frontiers in Immunology

REVIEW
published: 13 July 2021
doi: 10.3389/fimmu.2021.685978

Macrophages, as a Promising Strategy to Targeted Treatment for Colorectal Cancer Metastasis in Tumor Immune Microenvironment

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Specialty section:
This article was submitted to Cancer Immunity and Immunotherapy, a section of the journal Frontiers in Immunology

Received: 26 March 2021
Accepted: 24 June 2021
Published: 13 July 2021

Citation:
Zhang Y, Zhao Y, Li Q and Wang Y (2021) Macrophages, as a Promising Strategy to Targeted Treatment for Colorectal Cancer Metastasis in Tumor Immune Microenvironment. Front. Immunol. 12:685978. doi: 10.3389/fimmu.2021.685978

The tumor immune microenvironment plays a vital role in the metastasis of colorectal cancer. As one of the most important immune cells, macrophages act as phagocytes, patrol the surroundings of tissues, and remove invading pathogens and cell debris to maintain tissue homeostasis. Significantly, macrophages have a characteristic of high plasticity and can be classified into different subtypes according to the different functions, which can undergo reciprocal phenotypic switching induced by different types of molecules and signaling pathways. Macrophages regulate the development and metastatic potential of colorectal cancer by changing the tumor immune microenvironment. In tumor tissues, the tumor-associated macrophages usually play a tumor-promoting role in the tumor immune microenvironment, and they are also associated with poor prognosis. This paper reviews the mechanisms and stimulating factors of macrophages in the process of colorectal cancer metastasis and intends to indicate that targeting macrophages may be a promising strategy in colorectal cancer treatment.

Keywords: colorectal cancer, metastasis, macrophages, targeted treatment, signaling pathways

INTRODUCTION

Colorectal cancer (CRC) is the third most common malignancy and the second leading cause of cancer deaths in the world (1). Approximately 50% of CRC patients develop metastatic diseases (2). CRC has a high probability of metastasis following its diagnosis (3, 4), and metastases especially involve the liver (5). Cytotoxic drug chemotherapy is usually the first choice for metastatic CRC (mCRC), but it is accompanied by numerous side effects, and the prognosis is unsatisfactory. Targeted therapy also currently plays a significant role in the treatment of mCRC, while chemotherapy combined with targeted therapy for mCRC is the first-line therapy in clinical treatment (6, 7). Due to the advantages of targeted therapy, such as precision, high efficiency, significantly less toxicity than chemotherapy, and convenient oral administration (8), targeted therapy will become a promising treatment approach. The discovery of novel molecular biomarkers will likely be of great significance for the treatment of mCRC (9, 10). Immune checkpoint inhibitors, including anti-programmed cell death 1 (PD-1), anti-programmed cell death ligand 1 (PD-L1)

monoclonal antibodies (MAbs) and CTL-associated antigen 4 (CTLA4) blockade have been confirmed to improve the overall survival rate of patients in different cancer types (11, 12).

The tumor immune microenvironment (TIME) is complex and diverse and mainly includes tumor cells, immune cells, antigens, and cytokines (13). Recently, investigations regarding the TIME have received great interest. Macrophages are one of the most important cells in the TIME. Macrophages promote or inhibit tumor invasion and metastasis through the interaction between various molecules and signaling pathways (14–16). Studies have shown that the high immunoactivity defined by the microsatellite instability (MSI) subtype CRC is associated with the high degree of infiltration of M1 macrophages (17). Therefore, molecular targeted therapy directed at the CRC immune microenvironment is a focal point in the treatment of tumors; targeting macrophages potentially exerts long-term effects for the treatment of mCRC.

TUMOR IMMUNE MICROENVIRONMENT

The TIME is formed by the interaction between tumor cells, tumor-infiltrating immune cells, epithelial cells, fibroblasts, blood vessels, chemokines, and cytokines (18, 19). In the TIME, adaptive immune effector T cells (20), and innate immunity effector cells, macrophages (21), NK cells (22), and other cells promote the inflammatory response and are involved in antitumor effects. In contrast to antitumor immunity, tumor-associated macrophages (TAMs) (23), and myeloid suppressor cells (MDSCs) (24), and regulatory T cells (Tregs) (25) as immunosuppressive cells also play an immunomodulatory effect in tumor immunity, facilitating the metastasis of tumors. The occurrence and development of tumors are closely associated with antitumor immune cells in the TIME. Conversely, immune cells can also be influenced by products of tumor cells such as cytokines (26) and exosomes (27), and integrate with signaling pathways by activating immune evasion mechanisms, which further induce tumor metastasis.

In the TIME associated with CRC, various immune cells interact with each other to promote or inhibit the growth, invasion, and metastatic potential of CRC (**Figure 1**). Dendritic cells (DCs) activate T cells through the combination of the major histocompatibility complex (MHC) and T cell receptors (28). NK cells can kill tumor cells directly through antibody-dependent cell-mediated cytotoxicity (ADCC) (29), and T cells can kill tumor cells directly through cytotoxicity (30). Both NK cells and T cells can kill tumor cells through the Fas/FasL pathway, the perforin-granzyme pathway (31), and by releasing tumor necrosis factor (TNF) (32). MDSCs can mediate the development of M2 macrophages and Tregs, which depend on IL-10 (33, 34). T cells promote tumor immunity by secreting IFN-γ (35), while Tregs inhibit the immune effects of T cells *via* the PD-1/PD-L1 axis (36). Macrophages can promote or inhibit tumor immunity by polarizing into different types and play a critical role in the tumor microenvironment (TME). Furthermore, mutual transformation of macrophages regulates the TIME in CRC.

THE ROLE OF MACROPHAGES IN THE METASTASIS OF CRC

Macrophages can differentiate into different subtypes in the TIME and may play dual roles (37). They produce various molecules that interact with other immune cells and tumor cells and further affect the progression in CRC (38). Under the influence of different cytokines and exosomes, three types of macrophages can be identified: naive M0, the M1 subtype with pro-inflammatory effects, and the M2 subtype with immunosuppressive effects (39, 40), which promote or inhibit the progression of CRC.

Antitumor Effects of Macrophages

The classical activated macrophages are defined by the M1 macrophages whose surface markers are CD86, iNOS, and TNF-α (41–43), M0 macrophages polarize into M1 macrophages under the effect of LPS and IFN-γ (44), which are active against pathogen infection and play a significant function in immunity. M1 macrophages can inhibit tumor development by releasing tumor-suppressing molecules, including TNF-α (45). M1 macrophages also induce tumor cell apoptosis through phagocytosis (46), ADCC (47), and release of TNF and nitric oxide (NO) (48). Studies have shown that M1-type macrophages can promote tumor immunity by recruiting cytotoxic T cells (49).

