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Biological Roles and Clinical Therapeutic Applications of Tumor-Associated Macrophages in Colorectal Liver Metastasis

Shenghao Li[1,2,*], Liyuan Hao[1,2,*], Xiaoyu Hu[2]

[1]School of Clinical Medicine, Chengdu University of Traditional Chinese Medicine, Chengdu, Sichuan Province, People's Republic of China; [2]Department of Infectious Diseases, Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, Sichuan Province, People's Republic of China

*These authors contributed equally to this work

Correspondence: Xiaoyu Hu, Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, Sichuan Province, People's Republic of China, Email xiaoyuhu202206@163.com

Abstract: Colorectal cancer (CRC) commonly metastasizes to the liver, and this poses a significant clinical challenge. Tumor-associated macrophages (TAMs), key players within the TME, play a significant role in promoting CRC metastasis by secreting various chemokines, growth factors, and cytokines. This review not only aims to enhance our knowledge of TAMs' functions in CRC progression and metastasis but also examines innovative therapeutic strategies to address the clinical problem of colorectal liver metastasis (CLM). By targeting TAMs, we may be able to develop more effective treatments and offer hope to patients suffering from this devastating disease.

Keywords: colorectal liver metastasis, macrophage, TAMs

Introduction

Colorectal cancer (CRC) ranks as the third most prevalent cancer worldwide and is the second leading cause of cancer-related mortality.[1] While early-stage CRC can often be effectively managed with radical interventions,[2] a significant portion of patients (25–50%) with early-stage disease eventually develop metastatic CRC,[3] with the liver being the most common site for metastasis.[4] The high incidence of liver metastasis in CRC can be attributed to the liver's anatomical position, as it receives blood from the gastrointestinal tract via the portal vein, facilitating the spread of CRC cells.[5] Once CRC metastasizes, the prognosis worsens significantly, with a notable decline in the 5-year survival rate,[6] highlighting the urgent need to understand the mechanisms underlying CRC metastasis for the development of effective clinical treatments.

In recent years, extensive research has been conducted on colorectal liver metastasis (CLM), which is influenced by both the complex interactions between cancer cells and their surrounding microenvironment and inherent changes within cancer cells. The "seed and soil" theory, proposed by Stephen Paget in 1889, emphasizes the role of the microenvironment in metastasis, suggesting that tumor cells (the "seed") require a supportive environment (the "soil") to establish and grow in metastatic sites.[7] The interactions between tumor cells and the tumor microenvironment (TME) are thus critical for metastasis.[8,9] The TME consists of various non-cancerous cells, including fibroblasts, and immune cells, along with non-cellular components such as the extracellular vesicles (EVs), cytokines and extracellular matrix (ECM).[10] A growing body of evidence suggests that the TME plays a crucial role in the initiation, progression, and metastasis of CRC.[11,12]

Among the immune cells in the TME, tumor-associated macrophages (TAMs) are particularly important.[13] TAMs contribute to tumor growth, metastasis, immunosuppression, and angiogenesis through the secretion of a range of cytokines and chemokines.[14–16] TAM subtypes carry out diverse functions and can dynamically alter their behavior in

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response to various signals from cancer cells or the TME. Research indicates that TAMs are closely linked to the occurrence of CLM and influence 5-year survival rates. Patients with higher pre-invasive macrophage infiltration tend to have better 5-year survival rates following radical resection compared to those with lower infiltration. Moreover, TAMs infiltration at the invasive front is associated with better outcomes in CLM and overall survival.[17] In patients with CLM, TAMs comprise at least two major cell subtypes, namely large TAMs (L-TAMs) and small TAMs (S-TAMs). Notably, a S-TAM has a more favorable prognosis compared to a L-TAM. The 3-year disease-free survival (DFS) rates for S-TAMs and L-TAMs are 60% and 8.5%, respectively.[18] On the other hand, the accumulation and activation of Kupffer cells (KCs) in the peritumoral area of CLM patients correlate with poor prognosis.[19] TAMs interact with other immune cells in the TME and with tumor cells, shaping a microenvironment conducive to CLM. Despite the progress in understanding TAMs and their role in CLM, there are still challenges and gaps in current treatments. For example, targeting TAMs for therapeutic intervention may face difficulties in achieving specific delivery and avoiding off-target effects. Additionally, the complex nature of the TME and the dynamic interactions between different cell types make it challenging to develop effective therapeutic strategies. However, the potential for therapeutic interventions targeting TAMs in CLM remains significant. This review provides an overview of TAMs, discussing their definition, origin, and polarization, with a particular emphasis on their role in promoting CLM. Additionally, it explores therapeutic strategies that target TAMs in CLM patients.

Definition and Origin of TAMs

As a type of innate immune cell, macrophages primarily originating from monocytes, and they play a vital part in defending the host against pathogens, regulating tissue homeostasis, and maintaining structural integrity. Blood monocytes, which derive from bone marrow hematopoietic stem cells, are the primary source of these cells.[20–22] Many resident macrophages, such as Kupffer cells, alveolar macrophages, and brain macrophages, originate from yolk sac progenitors and undergo in situ proliferation or differentiation.[23] Macrophages that infiltrate tumor tissues or populate the solid tumor microenvironment are classified as TAMs.[24] As essential components of the tumor microenvironment, TAMs influence tumor progression by promoting growth, modulating immune responses, facilitating tumor angiogenesis, enhancing metastasis, and contributing to drug resistance.[25,26] TAMs are drawn to and activated by diverse signals within the TME, where they perform multiple roles in cancer progression, such as initiating and promoting tumors, modulating the immune response, facilitating metastasis, and supporting angiogenesis.

