OncoTargets and Therapy

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REVIEW

Emerging Role of Immunotherapy for Colorectal Cancer with Liver Metastasis

This article was published in the following Dove Press journal:
OncoTargets and Therapy

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Abstract: Colorectal cancer (CRC) is the third most common malignant tumor in the world and the second leading cause of cancer-related deaths, with the liver as the most common site of distant metastasis. The prognosis of CRC with liver metastasis is poor, and most patients cannot undergo surgery. In addition, conventional antitumor approaches such as chemotherapy, radiotherapy, targeted therapy, and surgery result in unsatisfactory outcomes. In recent years, immunotherapy has shown good prospects in the treatment of assorted tumors by enhancing the host's antitumor immune function, and it may become a new effective treatment for liver metastasis of CRC. However, challenges remain in applying immunotherapy to CRC with liver metastasis. This review examines how the microenvironment and immunosuppressive landscape of the liver favor tumor progression. It also highlights the latest research advances in immunotherapy for colorectal liver metastasis and identifies immunotherapy as a treatment regimen with a promising future in clinical applications.

Keywords: immunotherapy, colorectal cancer, liver metastasis, tumor immune microenvironment, immune checkpoint inhibitors, deficient DNA mismatch repair

Introduction

Colorectal cancer (CRC) ranked third with 1.8 million new cases (10.2%), and second (9.2%) in mortality with an estimated 881,000 deaths recorded worldwide in 2018.[1] Due to its atypical clinical symptoms in the early stages, CRC is often ignored, leading to a delay in diagnosis and treatment.[2] Considering that the liver is the main filter for intestinal venous drainage, CRC patients often develop colorectal liver metastases (CLM).[3] Synchronous CLM occurs in approximately 15% of patients at the initiation of therapy, and approximately 50% of patients during follow-up.[4] Surgical resection is the standard treatment for patients with limited disease CLM, but most patients are not suitable for surgery because of extensive disease, tumor multiplicities, concomitant major systemic diseases, or poor hepatic functional reserve.[5] Only 20–30% of patients have operable tumors with a chance of cure.[6,7] In untreated CLM, the median survival duration is 7.5 months, and the 5-year survival rate is <1%. Even after surgery, the 5-year survival rate is between 20 and 45%, with a recurrence rate of 60%.[8]

Patients with CLM who are not eligible for resection have a poor clinical outcome, highlighting the need for novel therapeutic strategies. The combination of radiotherapy, chemotherapy, and traditional Chinese medicine has improved the prognosis and prolonged the median survival rate in these patients.[9] Although the introduction of cytotoxic agents have improved the survival in CRC patients and

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DovePress
http://doi.org/10.2147/OTT.S271955

provided good symptomatic relief, the prognosis in the setting of metastatic CRC (mCRC) remains poor.[10] A systematic review of combination treatment (5-fluorouracil, oxaliplatin, and irinotecan) for mCRC led to a conclusion that conventional chemotherapeutic agents had limited utility.[11] Patients with mCRC who were administered this triple chemotherapy regimen in conjunction with bevacizumab had a 3-year overall survival of approximately 40%.[12] Therefore, there is a dilemma on choosing the best treatment regimen among the currently available conventional therapeutic strategies that can improve the unfavorable long-term outcomes in patients with mCRC.

In recent years, immunotherapy has achieved a certain degree of success in the treatment of advanced solid tumors.[13] The purpose of immunotherapy is to enhance the anti-tumor effect of patient's own immune system by augmenting the innate immunity and antitumor function of T cells, and by targeting immunosuppressive tumor-associated macrophages.[14] Immunotherapies include immune checkpoint inhibitors (ICIs); cancer vaccines; and other biotherapeutics such as chimeric antigen receptor (CAR) T cells. These therapies are effective in treating a variety of cancers,[15] and mCRC, especially mCRC with deficient DNA mismatch repair (dMMR) genes.[16] DNA mismatch repair (MMR) is an evolutionarily conserved DNA excision-resynthesis that preserves genomic integrity by correcting mismatched bases that have evaded the proofreading activity of DNA polymerase during DNA replication.[17] Therefore, when this system is deficient, replication errors, including frameshift mutations and single-nucleotide variants accumulate,[18] resulting in the production of microsatellite instability (MSI).[19] Mechanistically, dMMR contributes to an accumulation of numerous genomic mutations, which generates multiple neoantigens and obvious response to immune therapy.[18,20] It has been reported that reported that patients with dMMR colon cancer showed a stronger response to immunotherapy, including regimes containing programmed cell death 1 (PD-1) plus cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) inhibitors, than those with MMR-proficient (pMMR) tumors.[21] The US Food and Drug Administration (FDA) approved PD-1 antibodies for CRC with high MSI or dMMR levels, including pembrolizumab and nivolumab.[22] In May 2017, the FDA further approved pembrolizumab for patients with microsatellite instability-high (MSI-H) or those with dMMR metastatic solid tumors after the failure of the initial treatment.[23]

