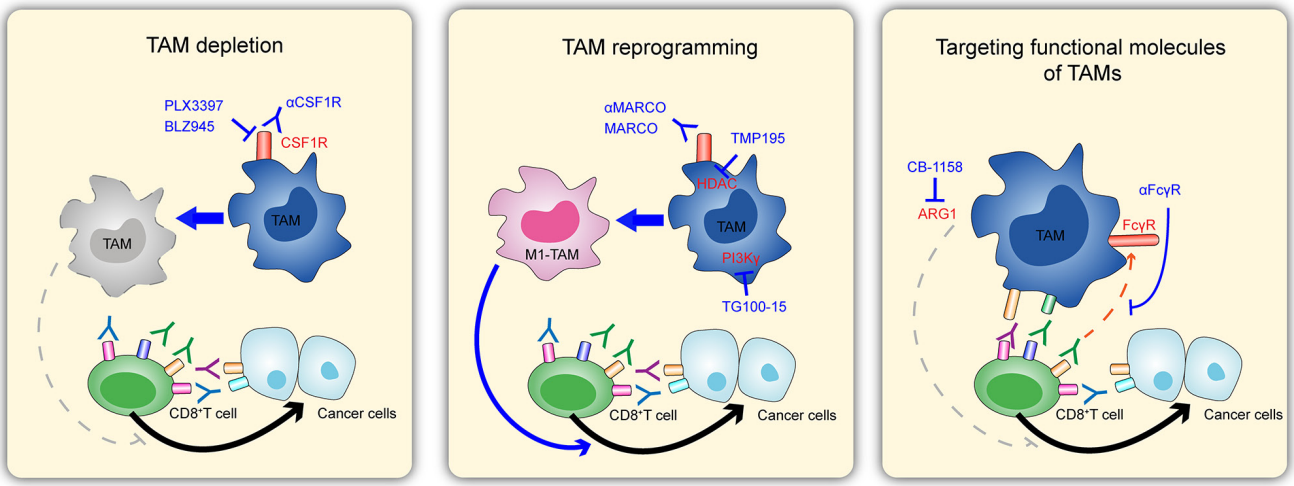

Figure 2. Potential strategies for treating melanoma by targeting macrophages. (Left) Depleting TAMs by regulating CSF1R receptors, thereby interfering with tumor killing by CD8+ T cells. (Middle) Enhanced tumor killing by CD8+ T cells by reprogramming TAMs to promote M1-type polarization of macrophages. (Right) Regulation of tumor killing by CD8+ T cells by targeting functional molecules of TAMs. This figure has been adapted from Fig. 1C of the article 'Targeting Tumor-Associated Macrophages as a Potential Strategy to Enhance the Response to Immune Checkpoint Inhibitors' (100). Front. Cell Dev. Biol., 04 April 2018 | https://doi.org/10.3389/fcell.2018.00 © 2018 Cassetta and Kitamura. ARG1, arginase 1; CSF1R, colony-stimulating factor 1 receptor; FcγR, Fc-γ receptor; HDAC, histone deacetylase; MARCO, macrophage receptor with collagenous structure; PLX3397, pexidartinib; TAM, tumor-associated macrophage.

melanoma to chemotherapeutics. It has been reported that the combination of transforming growth factor-β (TGF-β) and TGF-β receptor (TGF-βR) contributes to the drug resistance and invasiveness of tumor cells and weakens the antitumor immune response (71). A study has demonstrated that the blockade of TGF-βR can trigger reprogramming into an anti-tumor M1-TAM phenotype, thereby increasing the efficacy of doxorubicin chemotherapy (72). Macrophages have also been demonstrated to secrete TNFα, inducing melanoma resistance to MAPK pathway inhibitors (59). The aforementioned studies indicate that macrophages may be involved in melanoma resistance to multiple drugs. Further research is required to explore the mechanism by which macrophages cause drug resistance.

4. TAM-targeting therapies in melanoma

Targeting TAMs can improve antitumor immune responses (73). Given these profound effects exerted by macrophages on the progression of melanoma and several other tumors, targeting macrophages is considered a promising potential therapeutic strategy. Conventional therapies, including surgery, chemotherapy, radiotherapy and targeted therapy, in addition to reducing or reprogramming TAMs, are the two primary approaches to melanoma treatment (74). The current TAM-related approaches for melanoma treatment are described subsequently (Fig. 2).

Reducing the number of TAMs in melanoma: Deleting or inhibiting recruitment. Direct deletion of TAMs is an attractive option based on the idea that removing a tumor would improve the prognosis of a patient with melanoma. For instance, colony-stimulating factor 1 receptor (CSF1R) can control the differentiation, proliferation and survival of macrophages (75), and is present in the vast majority of macrophages. Targeting CSF1R seems to be an effective method for depleting TAMs in tumors, therefore, it has been studied in different tumors (74). In some tumor types, clinical trials have indicated that targeting CSF1R, or combining it with other therapies, can result in improved treatment outcomes (74). In addition, there is currently a clinical trial targeting the CSF1R axis in melanoma; this is, howwver, unable to provide definitive conclusions at this time (76).

Reducing the number of TAMs in the TME by inhibiting their recruitment is another approach to melanoma treatment (77). For example, the CCL2-C-C motif chemokine receptor 2 axis often recruits monocytes, causing TAM expansion, and inhibition of CCL2 can delay tumor progression in a number of experimental tumor models, including melanoma. However, the studies on this approach are insufficient, and more evidence is required.