Tumor-Promoting Effects of Macrophages

Macrophages in the TIME are often called TAMs; M0 macrophages are mostly polarized into M2 macrophages (50) following induction by IL-4, IL-10, and IL-13 (51–53). CD163, CD206 and Arg1 are common surface markers of M2 macrophages (54–56). TAMs are classified as M2 macrophage activation subtype, which can promote the development of CRC in the TIME (57). A research showed that TAMs increased significantly in hepatic metastatic tumors of colorectal cancer (58). TAMs contribute to angiogenesis (59), promote epithelial–mesenchymal transition (EMT) of tumor cells (60), and activate immunosuppression (61), promoting the metastasis of CRC. Comfortingly there is evidence that macrophages have plasticity (62, 63), and various stimuli, including drugs or M1 exosomes, can cause macrophages to alter their phenotype and reprogram M2 toward M1, inhibiting tumor development (64, 65). Therefore, targeting macrophages will be a potential strategy for the treatment of colorectal cancer metastasis.

SIGNALING PATHWAYS INVOLVING MACROPHAGES IN CRC METASTASIS

The immune cells in the TIME regulating CRC development are achieved by the secreted immune molecules and CRC cell-surface receptors, activating the intracellular signaling pathways involving macrophages, including Wnt/β-catenin, NF-κB, PI3K/AKT, JAK/STAT3, MAPK, and TGF-β/Smad signaling pathways **(Figure 2)**.

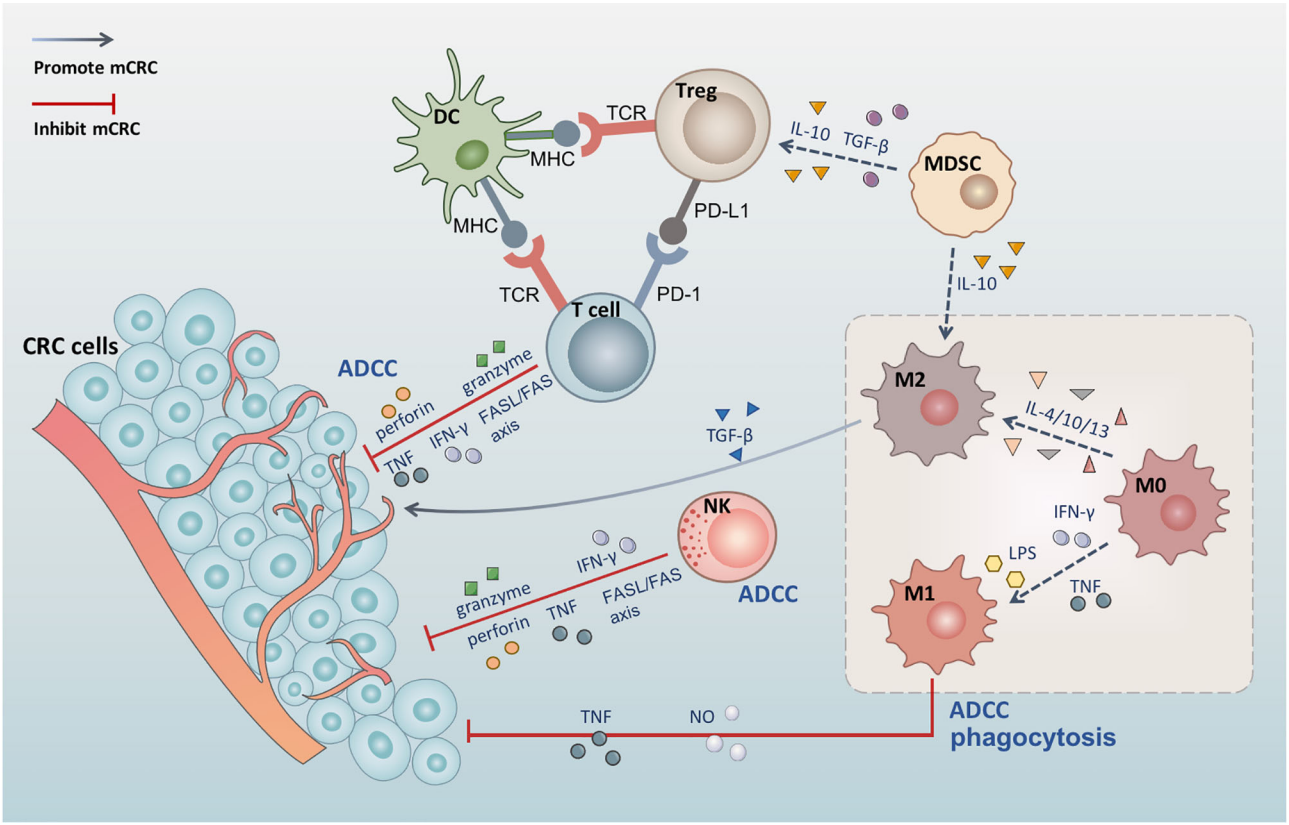

FIGURE 1 | The tumor immune microenvironment of colorectal cancer. DC activates T cells through the combination of the MHC and T cell receptors. Tregs inhibit the immune effects of T cells *via* the PD-1/PD-L1 axis. Both NK cells and T cells can kill tumor cells through the Fas/FasL pathway, the perforin-granzyme pathway, and by releasing TNF and IFN-γ. MDSCs can mediate the development of M2 macrophages and Tregs, which depends on IL-10, and produce TGF-β to induct Tregs. M0 macrophages polarize into M1 macrophages under the effect of LPS, IFN-γ, and TNF, while polarizing into M2 macrophages following induction by IL-4, IL-10, and IL-13. M1 macrophages also induce tumor cell apoptosis through phagocytosis, ADCC, and the release of TNF and NO. M2 secretes TGF-β and contributes to angiogenesis, promotes tumor cell EMT, and induces immunosuppressive microenvironment.