However, immunotherapy provides limited or no clinical benefits to most patients owing to the inhibitory impact of tumor immune microenvironment (TME).[24] Some patients fail to develop an initial response to treatment with ICIs. Although the mechanism of drug resistance has been proposed to be due to functional changes in many signaling pathways, this process is still not fully understood. Currently known mechanisms of tumor cells evading immune surveillance include the destruction of the original processing mechanism of the antigen presentation machinery (APM) or the expression of HLA complexes (HLA class I heavy chains or 2-microglobulin [B2M]), resulting in defects in antigen processing. Studies have shown that MSI tumors with B2M mutations in CRC patients were resistant to anti-PD-1 monoclonal antibodies. Mutations in the *JAK1* and *JAK2* genes are another mechanism that leads to immune evasion. These genes encode kinases downstream of the IFN-γ receptor and are necessary to mediate the IFN-γ signaling pathway. Mutations in these genes were found in CRC patients resistant to PD-1 inhibitors.[25,26] There is also a view that resistance to immunotherapy is a special form of Darwin's natural selection, which originated from the selection of genetic or epigenetic features in tumor masses before therapeutic intervention. The main driver of tumor cell immune resistance mutations generated by this mechanism appears to be the genomic instability in transformed cells.[27] Here, we will discuss the emerging role and potential challenges of immunotherapies in patients with CLM.

Biology of the Metastatic Colorectal Cancer

Colorectal carcinogenesis is a process that is accompanied by high heterogeneity and accumulation of somatic molecular mutations, which is impacted by several factors, including exposures to intestinal pathogens and host immunity.[28] As previously noted, a major determinant of CRC-related morbidity and mortality is distant metastasis,[29] with liver metastasis being the most common metastatic event and a dominant factor influencing survival. Immune escape is responsible for impairment of anti-tumor immunity during the initiation and metastasis in CRC.[30] The tumor immune microenvironment (TME) consists of the neighboring tissues that surround the tumor, which include blood vessels, immune cells, fibroblasts, the extracellular matrix, and signaling molecules. The synergistic effect of malignant cells, immune and non-immune

stromal cells together with soluble and insoluble factors in TME is the basis for the progression of CRC. The TME can trigger CRC progression by either recruiting immunosuppressive immune cells, inducing other immunosuppressive mechanisms, or inducing chronic inflammation.[30,31] It has been proposed that metastatic spread of CRC can be mediated by multiple interactive mechanisms in the TME, which includes acquisition of aberrant immune phenotypes (NT5E/CD73, CD68, and CD163) via generation of immunosuppressive mediators.[30]

Desmoplastic, pushing, replacement, and two rarer patterns (sinusoidal and portal) are the histologic growth patterns (HGPs) in liver metastases. The different interfaces between tumor cells and adjacent normal liver parenchyma define the HGPs.[32] A recent study showed high HGP concordance within metastasis (95%) when classifying HGPs as desmoplastic (dHGP) or non-desmoplastic (non-dHGP).[33] The dHGP is characterized by angiogenesis and a peripheral fibrotic rim, whereas non-angiogenic HGP is characterized by the co-option of endogenous sinusoidal hepatic vasculature. The main characteristics of tumors with dHGP include angiogenesis and the fibrotic reaction (desmoplasia) that surround the metastases.[34] Another critical step in tumor metastasis and invasion is thought to be through epithelial-mesenchymal transition (EMT).[35] EMT is a developmental program in cancer cells that can activate cancer cells for invasion and metastasis. During the EMT process, epithelial cells undergo morphological changes, leading to an aggressive migration phenotype that is characteristic of mesenchymal cells.[36] CRC characterized by EMT is known to have an increased vascular invasion and metastasis and a poor prognosis.[35]

Additionally, the expression level of PD-1 in CD8+ T cells in CRC specimens TME is higher than that in CD8+ T cells in tumor-free lymph nodes.[37] Therefore, the PD-1/programmed death-ligand 1 (PD-L1) inhibitors have become a treatment option for CRC.[37] Furthermore, an increasing number of clinical data have demonstrated that immune cell populations can influence the immune response and clinical outcomes in CLM patients.[38] For example, the progression of CRC is closely related to tumor-infiltrating lymphocytes (TILs). Compared with the widely used tumor staging and lymph node metastasis, TILs can provide more accurate clinical predictions.[39] A greater density of TILs in the primary tumor was correlated with better survival[40] in CRC patients, particularly in those with dMMR tumors.[41]