Activating macrophages in melanoma. It has been confirmed that among the tumor cells, TAMs can have antitumor effects and suppress tumor growth by activating immune responses, although other TAMs promote tumors (78). This suggests that TAMs are flexible and reprogramming them to treat tumors would be a reasonable therapeutic approach. Several studies have focused on this topic (79,80). Evidence has demonstrated that melanoma cells can block macrophage activation by suppressing toll-like receptor (TLR) signaling (81). A clinical study has been performed to test the efficiency and safety of TLR7 ligands (852A) in the treatment of melanoma (82). Combining an agonist of TLR (3M-052 for TLR7/8), which polarizes macrophages towards a pro-inflammatory phenotype, with a checkpoint blockade is more efficient than a checkpoint blockade alone in the treatment of B16-F10 melanomas (82). Targeting the macrophage receptor with collagenous structure (MARCO) with anti-MARCO antibodies could also improve the efficiency of immunotherapy (anti-CTLA4) in a B16 melanoma mouse model (83).

Among various stimulating factors, GM-CSF is widely known to induce macrophages to become tumoricidal not only in melanoma but also in various other tumors, and has been approved for the treatment of unresectable stage IIIB-IVM1a melanoma under certain circumstances (in those who received treatment with GM-CSF as part of combination therapy or in an adjuvant setting) (84). For example, GM-CSF combined with ipilimumab resulted in longer overall survival and lower toxicity, but no difference in progression-free survival was observed (85). By using an indirect treatment comparison in melanoma, a systematic review has revealed that GM-CSF shows improved therapeutic effects compared with glycoprotein peptide vaccines and is at least as good as dacarbazine (86). However, the tumoricidal role of GM-CSF may also not be related to macrophages, because it is also involved in the development and maturation of dendritic cells (DCs) and in the activation and proliferation of T cells (87). The different dependencies of GM-CSF on macrophages, DCs and T cells still remain unclear. IFN-γ, monocyte chemoattractant protein-1, IL-1β and galectin-9 have also been reported as macrophage activators that inhibit tumor growth (88). However, there is still a lack of clinical trials to validate treatment options.

Other approaches: Adoptive macrophage therapy. Adoptive cellular therapy and chimeric antigen receptor (CAR) T cells have achieved marked success in the treatment of lymphoma and leukemia, among others (89,90). Therefore, the adoptive transfer of engineered active macrophages may also be a feasible approach for melanoma treatment. These macrophages may become cytotoxic to tumor cells after artificial administration of special drugs, cytokines and even gene editing (91,92). In 1974, Fidler (93) demonstrated that intravenous injection of specifically activated macrophages by supernatants from lymphocytes can decrease lung metastases of melanoma. Another study also demonstrated the efficiency of the adoptive transfer of activated macrophages (using GM-CSF or muramyl dipeptide) (94,95). However, this is far from any clinical application of adoptive macrophage therapy, as the mechanism of action of adoptive macrophage therapy is not fully understood. Notably, an increasing number of applications of CAR-macrophages in tumors have been reported. Zhang *et al* (96) developed induced pluripotent stem cells, which have been derived from engineered CAR-macrophages that can be used to kill cancer cells. Additionally, Chen *et al* (97) have reported that CAR-macrophages could be used as a novel immunotherapy candidate against solid tumors. Furthermore, Klichinsky *et al* (98) have demonstrated that CAR-macrophages could induce a pro-inflammatory TME and boost antitumor T cell activity in two solid tumor xenograft mouse models. However, the application of CAR-macrophages in melanoma has not yet been reported and could be a potential future research direction.

5. Conclusion and future perspectives

TAMs can be classified as M1 or M2 macrophages. M1 macrophages can activate the adaptive immune system, whereas M2 macrophages have pro-tumor abilities. The present review aims to explore the current knowledge on the role of TAMs in melanoma development through the regulation of proliferation, invasion, metastasis, angiogenesis and chemical resistance of melanoma. Macrophage function and polarization are regulated by multiple TME-based factors. The TAM-activating molecules listed in Table I are expected to be potential candidates for targeted intervention in melanoma progression. Interestingly, the crosstalk between TAMs and other immune cells is also an important mechanism that affects tumor proliferation, invasion and metastasis. Furthermore, the participation of exosomes in the polarization process of TAMs is expected to become a future research topic. Notably, macrophages can adopt different activation states, and the repolarization of TAMs into antitumor M1 macrophages is a promising therapeutic option.

The present review describes three macrophage-based melanoma treatment strategies: Depletion of TAMs in melanoma, activation of macrophages in melanoma and adoptive macrophage therapy. However, the mechanism of action of macrophages in melanoma is not yet fully understood. Notably, an increasing number of applications of CAR-macrophages have been reported in several tumors, including leukemia (96), ovarian cancer (98) and breast cancer (99), but not in melanoma. Therefore, this could be a potential future research direction. Further exploration of the role and mechanism of TAMs in the occurrence and development of melanoma may provide a basis for improved treatment of melanoma.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

QZ conceived, wrote and reviewed the manuscript. TF wrote the manuscript. SW, SC and HY participated in performing the literature review and drawing Figs. 1 and 2. MT and YC were involved in reviewing the manuscript, agreed to be accountable for all aspects of the work and provided final approval of the version to be submitted. YC acquired the funding. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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