Polarization and Influencing Factors of TAMs

Macrophages can polarize into two distinct states: the classically activated M1-type (pro-inflammatory) and the alternatively activated M2-type (anti-inflammatory) (Figure 1).[13] M1 macrophages are known for promoting the Th1 immune response and their ability to engulf and destroy tumor cells.[27] These macrophages are typically induced by cytokines such as interferon-gamma (IFN-γ), lipopolysaccharide (LPS), or tumor necrosis factor-alpha (TNF-α). Within the TME, M1 macrophages release various inflammatory factors, including interleukins (IL-1, IL-6, IL-12, IL-23), C-X-C motif ligand 10 (CXCL-10), and TNF-α, which enhance the anti-tumor immune response and help recruit and activate other immune cells to target the tumor.[28] Conversely, M2 macrophages, which arise under the influence of cytokines like IL-4, IL-10, IL-13, or glucocorticoids, secrete anti-inflammatory growth factors such as vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF-β), and matrix metalloproteinases (MMP-2, MMP-9), along with cytokines like IL-4, IL-10, and IL-13. These factors play crucial roles in promoting epithelial-mesenchymal transition (EMT), angiogenesis, and immunosuppression, which can ultimately lead to tumor progression and poor treatment outcomes.[29] Additionally, due to the diverse cytokines they produce and their varied functions, M2 macrophages can be further subdivided into M2a, M2b, M2c, and M2d subtypes.[30] Interestingly, macrophage polarization between the M1 and M2 states is not fixed, and they can transition from one state to another, highlighting the potential of macrophages as therapeutic targets. While M1 and M2 are the most commonly studied macrophage phenotypes, macrophage polarization is not limited to these two states.

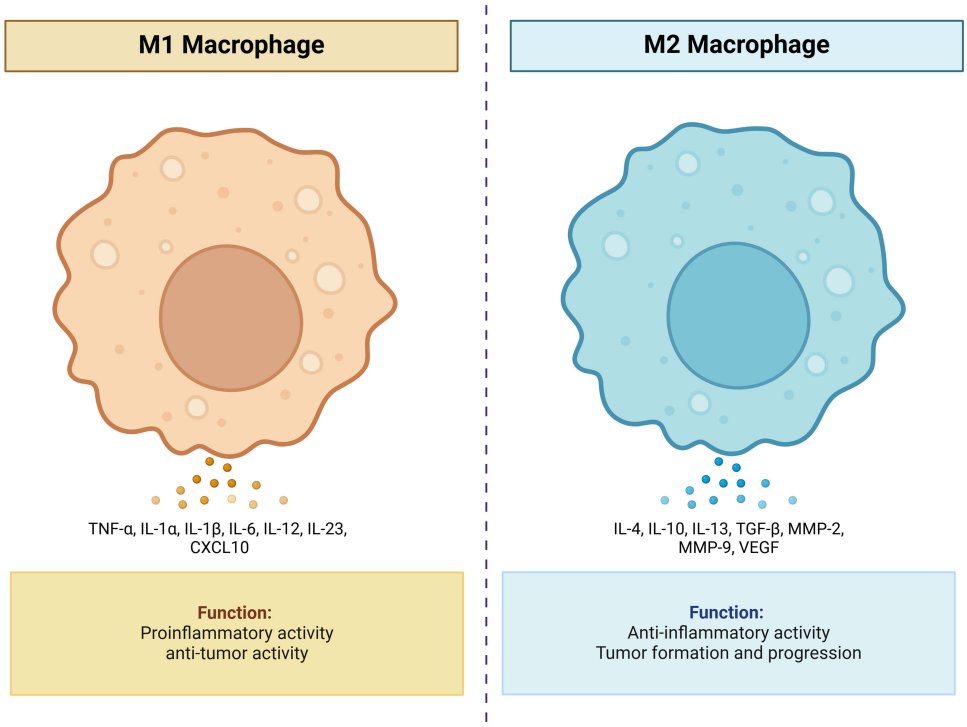

Figure 1 Functional Characteristics of M1 and M2 Macrophages in the Tumor Microenvironment. M1 macrophages produce pro-inflammatory and anti-tumor cytokines (TNF-α, IL-1α, IL-1β, IL-6, IL-12, IL-23, CXCL10). M2 macrophages secrete anti-inflammatory and pro-tumor factors (IL-4, IL-10, IL-13, TGF-β, MMP-2, MMP-9, VEGF), promoting tumor growth and progression. Created in BioRender. Hu, X. (2024) BioRender.com/k99w361.

The Role of TAMs Polarization in CLM

TAMs are integral to the development of CLM (Figure 2). Research indicates that different macrophage subtypes exhibit unique functions and distribution patterns within the liver metastasis microenvironment. Specifically,