Additionally, the formation of a pre-metastasis niche provides a supporting microenvironment for tumor cells to spread from the outside. The Features of the pre-metastasis niche include inflammation, immunosuppression, angiogenesis, etc.[42] Tumor-derived secreted factors, extracellular vesicles, bone marrow-derived cells, immunosuppressive cells, and host stromal cells constitute the pre-metastasis niche. Tumor exosomes can inhibit the recruitment of immune cells, increase angiogenesis in the pre-metastatic niche, and increase vascular permeability. Exosomes secreted by CRC cells promote angiogenesis and destroy vascular endothelial cell connections.[43] Recent evidence confirmed that miR-19a can inhibit CRC angiogenesis by targeting *KRAS* and *VEGFA*; exosomes secreted by CRC cells can trigger the downstream regulator *PEAK1* of the *EGFR/KRAS* pathway to reduce cell proliferation, migration, and invasion.[44] In CRC, tumor-derived exosomes facilitate the creation of pre-metastatic niche by modulating immune surveillance. Moreover, the liver inflammation microenvironment can be induced by CRC exosomes. Exosomes activate a pro-inflammatory phenotype in macrophages, which form an inflammatory pre-metastatic niche and promote liver metastasis.[45] Although the important role of pre-metastasis niche formation in CRC metastasis has been confirmed by many studies, the mechanism of CRC cells, inducing pre-metastasis niche formation, still needs further research.[42]

The immune system plays an essential role in the occurrence of tumors, suggesting a promising healing strategy for treating cancers, including CRC.[46] The clinical outcomes in all solid tumors, including CRC, are mainly impacted by the host's immune system.[47] A complicated interaction exists between immune cells and malignant cells within the TME of mCRC. The immune infiltrating cells in mCRC constitute both immune-supportive and immunosuppressive cells, and their relative ratio impacts the overall immune state of the tumor.[48] Increased level of immunosuppressive cells, such as myeloid derived suppressive cells (MDSCs), T-regulating cells (Tregs), type 2 (M2) macrophages, N2 neutrophils, and alternative cancer-related cell brands, including reduced antigen presentation, contribute to immunosuppression and eventual immune evasion in CRC.[49] For example, tumor-infiltrating neutrophils suppressed in vitro activated T cell proliferation via activation of the TGFβ signaling pathway in CRC.[50] Notably, neutrophil subsets can have either a protumor (N2) or antitumor (N1) phenotype by mediating TGFβ signaling.[30] Additionally, enhanced tumor invasion and growth is induced via MDSCs, extracellular matrix

remodeling, and angiogenesis mediated by M2 tumor-associated macrophages (M2-TAMs) in CRC.[30] Collectively, all of these cells are linked to cancer progression and unfavorable prognosis.[51]

Based on the genetic alterations, CRC can be divided into two clinically relevant subgroups: pMMR/microsatellite-stable (MSS) and dMMR/MSI.[52] In CRC, MMR deficiency leads to the accumulation of numerous insertions/deletions at DNA microsatellites, the repetitive DNA sequences with repeated units of 1–6 base pairs. This defect and the resulting MSI lead to genomic mutations that exhibit high levels of immunogenic tumor neoantigens targeted by the immune system.[53] This is linked to a high mutational burden in MSI cancers, the mutation rate of MSI is usually 10–50 times higher than that of MSS cancer.[54] Approximately 15% of CRC cases display MSI.[55] DMMR or MSI-H CRC are more responsive to ICIs because there are more somatic mutations; therefore, these patients experience more clinical benefit.[22,56,57] MSI-H tumors also possess a high level of TILs.[58] This is likely because MMR defects produce frame shift mutations, which result in the synthesis of truncated proteins contributing to antitumor adaptive immunity mediated by T cells, and this leads to a better response to immunotherapies and better prognosis.[59] Therefore, the altered mutation spectrum and molecular heterogeneity can be considered for therapy modification and patient stratification to avoid treatment-related toxicities.

However, the biological properties of liver metastases are reported to be distinct from those of the primary colorectal tumor.[60] CLM, a selective, non-random process, constitutes multiple steps, including local remodeling of the liver microenvironment.[44] MCRC cells enter the circulation by undergoing EMT to evade the host's defense system, survive in the blood circulation, and then disseminate and grow in the liver.[61] Immune dysfunction and immune evasion are the key mechanisms of CRC tumorigenesis and response to therapy.[62]

Therefore, from the available evidence, it can be concluded that the recruitment of immunosuppressive cells and other mechanisms of CRC tumorigenesis are pivotal to remodeling the TME and programming malignant cells toward a metastatic phenotype (Figure 1). This emphasizes the importance of the host CRC microenvironment in liver metastasis and highlights the potential of immunotherapy, including ICIs, in CRC, especially in dMMR CRC.