Wnt/β-Catenin Signaling Pathway

The wnt/β-catenin signaling pathway promotes the development of CRC by regulating the growth, differentiation, and migration of tumor cells (66). Abnormalities of the wnt signaling, such as the APC gene mutation, are common in CRC, which can promote the development of CRC (67). A global transcriptome immune classification experiment for CRC solid tumors showed that most patients had APC mutations (68). Activation of the wnt signaling pathway leads to granulocyte recruitment and tumor invasion, and abnormal wnt signaling directly alters the antineoplastic activities of effector T cells, helper T cells, and Tregs, suppressing tumor immunity (69). In highly proliferative colorectal tumors, wnt/β-catenin signaling is activated and abundant β-catenin accumulates in the nucleus, accompanying the immune cell infiltrates including TAMs (70).

NF-κB Signaling Pathway

Nuclear factor-κB (NF-κB) is a critical molecule underlying the relationship between inflammation and tumor immunity and is involved in the growth and development of CRC (71). Various extracellular factors including proinflammatory cytokines, LPS, and growth factors lead to IκB protein phosphorylation and ubiquitination and then degradation, freeing NF-κB/Rel complexes and transferring into the nucleus for transcription, promoting EMT, angiogenesis, and metastasis (72, 73).

Transcription factors mediated by NF-κB are associated with MDSC activation (74). NF-κB expression is upregulated in CD4+TIM-3+ tumor-infiltrating lymphocytes, inducing the expression of inflammatory factors and T cell failure, which in turn further facilitates the metastasis of CRC (75). The NF-κB pathway is activated in P2X7R overexpressed CRC cells, cytokines increasing leads to the recruitment of TAMs (76). TAMs can significantly upregulate the vascular endothelial growth factor-A (VEGF-A) of CRC cells and activate the NF-κB signaling pathway by secreting IL-6 and IL-8, promoting CRC metastasis (77). In patients with CRC, high levels of p50-NF-κB + TAMs at the invasive margin are associated with poor prognosis following surgical intervention for excision of tumors. The p50-NF-κB + TAMs participate in the development of CRC by reducing recruitment and antitumor activity of T cells, which confirms that the NF-κB pathway is a significant signaling pathway promoting CRC metastasis (78). However, the role of

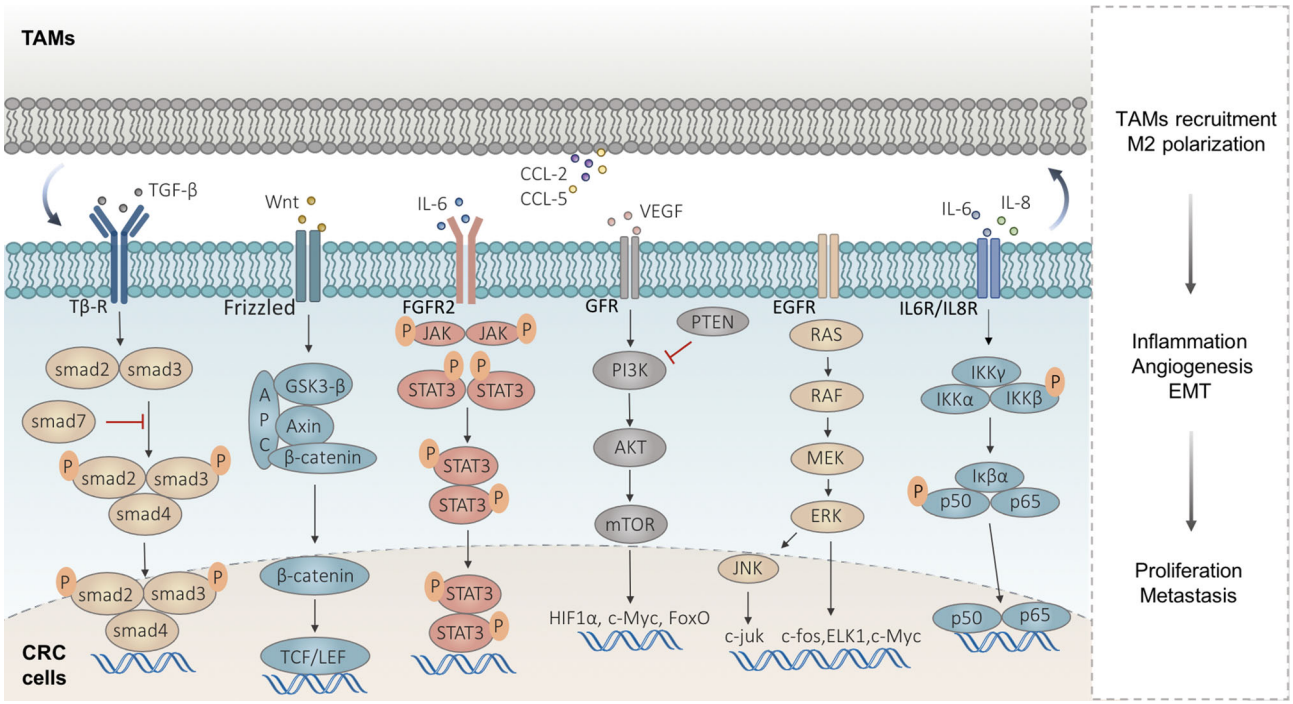

FIGURE 2 | Signaling pathways implicated in macrophages in CRC metastasis. Wnt/β-catenin signaling is activated in highly proliferative CRC accompanied by the TAM infiltrates. TAMs upregulate the VEGF-A of CRC cells and activate the NF-κB signaling pathway by secreting IL-6 and IL-8. TAMs produce IL-6 to activate the JAK/STAT3 signaling in CRC cells and lead to the EMT, which in turn lead to the CCL2/5 secreted by CRC cells, to promote macrophage recruitment. Some exosomes carrying miRNAs derived from CRC cells regulate PTEN, by activating the PI3K/Akt signaling to induce M2-like TAM polarization, and in turn, TAMs promote CRC metastasis by enhancing EMT and secreting VEGF. The abnormal activation of the MAPK signaling pathway can induce tumor cell proliferation and participates in the development and metastasis of CRC. TAMs facilitate the EMT program involved in the CRC metastatic process *via* TGF-β/Smad signaling.

NF-κB in colorectal cancer remains controversial. A research found that for macrophages, the activation of NF-κB can promote M1-polarization, so as to play an antitumor effect (79).

JAK/STAT3 Signaling Pathway

In the TIME, the abnormal activation of the JAK/STAT3 signaling contributes to an immunosuppressive tumor microenvironment, which promotes tumor growth and metastasis (80). Studies have indicated that TAMs produce pro-inflammatory cytokine IL-6 to activate the JAK/STAT3 signaling in CRC cells and lead to epithelial–mesenchymal transition (EMT) involved in tumor progression (81), which in turn leads to the CCL2 secreted by CRC cells, to promote macrophage recruitment, while, inhibition of CCL2 or IL6 can break this crosstalk (82).