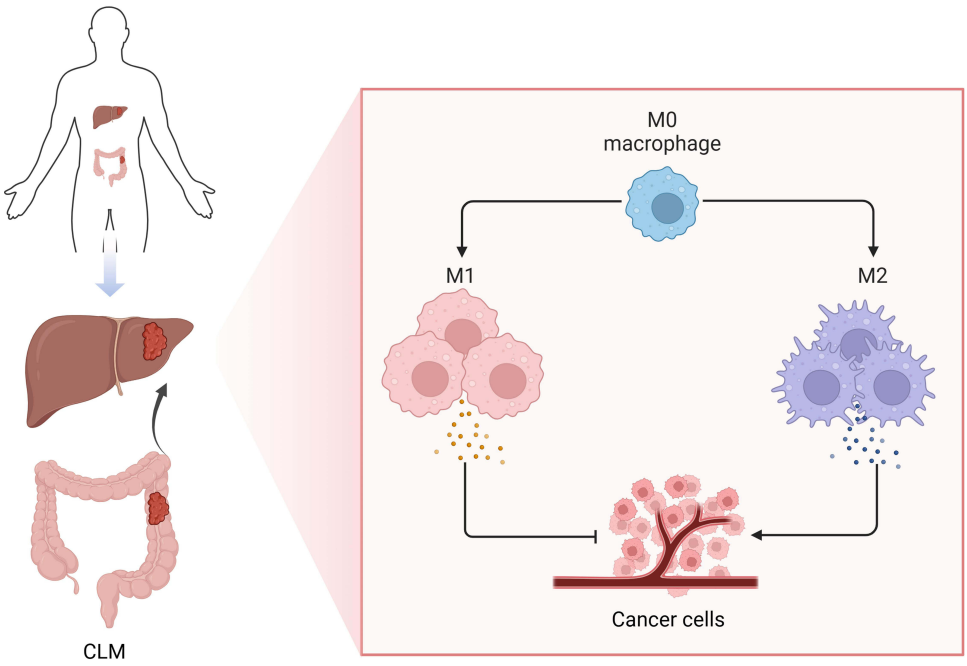

Figure 2 The role of TAMs in CLM. M0 macrophages can differentiate into M1 or M2 types. M1 macrophages are pro-inflammatory and anti-tumor, inhibiting cancer cell growth. M2 macrophages are anti-inflammatory and pro-tumor, promoting cancer growth and metastasis. The diagram illustrates colorectal cancer metastasis from the primary site to the liver and its interaction with macrophages. Created in BioRender. Hao, L. (2024) BioRender.com/b44g866.

$CD68^{+}CD163^{+}CD206^{neg}$ M2-type macrophages predominate in CLM, particularly around the tumor periphery. Patients with higher levels of $CD68^{+}MRP8\text{-}14^{+}CD86^{neg}$ M1-type macrophages in the tumor center had worse overall survival compared to those with lower M1 macrophage levels in this region.[31] The expression patterns between the tumor invasive front (TF) area (<150 μm from the tumor center) and the peritumoral (PT) region (≥150 μm from the tumor center) differ between primary CRC and liver metastases. Notably, CD163 expression is more pronounced in the PT region than in the TF region. Additionally, CD163 expression is higher in liver metastases compared to primary sites, with $CD68^{-}CD163^{+}$ macrophages being the dominant type in liver metastases.[32] Studies suggest that the impact of TAMs on liver metastasis in colorectal cancer is determined by the number and proportion of functional M1 and M2 subtypes, rather than the total TAMs count. The number of M2 macrophages and the M2/M1 ratio are more accurate predictors of CLM.[33] When connective tissue proliferates, there are more clusters of CD68-positive M1 macrophages and fewer CD206-positive M2 macrophages at the tumor-liver parenchyma interface. A better long-term prognosis is associated with fibrous tissue hyperplasia at the invasion front of metastatic tumors.[34] Consequently, further research into the roles and mechanisms of different TAM subtypes in CLM is essential for precise treatment strategies. These results indicate that in CLM, $CD68^{+}CD163^{+}CD206^{neg}$ M2-type macrophages are dominant around the tumor. Moreover, a higher level of $CD68^{+}MRP8\text{-}14^{+}CD86^{neg}$ M1-type macrophages in the tumor center is associated with a poorer overall survival rate. At the same time, it is pointed out that the number of M2 macrophages and the M2/M1 ratio are more accurate predictors for CLM. However, there is a potential conflict. Conclusions from different studies on which subtype of macrophages has what impact on prognosis under what circumstances may vary. For example, other studies may find different combinations of surface markers or in different tumor microenvironment regions, and the relationship between macrophage subtypes and prognosis is not so absolute. For critical evaluation, differences in research samples need to be considered, including sample size, individual differences of patients (such as age, gender, underlying diseases, etc.) and tumor staging and grading. Different sample characteristics may lead to different distributions and functional manifestations of macrophage subtypes, thus affecting the judgment of prognosis. In addition, the accuracy and specificity of research methods are also crucial. For example, surface markers used to identify macrophage subtypes may have cross-reactions or inaccurate situations, which may lead to misclassification of macrophage subtypes and further affect the conclusion on their relationship with prognosis.

TAM polarization is influenced by various cytokines, growth factors, chemokines, and other signals from tumor and stromal cells, playing a key role in CRC progression and metastasis. The CCL1-CCR8 axis, for example, is a positive regulator of CRC progression. Decreased CCL1 expression in TAMs at liver metastatic sites may contribute to the slower tumor progression in CRC, allowing time for radical resection of metastases.[35] Inhibiting TCF4 or CCL2 in tumor cells prevents CLM, while blocking the TCF4-CCL2-CCR2 axis significantly reduces CLM by hindering TAMs accumulation and M2 polarization in the tumor microenvironment.[36] The CCL2/CCR2 chemokine axis supports the recruitment of immunosuppressive TAMs to liver metastatic sites in CRC patients, thereby promoting tumor growth. Targeting CCR2 can reduce TAM accumulation in liver metastases and restore anti-tumor immunity.[37] CXCL16 suppresses CLM by promoting TNF-α secretion from TAMs, which induces tumor cell apoptosis.[38] High levels of CDC42 cargo from CRC cell-derived extracellular vesicles (CRC-EVs) are delivered to macrophages, activating NOD1 and subsequent phosphorylation of RIP2, leading to the release of downstream cytokines and chemokines, thus promoting CRC metastasis.[39] Human umbilical cord mesenchymal stem cells (hUCMSCs) secrete exosomes carrying miR-1827, which are delivered to CRC cells, inhibiting macrophage M2 polarization and preventing CLM.[40] Loss of Ndrg2 inhibits CLM by activating the NF-κB pathway, which regulates macrophage polarization towards the M1 phenotype.[41] Collagen triple helix repeat containing 1 (CTHRC1), an intrinsic marker of CRC metastasis, regulates macrophage polarization towards the M2 phenotype via the TGF-β signaling pathway, promoting CLM.[42] Orosomucoid 1 (ORM1) mediates tumor immune tolerance by inducing macrophage M2 polarization, thereby advancing CRC progression and liver metastasis.[43] Osteoprotegerin (OPG) expression inhibits macrophage migration by blocking the RANKL-RANK pathway, and its downregulation in CRC cells promotes liver metastasis by activating TAMs.[44] STING activation promotes the nuclear translocation of TFEB by activating IRG1, inhibiting M2 macrophage polarization, and consequently suppressing CLM.[45] Additionally, KCs activation and their phagocytosis of tumor cells can reduce CRC cell metastasis to the liver.[46] KCs also induce liver metastasis via TGF-β1 signaling through the angiotensin II subtype receptor 1a (AT1a)