Immunotherapy as an Emerging Treatment for Cancer

Tumor cells have the capacity of growing exponentially and spreading rapidly, partly through inhibiting, evading,

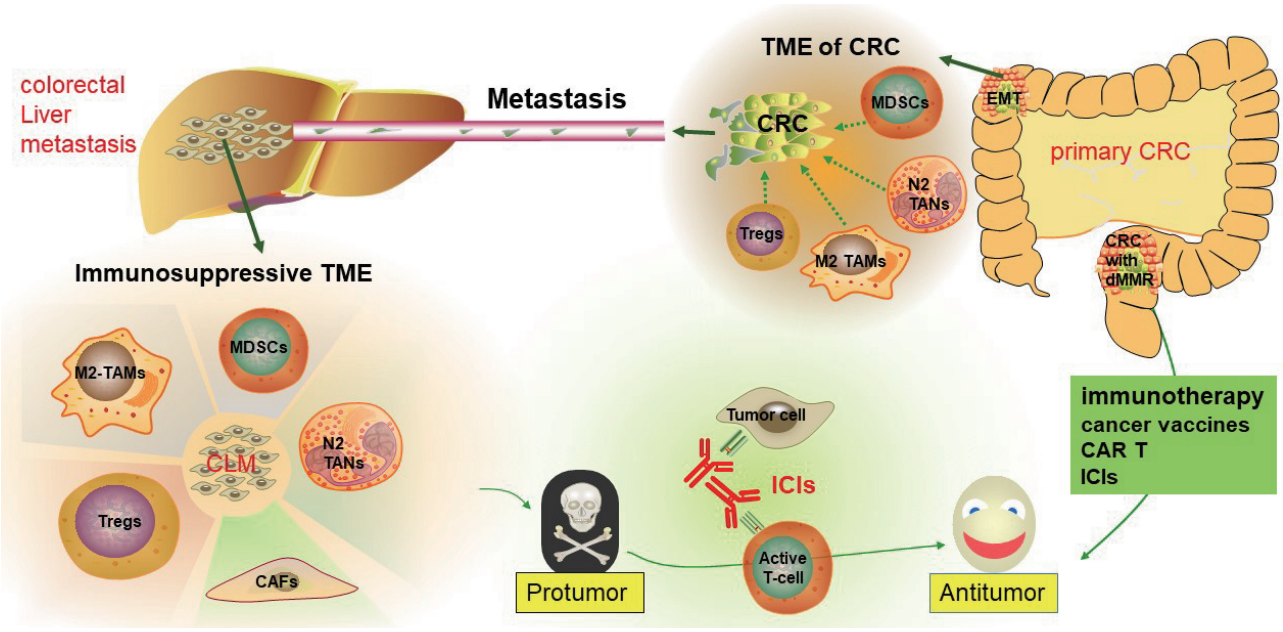

Figure 1 The immunosuppressive microenvironment of the liver and colorectal cancer contributes to liver metastasis and poor survival in colorectal cancer, which can be treated using immunotherapy.

Abbreviations: CAFs, cancer-associated fibroblasts; CAR-T, chimeric antigen receptor T cell; CRC, colorectal cancer; EMT, epithelial-to-mesenchymal transition; M2-TAMs, M2-type tumor-associated macrophages; TANs, tumor-associated neutrophils; TME, tumor immune microenvironment.

and exploiting the host immunity.[63] In the recent few years, immunotherapy has shown impressive efficacy and has revolutionized the therapeutic landscape of multiple malignancies by strengthening immune responses.[64] Unlike conventional cancer therapies, immunotherapy potentiates the patient's immune system or modulates the TME instead of directly targeting tumor cells.[13] Malignant cells can escape the antitumor immunity; therefore, the major aim of immunotherapy, such as ICIs, is to block the immune evasion mechanisms of tumor cells,[65] thereby suppressing tumor progression, relapse, and metastasis.