On the other hand, IL-6 can upregulate other inflammatory factors such as CCL2 and CCL5, and then recruit macrophages (83, 84). Furtherly, the recruited macrophages in turn secret IL-6 to activate JAK2/STAT3 signaling, promoting tumor metastasis (85). CCL2 significantly increases the number of MDSCs and M2-like TAMs mediated by STAT3, suppresses T cells, and promotes immune evasion in CRC (86). All the above studies have shown that CRC cells and TAMs influence each other to promote tumor development.

Phosphatidylinositol 3 Kinase/AKT Signaling Pathway

The phosphatidylinositol 3 kinase (PI3K)/AKT signaling pathway is one of the most activated pathways in human cancer (87, 88), which can regulate the immunosuppressive microenvironment, promoting immune cell exhaustion and inhibiting antitumor activity (89). PD-1/PD-L1 blockade can rescue depleted CD8+ T cells *via* the PI3K/Akt/mTOR signaling pathway (90). Tumor cells overexpressing T cell immunoglobulin mucin-4 (TIM-4) activate PI3K/AKT/mTOR signal transduction and recruit TAMs, promoting proliferation and tumor matrix remodeling in CRC (91). Some exosomes carrying miRNAs (miR-25-3p, miR-130b-3p, miR-425-5p) derived from CRC cells regulate PTEN by activating PI3K/Akt signaling to induce M2-like TAM polarization, and in turn, TAMs promote CRC metastasis by enhancing EMT and secreting vascular endothelial growth factor (VEGF) (92). In addition, inhibition of AKT can effectively limit the differentiation of T cells and enhance antitumor effects *in vivo* (93).

MAPK Signaling Pathway

The mitogen-activated protein kinase (MAPK) signaling pathway is one of the most important bridges for converting extracellular signals to intracellular responses (94). As the key

pathway participating in cell proliferation, the abnormal activation of the MAPK signaling pathway can induce tumor cell proliferation and participate in the development and metastasis of CRC (95, 96). The homeoprotein Six1 is associated with poor prognosis in CRC (97). Six1 overexpression promotes CRC growth and metastasis by stimulating angiogenesis and recruiting TAMs, accompanied by MAPK activation in CRC cells (98). In addition, a study demonstrated that TAMs interact with CRC cells inducing EMT in CRC cells by activating the MAPK pathway in TAMs, and then promoting the metastasis of CRC (77). The crosstalk in TAMs and CRC cells reveals the significant role of TAMs in the development of CRC, which provides a powerful argument for targeting TAMs in CRC treatment.

TGF-β/Smad Signaling Pathway

In tumor stroma, M2-polarized TAMs secrete transforming growth factor-beta (TGF-β) *via* the miR-34a/VEGF axis and promote invasion and metastasis of CRC (99). There are also some studies that found that *in vitro*, cytokines induced M2 macrophages to produce TGF-β1 *via* the VEGF/VEGFR2 signaling pathway (57). TAMs facilitate the EMT program involved in the CRC metastatic process *via* TGF-β/Smad2,3–4/Snail signaling (100, 101). A multicolor histology analysis indicated that patients with poor clinical outcomes may also have infiltration of T cells in tumor tissues, but always with high TGF-β expression and high TAM density (102), which reveals the critical role of TAMs in CRC metastasis.

Generally, in the immune microenvironment, TGF-β and IL-6 are required for the development of Th-17 cells which produce IL-17 (103). The production of IL-17 is positively correlated with distant colon tumorigenesis (104). However, different from the local tumor immune response, studies have found that in non-tumor tissues of cancer patients, the increase of Th-17 cells and IL-17 can enhance M1 polarization while inhibiting M2 polarization (105), which indicates the complex role of TGFβ in human immunity.

MOLECULES RELATED TO MACROPHAGES IN CRC METASTASIS

Molecular targeted therapy is a promising method for CRC therapy, especially in mCRC. Macrophages play a pivotal role in the metastatic niche, and many molecules, including IL-4, IL-10, and IL-13, can promote macrophages to polarize into M2. In turn, M2 can secrete IL-6, IL-8, and other inflammatory factors to promote the proliferation and metastasis of cancer cells. Therefore, macrophages act as important mediators of the development of CRC. Targeting macrophages may provide a new strategy for the treatment of CRC. All molecules related to targeting macrophages are listed in **Table 1**.

Non-Coding RNA

Studies have demonstrated that non-coding RNA molecules play a pivotal role in the polarization process of TAMs. The overexpression of the long non-coding RNA (LncRNA) RPPH1 has been associated with advanced tumor-node-metastasis (TNM) stages and poor prognosis (106). Exosomes derived from CRC cells transport RPPH1 into macrophages and mediate M2-like polarization to promote CRC cell proliferation and metastasis (107). The lncRNA HLA-F-AS1 regulates the expression of profilin 1(PFN1) in CRC-derived EVs by inhibiting miR-375, and then, in turn, mediates the M2 phenotype polarization of macrophages (108), promoting the CRC metastasis.

Several miRNAs including miR-25-3p, miR-425-5p, and miR-130b-3p induce macrophage M2 polarization by activating the CXCL12/CXCR4 axis in CRC metastasis (92). The tumor-derived exocrine miR-934 can promote CRC liver metastasis by regulating the interaction between CRC cells and TAMs (110). In addition, exosomes carrying miRNA-106b-5p promote the M2-like polarization of macrophages and induce the EMT in CRC cells, which is implicated in the crosstalk between tumor cells and TAMs (131). Brahma-related gene-1 (BRG1) is the core subunit of switch/sucrose nonfermentable (SWI/SNF) family complexes (132). M2-macrophages-derived exosomes carry miR-155-5p and miR-21-5p to CRC cells and combine with the BRG1 coding sequence to downregulate the expression of BRG1, promoting the metastasis of CRC (109).

Thus, we can achieve the effect of blocking M2-like polarization or of blocking tumor from promoting secretion from TAMs by inhibiting the above non-coding RNA, thereby controlling the tumor-promoting effect of macrophages in the tumor microenvironment of CRC.