pathway.[47] In summary, TAMs play a pivotal role in CLM, with their polarization regulated by various cytokines, growth factors, chemokines, and other signals produced by tumor and stromal cells. Different signaling axes and factors distinctly influence TAMs accumulation and polarization, thereby affecting CLM progression. Furthermore, KCs activation and their tumor cell phagocytosis significantly contribute to suppressing CLM. These insights not only deepen our understanding of the mechanisms underlying CLM but also offer a theoretical basis and direction for developing novel targeted therapeutic strategies. The study lists the effects of various cytokines, growth factors, chemokines, etc. on the polarization of TAMs. For example, the CCL1-CCR8 axis, the TCF4-CCL2-CCR2 axis, CXCL16, and the CDC42 cargo carried by CRC-EVs all have regulatory effects on the polarization of TAMs and affect the progression of CLM. However, there are potential conflicts in the complex tumor microenvironment. Multiple factors interact, and different studies may have disagreements on the importance ranking or mechanism of action of certain factors. For instance, one study may emphasize the key role of a specific factor in the polarization of TAMs, while other studies may find that the role of this factor is masked or regulated by other factors. A critical evaluation shows that the tumor microenvironment is a highly complex system, and there are complex network relationships among various factors. When studying the effect of a single factor on the polarization of TAMs, it is difficult to completely exclude the interference of other factors. Therefore, it is necessary to comprehensively consider the synergistic or antagonistic effects of multiple factors. At the same time, the choice of experimental models will also affect the research results. In vivo models can better reflect the real physiological environment but are more difficult to control variables and in vitro models can better control the role of a single factor but may not be able to fully simulate the complex situation.

Metabolic Changes of TAMs

Macrophage metabolism plays a pivotal role in regulating their phenotypic polarization and function. The metabolic enzyme abhydrolase domain containing 5 (ABHD5) in macrophages has been shown to diminish the invasion and metastatic capabilities of CRC cells by inhibiting the production of matrix metalloproteinases (MMPs) through the NF-κB signaling pathway. This finding suggests that targeting ABHD5 in TAMs could serve as a potential therapeutic strategy for CRC.[48] RNA sequencing of various macrophage populations revealed that the LXR/RXR pathway is highly enriched in large macrophages, contributing to the upregulation of cholesterol metabolism, clearance receptors, MERTK, and complement genes.[49] At the infiltrative edge of CLM, there is a significant presence of pro-inflammatory monocyte-derived macrophages (MoMϕ) marked by the expression of SERPINB2, as well as more differentiated TAMs expressing glycoprotein nonmetastatic melanoma protein B (GPNMB).[50] $SERPINB2^+$ MoMϕ are indicative of early inflammatory responses, while $GPNMB^+$ TAMs are involved in matrix degradation, angiogenesis, and lipid metabolism pathways.[50] A high infiltration of $SERPINB2^+$ cells correlate with extended disease-free survival, whereas a greater density of $GPNMB^+$ cells is associated with poorer survival outcomes.[50] Moreover, studies indicate that taurocholic acid (TCA) facilitates CLM, with notable increases in the proportions of myeloid-derived suppressor cells (MDSCs), neutrophils, and macrophages in the liver following TCA injection.[12] The effective establishment of secondary liver metastases may also be attributed to macrophage-induced immunosuppression within the tumor microenvironment. Liver metastases show a significant upregulation of "bile acid metabolism" pathways.[51] These insights highlight the crucial influence of metabolic processes in the tumor microenvironment on CLM progression and suggest new potential targets for therapeutic intervention.

Role of miRNAs in TAM-Mediated CLM

Non-coding RNAs play a role in the progression of CLM by modulating TAMs. MicroRNAs (miRNAs), a broad category of small non-coding single-stranded RNAs, are recognized for their widespread presence and functional diversity, highlighting their significant biological roles. The decreased expression of miR-22 is associated with a supportive tumor microenvironment, which facilitates epithelial-to-mesenchymal transition and contributes to a cancer stem cell-like phenotype.[52] Up-regulation of miR-216b inhibited the growth of CRC tumors and M2-type polarization of macrophages in mice. Overexpression of miR-216b also reduced liver and lung metastasis of mouse tumor cells.[53] Furthermore, CRC cell-derived exosomal miR-934 was found to induce M2 macrophage polarization, thereby enhancing CLM through a CXCL13/CXCR5/NF-κB/p65 positive feedback loop.[54] In conclusion, non-coding

RNAs, especially miRNAs, are crucial in the progression of CLM by modulating TAMs. The dysregulation of miRNAs in the TME can influence critical processes, including epithelial-to-mesenchymal transition and macrophage polarization.