PD-1 and CTLA-4 are receptors that inhibit T cell responses to maintain peripheral tolerance, thereby permitting the tumor cells to grow rather than being eliminated by the immune system. ICIs can potentiate anticancer immune responses via suppression of the inhibitory receptors expressed on T lymphocytes and on the surface of malignant cells (PD-L1), including CRC cells.[66] PD-L1 interacts with PD-1 to induce rapid phosphorylation of SHP-1 and SHP-2 to exert an inhibitory effect.[15] ICIs are the antibodies against CTLA-4, PD-1, or PD-L1 that can restore antitumor immune responses, leading to impressive clinical responses in various cancers.[67,68] The most successful immunotherapeutic strategy is blocking the pD-1/PD-L1,[69] and this is linked to strong clinical responses in many cancers, such as skin carcinomas, head and neck carcinoma, advanced non-small cell lung cancer, and renal cell carcinoma.[70] CTLA-4 modulates T cell function by competing for shared ligands with CD28 receptors expressed by both CD4+ and CD8+ T cells. Accordingly, CTLA-4 blockade by anti-CTLA-4 antibodies can render CD28 ligands more available, permitting the activation of effector T cells and suppression the Tregs that modulate homeostasis,[71] thereby inhibiting tumor progression.[72]

It has been confirmed that higher levels of lymphocyte activation gene-3 (*LAG-3*), T cell immunoglobulin mucin-3 (*TIM-3*), and T cell immunoreceptors with Ig and ITIM domains (*TIGIT*) were expressed in colorectal tumor tissues.[73] Therefore, they are potential therapeutic targets for immune-mediated therapy because of their important role in tumor immune evasion.[73] They are highly expressed in T cells that are stimulated by persistent antigens.[74] TIM-3 is considered to be a key immune checkpoint for regulating T cell responses. The expression of TIM-3 on Tregs can form an immunosuppressive tumor microenvironment and promote immune evasion and tumor progression. TIM-3 in colorectal cancer tissues is significantly related to tumor lymph node/distant metastasis. The expression of TIM-3 in CRC TME predicts T cell failure and promotes tumor metastasis. Accurate characterization of TIM-3+ T cells should contribute to specific targeted therapy, enhance anti-tumor immunity, and improve clinical response.[75] The proliferation and invasion of CRC cells are directly related to TIM-3.[76]

LAG-3 is another important immune checkpoint of the immunoglobulin superfamily. It is expressed in a variety of immune cells and inhibits the proliferation and activation of T cells. It encodes a surface molecule that is selectively upregulated in Treg and is a key regulator that controls the maximum Treg activity.[77] LAG-3 binds to a major histocompatibility complex II (MHC class II) and causes a decrease in CD4+ T cell activity.[78] Many preclinical studies have confirmed that LAG-3-mediated signaling led to cytotoxic CD8+ T cells exhaustion. PD-1/PD-L1 treatment can activate LAG3 block and restore anti-tumor immunity.[79] In the colorectal adenocarcinoma model, the combination of anti-PD-L1 and anti-TIM-3 can enhance the anti-tumor activity of T cells. Similarly, in the MC38 colorectal adenocarcinoma model, the combination of anti-PD-1 and anti-LAG3 antibodies inhibited tumor advancement. Therefore, treatment regimens targeting LAG-3 may benefit patients with CRC.[80]

The inhibitory receptor TIGIT is a new target for immunotherapy. It is expressed in immune cells in colorectal cancer, including natural killer (NK) cells and CD8+ TILs.[81] TIGHT can inhibit NK cells and effector T cells, and also inhibit the immune response by promoting Treg.[82] The combined effect of anti-TIGIT antibodies and ICIs can enhance the function of T cells in tumors, thereby promoting tumor clearance. Many clinical studies are currently investigating the therapeutic effects of TIGIT block alone or in combination with PD-1 on various cancers.[83] Blocking TIGIT in NK cells can restore the NK cells' powerful effect in vivo and reverse their functional failure.[84]

The response rates to anti-PD-1/PD-L1 antibodies and anti-CTLA-4 antibodies in CRC patients were far from satisfactory. However, these new-generation immune checkpoints are promising therapeutic targets for clinical application.[85]

Adoptive T cell therapy (ACT) is applied to anti-tumor therapy through the treatment of transferred T cells.[86] This process includes isolation of TILs with antitumor activity, followed with in vitro extensive expansion, activation, and subsequent administration to the patients.[87] In an

adaptation of this method, T cells with genetically engineered neoantigen-reactive T cell receptors (TCRs) were generated to enhance the efficiency of this strategy.[88] The T cells used for ACT can be endogenous CD8+ T cells isolated from TILs or autologous circulating CD8+ T cells with genetically engineered antigen receptors.[89] TILs, a crucial component of the TME, have been reported to be related to therapeutic responses and clinical outcomes.[90] TILs capable of recognizing tumor antigens are responsible for the highly specific antitumor immune response. TILs are also less toxic than TCR-modified T cells or CAR-T cells. Moreover, TILs have heterogeneous specificity, indicating a pivotal advantage for counteracting immune escape.[63] Therefore, TILs can function as an ideal source of tumor-reactive TCRs for personalized cancer immunotherapy and can also act as vehicles for ACT therapies and TCR gene therapies.[91] Using TILs in ACT correlated with favorable overall response rates and sustained remission.[92] In addition, immune therapies targeting toll-like receptors exhibited antitumor activity in CRC via activation of anticancer immunity or suppression of oncogenic signaling pathways.[93]