Cytokines

Many cytokines are involved in the polarization of TAMs, some of them active in downstream signaling pathways to promote CRC metastasis. Studies have revealed that complex chemokine networks can affect cancer progression *via* the recruitment and activation of TAMs. The increased expression of CCL17 in DCs and M2-like TAMs in tumors induces an immunosuppressive environment; CCL17 expression has been used as a marker for M2-like immunosuppressive macrophage polarization (133). CCL5, secreted by TAMs, inhibits T-cell-mediated killing of CRC cells and promotes immune escape by stabilizing PD-L1 (113). In addition, CRC cells secrete VEGF-A and then stimulate TAMs to produce CXCL1 in primary tumors. The increased release of CXCL1 transfer to the liver *via* blood circulation recruits CXCR2-expressing MDSCs to form a pre-metastatic niche, promoting liver metastasis (114).

In addition, sST2, a soluble isoform of the IL-33 receptor (ST2), suppresses angiogenesis, macrophage infiltration, and macrophage M2 polarization induced by IL-33 (111). M2-polarized TAMs secrete TGF-β (100), which regulates the miR-34a/VEGF axis to facilitate CRC cell proliferation and invasion (99). Wnt5a is highly expressed in TAMs and can induce M2 polarization by regulating the secretion of IL-10, which is mediated by the CaMKII-ERK1/2-STAT3 pathway (117). Furthermore, $Wnt5a^{+}$TAMs promote CRC development which also depends on CCL2 secretion mediated by the CaMKII-ERK pathway (112).

TABLE 1 | Molecules related to TAMs in CRC metastasis.

Molecules	Types	Expression in mCRC	Mechanism	Effects in CRC	References
lncRNA RPPH1	Non-coding RNA	Up	Mediates the polarization of M2	Promote	(106, 107)
lncRNA HLA-F-AS1	Non-coding RNA	Up	Mediates the polarization of M2	Promote	(108)
miR-21-5p	Non-coding RNA	Up	Derived by M2-macrophages, downregulates the expression of BRG1	Promote	(109)
miR-25-3p	Non-coding RNA	Up	Activates CXCL12/CXCR4 axis, induces M2 polarization	Promote	(92)
miR-130b-3p	Non-coding RNA	Up	Activates CXCL12/CXCR4 axis, induces M2 polarization	Promote	(92)
miR-155-5p	Non-coding RNA	Up	Derived by M2-macrophages, downregulates the expression of BRG1	Promote	(109)
miR-425-5p	Non-coding RNA	Up	Activates CXCL12/CXCR4 axis, induces M2 polarization	Promote	(92)
miR-934	Non-coding RNA	Up	Promotes CRC liver metastasis by regulating the interaction between CRC cells and TAMs	Promote	(110)
sST2	Cytokine receptor	Down	Suppresses IL-33-induced angiogenesis, macrophage infiltration and polarization	Inhibit	(111)
CCL2	Cytokines	Up	Promotes the recruitment of macrophages	Promote	(82, 86, 98, 112)
CCL5	Cytokines	Up	Promotes the recruitment of macrophages	Promote	(83, 113)
CCL17	Cytokines	Up	Upregulates in M2-like TAMs, induces an immunosuppressive environment	Promote	(111)
CXCL1	Cytokines	Up	Secreted by TAMs, forms a pre-metastatic niche, promotes liver metastasis	Promote	(114)
TGF-β	Cytokines	Up	Secreted by TAMs, facilitates EMT in CRC	Promote	(101)
VEGF	Cytokines	Up	Augments the recruitment of TAMs	Promote	(98, 99, 115, 116)
CSF-1	Cytokines	Up	Augments the recruitment of TAMs	Promote	(98)
IL-1β	Cytokines	Up	Regulates the crosstalk between TAMs and CRC cells	Promote	(115, 116)
IL-6	Cytokines	Up	Upregulates CCL2 and CCL5, and then recruits TAMs	Promote	(81)
IL-10	Cytokines	Up	Induces the M2 polarization	Promote	(117)
LUM	Metabolites	Up	Regulates macrophage polarization	Promote	(118)
ABHD5	Metabolites	Down	Low-level expressed in migratory TAMs, upregulates the MMPs	Promote	(119)
PRL-3	Phosphatases	Up	Activates the MAPK pathway in TAMs to promote EMT	Promote	(77, 120, 121)
Shp2	Phosphatases	Up	Promotes the maturation of TAMs	Promote	(122)
KRS	proteases	Up	Induces M2 polarization of macrophages	Promote	(122)
CTSK	proteases	Up	Induces M2 polarization of macrophages	Promote	(123)
Gas6	protein	Up	Induces M2 polarization of macrophages	Promote	(122)
NLRC4	Inflammasome	Up	Regulates the crosstalk between TAMs and CRC cells	Promote	(116)
NLRP3	Inflammasome	Up	Regulates the crosstalk between TAMs and CRC cells	Promote	(115)
Wnt5a	Secreted protein	Up	Activates macrophages polarization	Promote	(112, 117)
S100A8	Calcium- and zinc-binding protein	Up	Activates the NF-κB pathway in macrophages	Promote	(124)
GRP78	Glucose regulated protein	Up	Upregulates by TAMs, promotes STAT3 phosphorylation	Promote	(125)
P2X7R	Purine receptor	Up	Leads to the recruitment of TAMs *via* NF-κB pathway	Promote	(76, 126)
LAYN	Hyaluronan receptor	Up	Activates macrophages polarization and associates with poor prognosis of patients	Promote	(127)
COX-2	Cyclooxygenase	Up	Promotes the differentiation of M2 macrophages and reduces the expansion of M1 macrophages	Promote	(128)
PGE2	Prostaglandin E2	Up	Promotes the differentiation of M2 macrophages and reduces the expansion of M1 macrophages	Promote	(128, 129)
IDO	Indoleamine 2,3-dioxygenase 1	Up	Promotes Tregs and M2 macrophages cooperative effects, leads to immunosuppression	Promote	(130)
KYN	Kynurenine	Up	Promotes Tregs and M2 macrophages cooperative effects, leads to immunosuppression *via* AHR axis	Promote	(130)

Therefore, we can regulate the immunosuppressive microenvironment by inhibiting the cytokine-induced macrophage M2-like polarization, decrease the recruitment of immunosuppressive cells, and enhance tumor immunity for CRC metastasis.