Cellular Interactions Between Macrophage and Components of TME

The complex interactions between macrophages, immune cells, and fibroblasts are vital in sustaining metastatic spread within the hepatic parenchyma. In patients with a high aspartate aminotransferase to platelet ratio index (APRI), there is an enrichment of inflammatory cancer-associated fibroblasts (CAFs) and SPP1-expressing macrophages. This enrichment is linked to the activation of malignant cells, the development of a fibrotic microenvironment, and a more suppressed T cell function.[55] Studies suggest that the increase in M2 macrophages and CAFs contributes to CLM progression.[56] Specifically, ANGPTL2 and SPP1 are notably enriched in CAFs and macrophages within CRC tissue, respectively, where ANGPTL2+ CAFs and SPP1+ macrophages facilitate CRC cell metastasis.[57] MYL9 in CAFs is implicated in modulating cytokine and chemokine secretion, which recruits M2 macrophages, inhibits dendritic cell activation, and fosters an immunosuppressive microenvironment.[58] $SPP1^{+}$ macrophages are especially predominant in liver metastasis.[59] Complement 5a (C5a) promotes tumor metastasis through its regulation of inflammation in CLM, with C5a receptor deficiency in metastatic colon cancer leading to reduced infiltration of macrophages, neutrophils, and dendritic cells.[60] The STING signaling pathway further plays a role in inhibiting CLM by promoting NK cell activity via 4–1BBL/4-1BB co-stimulation.[61] In summary, the interactions between macrophages, immune cells, and fibroblasts within the TME are pivotal in maintaining CLM, underscoring the potential of targeting these cellular interactions as a therapeutic approach for CLM management.

Crosstalk Between Macrophages and the Environmental Factors, Microbiota in CLM

KCs regulated by intestinal flora affect the occurrence and development of CLM. Liver metastases were observed in the Vanc group compared with the Coli group and the combined treatment group. vulgatus was more abundant in the Coli group before metastasis occurred. KCs might play a role in mediating the effects of gut microbiota on CLM. Specifically, P. mirabilis was found to enhance the migration of CT26 cells and promote CLM by decreasing the recruitment of KCs. In contrast, B. vulgatus appeared to inhibit CT26 cell migration and CLM by promoting the accumulation of KCs.[62] In summary, the crosstalk between macrophages and the microbiota plays a crucial role in CLM. These insights suggest that targeting the gut-liver axis and modulating specific microbial populations could provide strategies for managing CLM. The study found that cathepsin K (CTSK) is an important mediator of intestinal flora imbalance and CRC metastasis. Experiments found that when the intestinal flora is imbalanced, overexpression of CTSK leads to larger tumors and more metastases. CTSK can bind to TLR4 to stimulate the polarization of tumor-associated macrophages M2. It can also stimulate M2 macrophages to secrete cytokines to promote the metastasis of CRC cells. Clinically, overexpression of CTSK is associated with high M2 macrophages, CRC metastasis, and poor prognosis.[63] It is found that tumor-infiltrating bacteria such as Escherichia coli exist in CLM. They increase lactate production. Lactate inhibits nuclear factor-κB-gene binding (NF-κB) signal transduction through the lactylation of retinoic acid-inducible gene 1 (RIG-I), mediates M2 macrophage polarization, and affects the immunosuppressive activity of regulatory T cells (Tregs) and the anti-tumor activity of CD8 T cells. The RIG-I lactylation inhibitor screened by small molecule compounds can inhibit M2 polarization and make CLM sensitive to 5-fluorouracil.[64] In clinical practice, PRM1201 can increase the number of short-chain fatty acid-producing bacteria and the production of short-chain fatty acids in the feces of patients with CRC. The anti-metastasis effect is positively correlated with the recovery of short-chain fatty acids. Its anti-cancer metastasis mechanism is related to inhibiting histone deacetylation and epithelial-mesenchymal transition, highlighting the dependence on the microbiota.[65] Many studies have shown that the environment has an important impact on the treatment of CLM and may become a future development direction. Plexin B2 is a key host regulatory factor for liver colonization in mouse models of colorectal cancer and others. It interacts with related substances on tumor cells to upregulate KLF4 and promote tumor cells to obtain epithelial characteristics. Blocking the plexin-B2-semaphorin axis can prevent liver metastasis.[66] Chronic low-dose cadmium exposure can promote the invasion and metastasis ability of colorectal cancer cells in vivo and in vitro. Cadmium activates epidermal growth factor receptor (EGFR) in a non-classical way and promotes a continuous EGFR signal to trigger the protein kinase B (Akt)/mammalian target of rapamycin (mTOR)

cascade reaction. Blocking EGFR can eliminate the promotion effect of cadmium on liver metastasis of CRC cells.[67] In mice fed an ethanol-containing diet, metastasis occurs earlier and more severely. Alcohol can increase the expression of liver cytokines and factors related to the colonization of colorectal cancer cells by 1.5–3.0 times. Moreover, alcoholic liver injury is related to changes in liver localization and increased circulating levels of CEA released by CRC cells.[68] Therefore, research on lifestyle, immunity, and personalized molecular biomarkers as well as molecular pathological epidemiology is a promising direction. The influence of external factors is related to molecular pathology and can evaluate the efficacy and side effects of the therapy.[69,70]

Potential Mechanisms of TAMs to Promote CLM

CLM may have different growth patterns, angiogenesis characteristics, and immune microenvironments compared to the primary CRC. The potential of liver metastasis is related to the matrix composition of primary CRC. Differences in the expression patterns of type I and type IV collagen in CRC are associated with an increased risk of CLM.[71] CLM patients had significantly higher levels of circulating type IV collagen.[72] KCs may promote CLM by promoting ECM remodeling and promoting tumor cell invasion by CRC cells that evade initial phagocytosis.[73] Although numerous studies have explored the role and clinical relevance of TAMs in CRC, little is known about its role in CLM, which may be due to a different phenotype profile of macrophage expression in the liver.[74] Therefore, TAMs with different morphological and molecular fingerprints coexist in CLM and are associated with clinicopathological variables.[24] In patients with CLM, TAMs comprise at least two major cell subtypes, namely large TAMs (L-TAMs) and small TAMs (S-TAMs). The quantitative morphological characterization of TAMs can act as an easily quantifiable correlate of functional diversity with significant prognostic implications. TAMs can be used to reliably stratify patient outcomes and predict recurrence. Notably, a S-TAM has a more favorable prognosis compared to a L-TAM. The 3-year disease-free survival (DFS) rates for S-TAMs and L-TAMs are 60% and 8.5%, respectively.[18]

TAMs play crucial roles in various aspects of tumor progression, particularly in CLM. The mechanisms by which TAMs contribute to tumor liver metastasis are intricate, as they are implicated in almost every stage of metastasis.