The use of CAR-T cells as an ACT method, or obtaining antigen-specific T cells through genetic engineering,[94] holds an edge over the previous approaches using these modifications.[87] CAR-T cells have shown promising curative effect, especially in hematological malignancies.[95] CAR-T therapy has been approved in patients harboring B cell malignancies,[96] and the FDA recently approved anti-CD19 targeting CAR-T cell therapy for both acute lymphoblastic leukemia and diffuse large B cell lymphoma.[97] The capacity of CAR-T cells, including their improved expansion to tumor loci, long-lasting in vivo persistence, and synergy with the endogenous immune response, may permit this living, replicating therapy to trigger prolonged antitumor control in patients.[98] Outside of the hematological malignancies, the therapeutic potential of CAR-T cells in solid cancers has recently been confirmed. However, further research is required to thoroughly utilize CAR-T cells in the management of solid cancers. Solid cancers are more complicated than hematological malignancies, which makes it difficult for T cells to eradicate tumor masses and maintain lasting regression.[99]

Recently, CAR-T cell therapy has produced great success in the treatment of CLM in preclinical models.[100] In a carcinoembryonic (CEA) positive mouse model of colon cancer, recombinant lentivirus-modified peripheral lymphocytes with a chimeric T cell receptor demonstrated antitumor efficacy.[101] In another CRC mouse model, intraperitoneal administration of CAR-T cells in conjunction with reduction of MDSCs and Tregs also showed antitumor potential.[100] CAR-T cells against other targets, CD133, CYAD-101, EGFR, and IL-12, have also demonstrated potential antitumor activity in preclinical and clinical environments.[100] In a Phase I clinical study, CART72 cells, the first-generation CAR-T cells, showed a good safety profile by intravenous injection or direct hepatic artery administration in patients with CLM.[102] Another Phase I study reported that CEA CAR-T cell therapy was tolerable and had efficacy in 8 of 10 refractory and relapsed CEA-positive patients with CLM.[103] However, a durable response to CAR-T cell therapy is only observed in a small number of CRC cases harboring CLM due to limitations such as augmented toxicities, recurrence, limited trafficking, and an unfavorable TME.

After the first successful attempt at cancer immunotherapy by Dr. William Coley, bacillus Calmette-Guérin, a live attenuated vaccine against *Mycobacterium tuberculosis*, was evaluated for treatment of bladder cancer.[104] This encouraged the investigation and approval of cancer vaccines as a type of cancer immunotherapy by the FDA.[105] Cancer vaccines are designed to induce an intense immune response to one or more tumor-specific antigens, thereby driving antitumor cytotoxicity.[106] This is achieved by injecting cancer-specific elements into patients to stimulate an immune attack that is highly specific to the patient's tumor cells.[107] Notably, vaccines can influence the TME by modifying antigen-specific B and T cell responses.[65,108] The different impacts of varied levels of neoantigen clonality on TME might offer opportunities to refine the targets used in the vaccines, indicating the valuable role of adjuncts in the treatment of CRC.[109] Other potential therapeutic strategies, such as hepatic macrophages and Tregs, were also found to be rational targets for antitumor intervention.[30,110]

Despite the establishment of multiple emerging therapeutic strategies in CLM, the lack of consistently successful clinical outcomes highlights the necessity of further research to develop novel treatment approaches or enhance the efficacy of existing strategies to improve clinical benefits.

The Hepatic Tumor Microenvironment

A tumor is a complex tissue constituting tumor cells and non-malignant components such as stromal fibroblasts,

inflammatory cells, vasculature, normal epithelia, extracellular matrices and secreted factors; all of these are considered to be the TME.[111] In the TME of the liver, non-malignant cells can promote immunosuppression and favor tumor growth by contributing to proliferation, invasion, migration, and metastasis of malignant cells;[112] that is, the liver is a unique organ in which a characteristic TME facilitates the capability of cancer cells to evade natural host defenses and augments their ability to grow and migrate, contributing to a skew toward tolerance.[113] The quantity and differentiation of infiltrated immune cells in the TME, especially immunosuppressive cells,[114] is closely associated with tumorigenesis and metastasis, as well as the cellular response to therapy.[115] Eynde and his colleagues noted that the densities of T/B cell infiltration differed in 603 distinct CLMs from the same case,[116] suggesting diverse patterns of genetic variations within these metastatic sites. With the increasingly wide utilization of immunotherapy, including ICIs, assessing the heterogeneity of the TME landscape and remodeling the TME have emerged as promising avenues to improve treatment efficiency and patient prognosis.[117] Furthermore, lymphocyte infiltration and plasma cell infiltration are linked to prolonged patient survival in CLM.[118,119]