Macrophage-Related Metabolites

Many metabolites are also related to the metastasis of CRC. These factors are involved in tumor progression and offer a new direction for mCRC treatment. A small leucine-rich proteoglycan lumican (LUM) regulates macrophage polarization in colorectal adenocarcinoma and induces immune escape in the microenvironment of CRC (118). TAMs play an important role in tumor invasion and can migrate with tumor cells during the process of tumor metastasis. TAMs exhibit a heterogeneous expression of the hydrolase domain containing the triglyceride hydrolytic activator 5 (ABHD5) which is expressed in low-level in migratory TAMs and

upregulate matrix metalloproteinases (MMPs) involved in CRC metastasis (119). According to the above, we consider that the regulation of Lum or ABHD5 can target TAMs to prevent the metastasis of CRC.

Phosphatases and Proteases

During the metastasis of CRC, phosphatases and proteases play an important role. It has been found that the protein tyrosine phosphatase-3 (PRL-3) (120) increases CCL26 secretion to stimulate TAM infiltration (121) and activates the MAPK pathway in TAMs, ultimately initiating EMT. On the other hand, PRL-3 can directly induce angiogenesis *via* NF-κB signaling (77). Tyrosine phosphatase 2 (Shp2), which contains two homologous domains of Src, is a non-receptor tyrosine phosphatase encoded by the PTPN11 gene and is positively correlated with tumor metastasis. Studies have shown that Shp2 promotes the maturation of TAMs by activating RAS, and it is associated with PD-1 signaling in T cells (134). KRAS-positive CRC cells secrete cytokines, including growth arrest-specific 6 (Gas6) and cause M2 macrophages polarization and infiltration. In addition, CAFs are activated by communication between CRC cells and TAMs, which remodels the environment of CRC metastasis for cancer cell dissemination (122, 135). Cathepsin K (CTSK) is a lysosomal cysteine protease, which is implicated in signal transduction in cancer cells. CTSK, secreted by CRC cells, induces the polarization of M2 macrophages and mediates the interaction between the gut microbiota imbalance and CRC metastasis, and CTSK overexpression in CRC predicts advanced progression and poor prognosis (123). Thus, it can be seen that the downregulation of these enzymes can block the interaction between TAMs and CRC cells and then inhibit the development of CRC.

Other Biomolecules

Many other biomolecules are also involved in TAM-mediated CRC metastasis. Both nucleotide-binding oligomerization domain (NOD)-like receptor C4 (NLRC4) and NOD-like receptor family pyrin domain containing 3 (NLRP3) are the main components of the inflammasome, which can increase TAM infiltration and IL-1β production, and promote CRC metastasis by regulating the crosstalk between TAMs and CRC cells (115, 116).

The P2X purine receptor 7 (P2X7R) expressed in tumors leads to the recruitment of TAMs *via* the NF-κB pathway, which facilitates the angiogenesis and the progression of CRC (76, 126). Tumor cells characterized by the overexpression of homologous protein Six1 can raise the recruitment of TAMs by increasing the expression of CSF-1, CCL2, CCL5, and VEGF, promoting CRC metastasis (98). S100 calcium-binding protein A8 (S100A8) can activate NF-κB signaling in macrophages and upregulate IL-1β and TNF-α in TME and augment the migration of CRC cells (124). LAYN, a cell surface hyaluronan (HA) receptor, may be used as a prognostic biomarker for CRC, and it is associated with immune infiltration including TAMs (127). Complement 5a expressed in CRC cells activates macrophage polarization, which in turn facilitates CRC liver metastasis *via* the NF-κB pathway (136).

M2-like macrophages have been reported to upregulate the expression of the glucose-regulated protein of 78 kDa (GRP78) in tumor cells, promoting STAT3 phosphorylation, leading to the downstream inflammatory factors including IL-1β and TNF-α upregulation, which facilitates tumor progression (137).

COX-1 and COX-2 are two isozymes of cyclooxygenase (COX). COX-2 has been found in high levels in CRC (125). Studies have confirmed that COX-2 is a promoting factor for liver metastasis of CRC, and it can convert arachidonic acid into prostaglandin E2 (PGE2) (138, 139). TAMs are the main source of COX-2 in intestinal tumors; PGE2-bound EP4 promotes the differentiation of immunosuppressive M2 macrophages and reduces the expansion of immunostimulatory M1 macrophages (128). PGE2 also enhances the tumor infiltration of M2 macrophages and promotes the development and metastasis of CRC (129).

In addition, Indoleamine 2,3-dioxygenase 1 (IDO) suppresses T cell immunity by catabolizing tryptophan into kynurenine (KYN) and promotes CD8+ T cell exhaustion (140). In IDO-expressing tumors, Tregs cooperate with M2-like macrophages, promoting immune suppression *via* Kyn-aryl hydrocarbon receptor (AHR) axis (130). Studies have shown that in tumor tissues, the levels of IDO1 and its catabolite KYN are higher in late stages (stages III and IV) than in early stages (stages I and II) of CRC patients (141). Also, IDO was found to be negatively correlated with the survival rate of patients (142).

From the above data, we infer that by interfering with these molecules in TIME, we can directly or indirectly block the crosstalk between TAMs and CRC cells and then inhibit the progression of the tumor.

CLINICAL DEVELOPMENT OF TARGETED THERAPY IN CRC METASTASIS

In recent years, the development and applications of clinical targeted drugs have been increasing. In the treatment of CRC metastasis, targeted drugs that have entered clinical application stage or clinical trials include bevacizumab, ramucirumab, cetuximab, panitumumab, trastuzumab, regorafenib, lapatinib, erlotinib, napabucasin, sym004, and pimasertib **(Table 2)**.

TAMs are one of the causes of tumor angiogenesis and tumor immune escape mechanisms, and targeted treatment of macrophages represents a new challenge and may become a novel strategy for cancer therapy. In the TME, the antiangiogenic drugs bevacizumab and ramucirumab can bind to human VEGF and block its biological activity (143–145); cetuximab and panitumumab bind to the epidermal growth factor receptor (EGFR), repolarize TAMs from M2-like to M1-like phenotypes, recruit myeloid effector cells such as M1 macrophages and PMN for tumor cell killing by ADCC (147–149), and inhibit angiogenesis and vascular endothelial permeability (162–164), and thus block M2 cell infiltration in the inflammatory environment and impede tumor development (165–167). HER2 is positively expressed in CRC, and some studies have shown that trastuzumab and lapatinib, drugs

TABLE 2 | Targeted drugs of the treatment in mCRC.