TAMs Promote Angiogenesis of Tumor Cells

TAMs significantly influence tumor angiogenesis, a process essential for supplying nutrients and oxygen to support tumor growth, invasion, and migration.[75] Located within the tumor microenvironment (TME), TAMs are key players in promoting cancer cell survival and progression by producing various inflammatory mediators, growth factors, cytokines, and chemokines.[76] For example, SHP-2 deficiency in macrophages has been shown to enhance liver tumor metastasis by activating the Ang/Tie2-PI3K/Akt/mTOR pathway in Tie2-expressing monocyte/macrophages, which in turn promotes tumor microvascular formation.[77] Research indicates that macrophage infiltration is positively correlated with blood vessel density specifically in liver metastasis patients.[78] Notably, while TAMs generally exhibit an M1-like phenotype, metastasis-associated macrophages (MAMs) tend to be more M2-like and are particularly effective in promoting angiogenesis.[78] In both murine models and human studies, a subset of MAMs expressing VEGFR1 has been identified as key contributors to angiogenesis and metastasis.[78] KCs, the liver-resident macrophages, are instrumental in the formation of liver metastases.[79] The activation of TGFα-EGFR signaling in colon cancer cells leads to macrophage recruitment, resulting in an increase in both blood and lymphatic vessel surface area and enhanced lymphatic metastasis.[80] Additionally, the CX3CR1 receptor in macrophages has been linked to tumor metastasis and poor prognosis by promoting the survival of angiogenic macrophages.[81] TAMs also release VEGF, a potent pro-angiogenic factor, contributing to blood vessel remodeling and the progression of CRC.[82] VEGF-A secretion by CRC cells stimulates TAMs to produce CXCL1, which, in premetastatic liver tissue, recruits CXCR2-positive myeloid-derived suppressor cells (MDSCs) to establish a premetastatic niche, ultimately driving liver metastasis.[83] Furthermore, OPN-positive macrophages are known to produce VEGF, thereby enhancing angiogenesis and potentially facilitating cancer cell metastasis to the liver.[84] In the TME, tumor-derived VEGF enhances the Syk signaling pathway in macrophages, leading to the assembly of the CARD9-BCL10-MALT1 complex.[85] CARD9 plays a pivotal role in directing macrophage polarization towards the M2 phenotype through NF-κB pathway activation.[85]

TAMs Play a Critical Role in Facilitating Tumor Metastasis and Invasion

Metastasis requires tumor cells to spread from the primary tumor to different organs.[86] Metastasis involves the dissemination of tumor cells from the primary site to distant organs, and the ability of these cells to invade new tissues is a key indicator of malignancy.[87] A pivotal process in this context is the epithelial-mesenchymal transition (EMT), where epithelial cells gain mesenchymal traits, enhancing their migratory and invasive potential.[87] TAMs contribute to the metastatic process of colorectal cancer (CRC) by fostering a microenvironment conducive to EMT, primarily through the activation of the TGF-β signaling pathway. This allows tumor cells to acquire mesenchymal characteristics, enabling them to detach from the primary site and invade new tissues.[88]

Further, TAMs are involved in promoting CRC cell migration, invasion, and metastasis by inducing the EMT process via the JAK2/STAT3/miR-506-3p/FoxQ1 axis, which also enhances the production of CCL2, facilitating macrophage recruitment to the tumor microenvironment.[89] The activation of NLRP3 signaling in TAMs further supports the migratory and invasive capabilities of CRC cells.[90] Moreover, cytokine TWEAK, secreted by Th17 cells, interacts with its receptor Fn14 on tumor cells, promoting EMT and, consequently, tumor migration and invasion.[91] High TWEAK expression levels have been correlated with poor prognosis in CRC patients.[91] Additionally, the interaction between $CD163L1^{+}$ macrophages and Th17 cells recruits Th17 cells through the CCL4-CCR5 axis, further contributing to the invasive potential of the tumor.[91] Interestingly, certain mechanisms within TAMs can also inhibit metastasis. For instance, Src homology 2 domain-containing tyrosine phosphatase 2 (Shp2) on TAMs promotes macrophage polarization towards the M1 phenotype, which is associated with anti-tumor effects, thus reducing metastasis.[92] Similarly, the α7 nicotinic acetylcholine receptor (α7nAChR) in TAMs suppresses CRC metastasis through the JAK2/STAT3 signaling pathway[93].

Induction of Premetastatic Niche Formation

Formation of a premetastatic niche (PMN) is crucial in facilitating metastatic tumor growth in distant organs by creating an environment conducive to tumor cell colonization. The PMN is characterized by features such as increased vascular permeability, lymphangiogenesis, extracellular matrix (ECM) remodeling, and an immunosuppressive milieu.[94] For instance, exosomal miR-203a-3p contributes to M2 macrophage polarization by downregulating PTEN and activating the PI3K/AKT pathway.[95] These M2 macrophages secrete CXCL12, which enhances tumor metastasis via the CXCL12/CXCR4/NF-κB pathway, thus establishing the PMN in CLM.[95] The tumor microenvironment (TME) in CLM is notably immunosuppressive, with myeloid cells, particularly myeloid-derived suppressor cells (MDSCs), being polarized to an M2 state and T cells becoming exhausted. Myeloid cells further interact with various TME components, influencing processes like angiogenesis, tumor cell invasiveness, and EMT, and thereby aiding in PMN formation and promoting CRC liver metastasis.[96] High levels of serum exosomal miR-203 are linked to distant metastasis and are an independent predictor of poor prognosis. This microRNA promotes distant metastasis by driving host M2 macrophages to form the PMN.[97] Similarly, miR-21-5p, abundant in CRC-derived small extracellular vesicles (sEVs), is pivotal in inducing a pro-inflammatory hepatic environment, facilitating CRC liver metastasis through the miR-21-Toll-like receptor 7 (TLR7)-IL6 axis.[98] Additionally, VEGFA from CRC cells triggers TAMs to produce CXCL1, which attracts CXCR2-positive MDSCs, whose accumulation in the liver furthers PMN formation and supports liver metastasis [99].