Several key factors contribute to the extravasation of CRC cells into the liver.[120] First, the blood circulation system in the liver is complicated and enriched. For example, CRC cells metastasize to the liver through the portal circulation, which accounts for 75% of the blood flow in the liver.[121] Simultaneously, the slow microcirculation in the hepatic sinusoidal vessels permits retention of malignant cells in the liver. Second, the lack of a basement membrane in the liver endothelium allows cancer cells to efficiently attach to microvascular endothelium for developing micrometastases. Finally, high levels of some surface molecules expressed on liver-resident cells contribute to malignant cell migration from the vascular lumen into the space of Disse.

The cell types in tumors mainly include cancer cells, endothelial cells, hepatic stellate cells, mesenchymal stem cells, cancer-related stromal fibroblasts, and immune cells.[122,123] Infiltration of immune cells, including macrophages, monocytes, lymphocytes, NK cells, and dendritic cells (DCs), into the tumor tissue at early stages of tumor progression is essential for targeting cancer. However, the antitumoral immune effects could be counteracted by the action of immunosuppressive cells within the developing TME, including MDSCs, Tregs, tumor-associated neutrophils, cancer-associated fibroblasts, and M2-TAMs.[112,122]

The hepatic microenvironment also constitutes various other cell types like liver sinusoidal endothelial cells (LSECs), hepatocytes, Kupffer cells (KCs), liver-associated lymphocytes, and hepatic stellate cells.[124] Because 75% of the blood supply of the liver comes from the intestine via the portal vein,[125] the liver is enriched with antigens and microbial products from the digestive tract. Moreover, due to its anatomical site, the liver is frequently exposed to pathogens as well as non-pathogenic stimuli.[126] The TME of the liver exerts a key effect in antitumor response by activating naïve T cells via presenting antigens by LSECs, KCs, DCs, and hepatocytes. However, the liver's immunosuppressive microenvironment promotes tolerance to numerous endogenous and exogenous intestinal bacteria and antigens and other endogenous and exogenous stimuli.[127] As a result, the liver cannot attack cancer cells.

LSECs, the initial cells encountered by metastatic cancer cells, can function with KCs as a physical platform for recruiting and anchoring immunological cells and are the gatekeepers for liver immunomodulation.[128] Hepatocytes can activate naïve CD8 T cells and promote the apoptosis and elimination of T cells in a Bim-dependent way, thereby triggering antigen-specific immunological tolerance.[129] Other mechanisms include defects in antigen presentation, recruitment of immunosuppressive cells, inhibition of NK cells, compromised function of CD4+ T cells, and upregulation of immune checkpoint signaling.[130] In CLM patients, the tumor-specific T cell response constitutes an expanded population of activated Tregs and MDSCs.[37] MDSCs are considered as the most important cell types for the organization and maintenance of local immune suppression in mCRC.[131]

Hepatic macrophages are a heterogenic population including both resident KCs and recruited monocyte-derived macrophages.[110] KCs, a subpopulation of macrophages with an immunosuppressive phenotype, control liver metastasis via regulating Dectin-2, a C-type lectin innate receptor.[132] Another critical determinant of CLM, TAMs, nurture malignant cell growth and metastasis by mediating immunosuppression and secreting pro-inflammatory factors, including IL-10 and TGFβ, eventually leading to poor prognosis.[38] TAMs can also express inhibitory receptors including PD-L1/2, B7-1/2, and the non-typical MHC-I proteins.[133] Therefore, TAMs may be

able to synergize with chemotherapy, radiotherapy, and unconventional targeted therapies.[38]

Secretory proteins can exert both antitumor and protumor effects during CLM.[61] For example, the tumoricidal activity of LSECs is partly determined by cytokines. Additionally, activated LSECs and KCs can also promote metastasis by triggering the expression of cytokines (namely, TNF-α and IL-1) and adhesion molecules (CD44, CD15s, and mucin among many others).[60] In addition, activated NK cells can stimulate KCs to release granulocyte macrophage colony stimulating factor and IFN-γ.[134] Multiple chemokine signaling pathways have been reported to be linked to liver metastasis, including those that involve CXCL1–CXCR2, CXCL12–CXCR, CCL2–CCR2, CCL15–CCR1, and CX3CL1–CX3CR1 chemokines.[135] Therefore, secreted factors, such as chemokines and cytokines, can remodel the TME of CLM by recruiting host-derived myeloid cells to promote metastasis and immune escape.