Targeted drugs	Types	Target	Mechanism	Association with macrophage	References
Bevacizumab	Human monoclonal IgG1 antibody	VEGF	Inhibits angiogenesis of tumor	Inhibits the infiltration of TAMs	(143–146)
Ramucirumab	Human monoclonal IgG1 antibody	VEGF	Inhibits angiogenesis of tumor	Inhibits the infiltration of TAMs	(144, 145)
Cetuximab	Human monoclonal IgG1 antibody	EGFR	Inhibits angiogenesis and vascular endothelial permeability	Repolarizes TAMs from M2-like to M1-like phenotypes	(147–149)
Panitumumab	Human monoclonal IgG2 antibody	EGFR	Inhibits angiogenesis and vascular endothelial permeability	Recruits myeloid effector cells such as M1 macrophages and PMN for tumor cell killing by ADCC	(47, 148)
Trastuzumab	Human monoclonal IgG antibody	HER2	Blocks the growth of cancer cells	Increases macrophage levels and phagocytosis	(146, 150)
Lapatinib	Human monoclonal IgG antibody	HER2	Blocks the growth of cancer cells	Reduces the content of TAMs in TIME	(150, 151)
Regorafenib	Multi-kinase inhibitor	VEGF	Inhibits angiogenesis of tumor	Reduces the content of TAMs, increase M1 polarization of macrophages	(152–154)
Erlotinib	EGFR tyrosine kinase inhibitor	EGFR	Blocks tumor growth by inhibiting the activity of tyrosine kinase	Reduces the content of TAMs, increases M1 polarization of macrophages	(155–157)
Napabucasin	Inhibitor of STAT3	STAT3	Inhibits tumor metastasis and recurrence	Reduces the polarization and infiltration of M2	(158, 159)
Sym004	Anti-EGFR Antibody Mixture	EGFR	Inhibits tumor growth and metastasis	Reduces the polarization and infiltration of M2	(160)
Pimasertib	MEK inhibitor	MAPK	Inhibits the development and metastasis of CRC	Reduces the polarization and infiltration of M2	(161)

targeting HER2, can inhibit tumor formation by increasing macrophage levels and phagocytosis, and by increasing the infiltration of immune cells, it exerts a therapeutic effect on CRC metastasis (83, 146, 150, 151).

Besides the mentioned monoclonal antibodies above, there are several targeted drugs proposed for the treatment of mCRC. Regorafenib, a multi-kinase inhibitor, not only plays an anti-angiogenesis role by inhibiting VEGF but also induces M2 to M1 TAM polarization (152–154). Erlotinib can inhibit the phosphorylation of intracellular tyrosine kinases associated with EGFR, reducing the content of TAMs (155–157). Napabucasin inhibits the STAT3, which is associated with tumor stemness. Due to the increasing evidence supporting the overexpression of STAT3 in CRC cells, it can be inferred that napabucasin may reduce the STAT3-mediated TAM infiltration and chemoresistance (158, 159). Furthermore, as previously mentioned, in the TIME, the activation of the MAPK pathway in CRC cells can promote the recruitment of TAMs. Pimasertib, a drug targeting MAPK, has also been shown to be effective in phase I clinical treatment of mCRC (161). Sym004, a dual-antibody mixture targeting non-overlapping EGFR epitopes, can inhibit the infiltration of macrophages in TIME, thus providing a good therapeutic approach for mCRC (160, 166). All the above-mentioned pre-clinical and clinical-stage drugs are implicated in macrophage, which suggests that the development of macrophage-targeted drugs have long-term clinical significance.

CLINICAL TRIAL DRUGS TARGETING TAMS IN METASTATIC CRC

There are drugs targeting macrophages to treat mCRC in clinical trials, either as a single therapy or in combination with chemotherapy or immunotherapy (**Table 3**). Studies have shown that the expression of PD-1 by TAMS can inhibit the phagocytosis of macrophages against tumors and tumor immunity (173). Also, macrophage colony-stimulating factor 1 (CSF-1) plays an important role in macrophage differentiation and angiogenesis (174). In the clinical research on the treatment of mCRC, there are many studies on the anti-CSF-1 receptor (CSF-1R) and anti-PD-1/PD-L1 targeted drugs. RG7155 (emactuzumab) is a humanized mAb that binds to CSF1R and blocks its dimerization. In mouse models of CRC, RG7155 treatment reduces the infiltrated TAMs and increase CD8 (+)/CD4(+) T cell ratio (168). Pexidartinib and Durvalumab are anti-CSF1R and anti-PD-L1 drugs respectively. Recently, clinical studies are evaluating the safety and activity of their combination in patients with advanced/metastatic CRC and clinically active pancreatic cancer (169).

In addition, granulocyte-macrophage colony stimulating factor (GM-CSF) can enhance the function of macrophages and other immune cells and improve the antitumor and anti-infective immunity of the body (175). GM-CSF is widely used in clinical research. A clinical trial demonstrated the safety and feasibility of the GM-CSF colon cancer vaccine administered to patients with mCRC and recommended that it is necessary to further study the efficacy and antitumor immunity of this vaccine (176, 177). JX-594 is recombinant vaccinia granulosa cell-macrophage colony stimulating factor (RAC VAC GM-CSF). It has been proved that intravenous infusion of Pexa-Vec (JX-594) is a safe and well-tolerated drug (170). At present, a phase 2 study of Pexa-Vec combined with irinotecan in patients with mCRC is

TABLE 3 | Current clinical trial drugs targeting TAMs in mCRC treatment.

Drug	Target	Inhibitor type	References
RG7155	CSF-1R	mAb	(168)
Pexidartinib	CSF-1R	Small molecule	(169)
JX-594	GM-CSFR	Small molecule	(170)
GVAX	GM-CSFR	Allogeneic colon cancer cell vaccine	(171, 172)
Durvalumab	PD-L1	mAb	(169)

currently under way (178). Drugs targeting epigenomes include DNA methyltransferase 1 inhibitor (DNMTi) and histone deacetylase inhibitor (HDACi). The trial of the second generation DNMTi guadecitabine combined with colon vaccine (GVAX) secreting GM-CSF in the treatment of advanced CRC showed that the treatment was well tolerated and had no accidental toxicity, but it is closely related to the order of administration sequence (171, 172). These drugs, which have entered into clinical trials, show the potential of targeting macrophages in the treatment of CRC metastasis.