Promotion of Tumor Cell Intravasation and Extravasation

TAMs also play a vital role in the intravasation and extravasation of CRC cells, processes crucial for metastasis.[100] Activation of EGFR is necessary for tumor cell intravasation[101], and TAM-derived EGF enhances CRC cell invasion and motility.[102] In CRC, TAMs promote tumor progression by remodeling the ECM.[103] VEGF further facilitates tumor cell extravasation by inducing endothelial gaps, as demonstrated by experiments with VEGF-expressing CT26 cells in mice.[104] Thus, TAMs with pro-inflammatory and pro-angiogenic traits are key players in promoting the metastatic spread of CRC cells through intravasation and extravasation.

Targeting TAMs in the Treatment of Metastatic CRC

Inhibiting TAMs Survival

Targeting TAMs represents a promising therapeutic strategy in managing metastatic CRC. Inhibiting TAM survival can hinder tumor proliferation, invasion, migration, and angiogenesis while enhancing anti-tumor immunity. Overview of macrophage-targeting drugs and their functions (Table 1). For example, blocking CSF1R with the inhibitor PLX3397 depletes M2 TAMs, boosts CD8 T cell infiltration, and improves responses to chemotherapy and immunotherapy, effectively curbing tumor growth and metastasis.[105] The drug TMP195 reduces F4/80+ TAM populations, which significantly inhibits the growth and vascularization of CLM.[106] Additionally, the combination of Oxaliplatin with Trifluridine/Tipiracil (FTD/TPI), an antimetabolite for treating chemotherapy-refractory metastatic CRC, can eliminate M2 TAMs in CT26 tumor-bearing mice.[107] Moreover, inhibiting FGL1 secretion, which is targeted by the FDA-approved antiseptic benzethonium chloride, enhances the effectiveness of anti-PD-1 therapy in liver metastasis by suppressing TAM activity.[108] Thus, inhibiting TAM activation is a beneficial strategy in the treatment of CRC metastasis.

Reprogramming TAMs

Reprogramming TAMs from an M2 to an M1 phenotype offers potential therapeutic benefits for patients with metastatic CRC. In CRC mouse models, anti-MARCO monoclonal antibodies can shift TAMs towards an M1 phenotype, enhancing anti-tumor activity and potentially inhibiting CRC metastasis.[109] Tasquinimod induces a shift from M2-like to M1-like TAMs, thereby modifying the TME to enhance immune regulation, inhibit angiogenesis, and suppress metastasis.[110] CCR5 antagonists have demonstrated the ability to repolarize TAMs to an M1 phenotype by affecting the STAT3/SOCS3 signaling pathway, showing promising anti-tumor effects in Phase I clinical trials for CLM.[111] M1-derived EVs containing oxaliplatin, retinoic acid, and Libidibia ferrea can induce TAM transition from M2 to M1 through the STAT3/NF-κB/AKT signaling pathway, effectively inhibiting metastasis in CRC mouse models.[112] Additionally, dual blockade of CTLA-4 and PD-L1 synergistically enhances CRC growth and metastasis inhibition by increasing $CD8^+$ and $CD4^+$ T cell counts, promoting Th1 responses, and inducing M1 macrophage polarization.[113] Nanodrugs like NCG inhibit MDSC differentiation, promote M1-like TAM polarization, disrupt the immunosuppressive barrier of tumor-associated fibroblasts, and increase effector T cell infiltration.[114] Dahuang Zhechong Pill reduces CLM by decreasing hepatic macrophage infiltration and M2 polarization, mitigating CCL2-mediated M2 skewing, and improving the fibrotic microenvironment.[115] Xiaoyaosan treatment significantly reduces the number of $CD11b^+F4/80^+$ TAMs and

Table 1 Overview of Macrophage-Targeting Drugs and Their Functions

Drug	Function	Reference
PLX3397	Depletion of M2 macrophages and increases $CD8^+$ T cell infiltration	[105]
TMP195	Inhibits $F4/80^+$ TAMs population	[106]
FTD/TPI	Depletes TAMs	[107]
Benzethonium chloride	Inhibits FGL1	[108]
Anti-MARCO	Increases M1 macrophages and decreases M2 macrophages	[109]
Tasquinimod	Leads to the transformation of M2 macrophages into M1 macrophages	[110]
CCR5 antagonist	Leads to the transformation of M2 macrophages into M1 macrophages	[111]
Oxaliplatin, retinoic acid, and Libidibia ferrea	Leads to the transformation of M2 macrophages into M1 macrophages	[112]
Anti CTLA-4 and anti-PD-L1	Induces polarization of macrophage M1	[113]
NCG(+) and anti-PD-L1	Induces polarization of macrophage M1	[114]
Dahuang Zhechong Pill	Decreases M2 polarization	[115]
Xiaoyaosan	Decreases $CD11b^+F4/80^+$TAM	[116]
Bufalin	Decreases M2 polarization	[117]
Macelignan	Decreases M2 polarization	[118]
NT157	Inhibits expression of CCL2 to inhibit recruitment of TAMs	[119]
AMG 820	Blocks the CSF1-CSF1R signaling to inhibit recruitment of TAMs	[120]
Grapefruit-derived nanovectors deliver miR-18a	Induction of IL-12	[121]

$CD11b^{+}GrloLy6Chi$ MDSCs, thereby inhibiting the progression of CLM.[116] Bufalin mitigates M2 polarization of KCs and downregulates IL-6 expression, inhibiting tumor metastasis.[117] Macelignan reduces IL-1β secretion by M2 macrophages, blocks NF-κB p65 nuclear translocation, and prevents IL-1β/NF-κB-dependent CRC metastasis through the ROS-mediated PI3K/AKT signaling pathway.[118] Therefore, influencing the polarization of TAMs is a promising strategy for the treatment of metastatic CRC.