DISCUSSION

Clinical studies have shown that patients with mCRC have short survival time and poor prognosis, which indicate that inhibiting the metastasis of CRC is the critical point to treatment (179). With the effective application of immune checkpoint inhibitors in the treatment of melanoma, the prospects of immunotherapy for the treatment of other cancer types, including CRC, have been gradually proposed. The inhibition of immune checkpoints can enhance the tumor immune response and inhibit tumor development. PD-1 and TIM-3 are two significant immunosuppressive molecules, which have a crucial effect on immune escape and tumor development (180). PD-1 exists on the surface of T lymphocytes and is bound by its ligand PD-L1 expressed on Tregs or tumor cells, causing the reduction of tumor immunity (181–183). PD-L1 expression is a suitable prognostic biomarker to predict the survival of patients with CRC. In stage I–III CRC patients, the upregulated expression of TIM-3 and PD-1 may predict poor prognosis (180). Furthermore, there are significant differences in expression between metastatic and primary tumors. PD-1 expression in tumor-infiltrating lymphocytes is a strong prognostic indicator for CRC patients following pulmonary resection for CRC metastasis (182). In addition, lymphocyte activation gene 3 (LAG3) is also an immune checkpoint protein (184). Blocking LAG3 can enhance tumor-infiltrating T cell response in patients with mismatch-repair proficient liver metastasis of CRC (185), which might be a newfound immunotherapy target for CRC liver metastasis. CTLA-4 expressed by T cells can also inhibit the activity of CD8+ T cells and tumor immunity. All the above-mentioned proteins are important immune checkpoints, which can suppress T effector cell proliferation and consequently inhibit tumor immunity (186). Clinically, PD-1 inhibitors are effective in mCRC with mismatch repair defects and high microsatellite instability (dMMR-MSI-H), which provides a rationale for the development and application of immunotherapy in mCRC (187). As one of the most abundant immune cell types in the TIME, TAMs have fundamental significance for their development as potential targets in tumor therapy. Studies have shown that the phagocytic ability of PD-1+ TAMs is decreased, thus PD-1 inhibitors also play a critical role in the targeted therapy of TAMs (173).

In TIME, some molecules, as cellular receptors, can directly target TAMs by inhibiting the development of CRC through their inhibitors. Some molecules, such as cytokines or non-coding RNA, that activate tumor-related signal pathways can promote the immunosuppression of TIME, increase the recruitment and infiltration of immunosuppressive cells, promote EMT and tumor angiogenesis, and indirectly promote the CRC metastasis through the crosstalk between macrophages and tumor cells. Also some molecules, as cell products, can induce the polarization of M2 macrophages and predict a poor prognosis in patients.

Researchers have identified a number of factors that regulate macrophages, and we have classified and summarized their findings. According to **Table 1**, we can regulate or block the molecules that interfere with TAM infiltration or M2-like polarization, so that their depletion and reprogramming can then inhibit CRC metastasis. At present, there are few studies on direct targeting of macrophages. With the rise of nanometer technology and its application in tumor treatment (188), we hypothesize that we can use the existing nanomaterial-targeting technology to identify the unique surface markers that directly and specifically bind to TAMs and to remove or block their synergistic invasion of CRC cells; however, this technology needs to be further investigated by researchers. Inhibitors or gene knockout methods can be used in *in vivo* and *in vitro* experiments to regulate related molecules, directly or indirectly inhibit the tumor-promoting effect of TAMs, and then induce targeted macrophages to interfere with the process of CRC metastasis.

At present, there are still numerous challenges for the development of macrophages as molecular targeted therapy for tumors. Most biomarkers associated with macrophages play an indirect role, but their effects are not completely clear. Furtherly, TAM-associated molecular targets and their therapeutic effects on CRC still require verification using experimental models. Tumor treatment that relates to macrophages has entered clinical application and the associated immunotherapeutic and targeted therapy has shown the effective potential to inhibit tumor metastasis, but its clinical application is still very limited and requires further exploration before its therapeutic benefits are expanded as an intervention for tumor metastasis.

Although targeted drug therapy has achieved a certain degree of therapeutic efficacy, these agents are not effective for all patients. Besides, prolonged treatment with targeted drugs may also result in drug resistance. Studies have shown that TAMs are associated with drug resistance to bevacizumab, and TAMs secrete IL-8 which induces drug resistance to lapatinib by activating EGFR signaling (189). In a phase III clinical trial, the addition of cetuximab to mCRC patients who were treated with chemotherapy combined with bevacizumab activated M2 macrophages and reduced the progression-free survival rate (190). Thus, targeted drugs require further experimental evaluation despite their potential benefits for the treatment of cancers.

Targeted drug therapy is also limited by the degree of toxicity during treatment. Adverse effects mainly include skin toxicity and gastrointestinal reactions. Thus, the control of side effects is also a key point to be considered in the development of targeted

drugs. Furthermore, exploration of therapeutic doses should also consider the maximal doses tolerated based on the condition of the patients in order to achieve the best therapeutic outcome and simultaneously to improve their quality of life during treatment. Therefore, before targeted drugs are fully applied in clinical practice, well-organized clinical trials are needed to fully elucidate the advantages of the approach and to determine ways to avoid side effects as much as possible. If these strategies can be applied to the human body after improvement, they can be used as supplementary strategies for routine treatment, which could prolong survival time and improve life quality of patients with advanced CRC.

In conclusion, CRC metastasis is a complex process associated with the interaction between tumor cells and their metastatic niche. In this paper, we described the feedback loop between CRC cells and TAMs in TIME during metastasis. As the main immune cells in the TIME, macrophages play a pivotal impact in the development of mCRC. Macrophages may exert tumoricidal effects as the M1 subtype and participate in tumor immunity. Conversely, macrophages also inhibit inflammatory reaction as in the M2 subtype and facilitate the development of mCRC. In the TIME of CRC, TAMs interact with cytokines, cell metabolites, and signaling pathways to regulate the TME of CRC. We summarized the biomolecular markers associated with macrophage activity in the mCRC TIME and provided an outline of the rationale for the development of novel molecular targeted therapy for mCRC. Accordingly, targeting TAMs is a promising strategy for CRC metastasis immunotherapy.

AUTHOR CONTRIBUTIONS

YinZ contributed to design the article structure, writing–review and editing, and draw the figures and tables. YiyZ contributed to writing-original draft and draw the figures and tables. QL contributed to conceptualization and funding acquisition. YW contributed to supervision, funding acquisition, and project administration. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the National Natural Science Foundation of China (81774095, 82074232, 82030118).

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.