Inhibiting TAM Recruitment

Inhibiting TAM recruitment involves targeting growth factors and chemokines that tumors use to attract TAMs, thereby promoting tumor growth.[122] NT157 reduces CRC cell migration and invasion and decreases metastatic lesion formation in the liver by inhibiting oncogenic cytokines, chemokines, growth factors, and TGF-β, consequently lowering TAM levels in the TME.[119] The anti-colony-stimulating factor 1 receptor (anti-CSF1R) monoclonal antibody AMG 820, used in combination with pembrolizumab, may inhibit TAM recruitment by reducing CSF-1R levels in metastatic CRC.[120] Moreover, miR-18a encapsulated in grapefruit-derived nanovectors (GNV) can inhibit liver metastasis by inducing M1 macrophages.[121] Therefore, targeting TAMs recruitment signals is a significant therapeutic strategy for treating CRC metastasis.

The above research presents various treatment strategies targeting TAMs, including inhibiting TAMs' survival, reprogramming TAMs, and suppressing TAMs recruitment. It also lists the action mechanisms of related drugs and methods and their effects in animal models. However, it is noted that translating these strategies into clinical success poses challenges. There is a potential conflict in that treatment strategies effective in preclinical studies may not work well in clinical trials. Different clinical trials may yield different conclusions, for instance, some drugs may be effective in certain patient groups but not in others. A critical assessment reveals that there are significant differences between preclinical studies and clinical trials. Preclinical studies are typically conducted in animal models, which may not fully mimic the complexity and diversity of human diseases. In clinical trials, factors such as individual differences among patients, tumor heterogeneity, and the complexity of combination therapy regimens all affect the treatment outcome. Additionally, factors like drug dose, administration route, and treatment duration need further optimization. For example, a drug may have an inhibitory effect on TAMs at a specific dose, but an excessively high or low dose may lead to reduced efficacy or adverse reactions.

Discussion

Liver metastasis is a predominant site of spread in CRC and remains the leading cause of death for these patients. Traditional approaches—chemotherapy, surgical resection, and local therapies-are commonly used to manage or control these metastatic tumors. However, the challenge of recurrence persists, emphasizing the need for deeper insights into the mechanisms of liver metastasis and the development of novel therapeutic strategies.

The TME has become a focal point in cancer research. This environment is characterized by complex interactions among cancer cells, endothelial cells, fibroblasts, and immune cells, all of which contribute to tumor growth and metastasis.[123] TAMs within this milieu can adopt either M1 or M2 phenotypes, playing a significant role in metastasis through mechanisms such as promoting angiogenesis. This makes TAMs promising targets for therapeutic intervention.

TAMs demonstrate considerable heterogeneity in their role in metastasis. Their interactions with cancer cells and other immune components within the TME are dynamic and multifaceted, influencing therapeutic responses. To comprehensively understand the mechanisms driving liver metastasis, researchers must investigate gene mutations unique to the liver TME and consider how these factors contribute to adaptive phenotypes. Advances in single-cell RNA sequencing, spatial transcriptomics, and high-throughput multi-omics are proving valuable in uncovering the detailed molecular landscape of liver metastasis. For instance, multi-omics studies have revealed that memory $CD8^{+}$ T cells, B cells, and $CTSB^{+}$ macrophages are prevalent at liver metastatic sites, forming crucial immune memory niches that support antitumor responses.[111] Single-cell analysis shows that primary CRC tumors have fewer myeloid cells, mainly monocytes and M1-type TAMs, whereas liver metastases are marked by an immunosuppressive TME with MDSCs, M2-type TAMs, and exhausted T cells.[83] These advanced techniques offer new insights into CRC metastasis and open avenues for enhancing CRC prognosis.

Despite promising preclinical data, translating TAMs-targeting strategies into clinical success has been challenging. Multicenter clinical trials are vital for advancing the diagnosis and treatment of liver metastasis. Merely blocking TAMs infiltration or inhibiting their cancer-promoting functions may not suffice for effective therapy. Combining TAMs-targeting agents with chemotherapy, radiotherapy, immunotherapy, and other treatments might provide more effective solutions for CRC progression and metastasis. Nonetheless, these combined approaches have yet to achieve significant clinical results. Further research is needed to assess their efficacy both as standalone treatments and in combination therapies.

This review outlines the origins, polarization, and functions of TAMs, underscoring their potential as therapeutic targets for liver metastasis. Despite the existing challenges, the rapid progress in biotechnology holds promise for identifying TAMs as a critical focus for future therapies. Continued research and clinical trials are essential to fully exploit the therapeutic potential of targeting TAMs in metastatic CRC.

In conclusion, while TAMs represent a promising target for treating liver metastasis, further investigation is crucial to define their roles in disease progression and refine therapeutic strategies. Ongoing exploration of TAMs biology and their interactions within the TME may lead to innovative treatments that could significantly improve outcomes for patients with liver metastasis.

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Disclosure

The authors declare that they have no competing interests in this work.

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