In conclusion, the immunosuppressive nature of the intrahepatic milieu might be responsible for the establishment of CLM and the aggressive pathology of the disease.[136] Therefore, targeting TME components, including immune checkpoints, immunoregulatory cells and their secretory factors, and the tumor structure,[122] may be an option to prevent and treat CLM.

Immunotherapy Solutions for Liver Metastases

Metastases have been and remain the main obstacle to a successful management of malignancies. Even among cancers that are sensitive to radiotherapy or chemotherapy, one of the principal pathways of treatment failure is metastasis. The previous treatments for CRC showed minimal improvement in the 5-year survival rate, even with relapses that followed a complete surgical resection,[137] especially among the patients with liver metastasis. Therefore, new treatment strategies are desperately needed. One such approach is immunotherapy.[106] Previous findings have confirmed that blocking protumorigenic interactions and cancer-stromal interplay via the accumulation of myeloid cells and T cells and blocking protumorigenic cytokine signaling by targeting CCR5, achieved clinical benefits among patients with advanced/metastatic CRC.[64]

Liver metastasis occurs in multiple cancers and is related to an unfavorable prognosis.[132] Among the main metastatic sites of CRC are also the liver and lungs. The liver's immunosuppressive microenvironment supports the generation of both pre- and pro-metastatic niches to develop hepatic metastases.[138] For instance, the treatment efficacy of CAR-T cells engineered against hepatic metastases can be undermined by the accumulation of MDSCs. Hepatic MDSCs can express PD-L1, and PD-L1 can inhibit the activation and proliferation of T cells by interacting with PD-1 on T cells. Reduced MDSC accumulation within the liver via PD-L1 blockade synergizes with CAR-T cell therapy to achieve antitumor responses. Moreover, blockade of molecules involved in MDSC biology and function augmented the efficiency of ACT against CRC metastases.[139]

Many ICIs are being evaluated in prospective trials of various cancer types, including CLM.[140] Clinical trials with pembrolizumab have confirmed that patients with dMMR mCRC pretreatment benefited from ICIs.[141] The immune-related objective response rates (ORRs) and progression-free survival rates in patients with MSI-H CRCs were 40% and 78%, respectively, and 0% and 11%, respectively, in those with MSS/pMMR CRCs.[142] Compared to MSI cancers, MSS cancers are less sensitive to immunotherapy alone, and novel combination approaches are being investigated to enhance therapeutic efficiency in patients who might be sensitive to combination treatments.[143] DMMR CRCs are prone to be heavily infiltrated by CD8+ T cells and highly expressed immune checkpoint molecules, including PD-L1.[144] Response Evaluation Criteria In Solid Tumors (RECIST) assessment was performed in 10 patients with dMMR CRC, with an objective response rate (ORR) of 40% and an objective remission rate of 0% in MMR-proficient (MMR-p) CRC. In dMMR CRC and pMMR CRC, the disease control rate at 12 weeks was 90% and 11%, respectively.[145] This might partly explain why anti-PD-1/PD-L1 therapies are correlated to antitumor responses in MSI-H or dMMR mCRC. The FDA has approved pembrolizumab and nivolumab for cases with dMMR or MSI-H mCRC.[146] Indeed, pembrolizumab is now approved by the FDA for the treatment of all dMMR-MSI-H metastatic solid tumors.[144]

The Phase II CheckMate 142 clinical trial evaluated the nivolumab in conjunction with or without ipilimumab in dMMR or MSI-H mCRC. Preliminary data revealed an ORR and disease control rate of 31% and 69%, respectively. When ipilimumab was added to nivolumab, the ORR and disease control rates of 41% and 78%, respectively, were noted.[141] Nivolumab has been approved for

patients harboring dMMR/MSI-H mCRC who experience disease progression following treatment of fluoropyrimidine, oxaliplatin, and irinotecan.[147] Targeting immune checkpoints has achieved good response in multiple tumor types, but response rates varied in CRC with distinct genomic alterations.[148] Among mCRC patients with MSI-H tumors, ICIs resulted in good responses to some extent. The ORR of pembrolizumab or nivolumab varies between 30% and 40%, and 50% of cases are controlled. These drugs have been approved for the second-line treatment of mCRC patients with dMMR/MSI-H tumors.[149] Additionally, ipilimumab plus nivolumab improved the ORR to 55% among these patients. Based on this data, ICIs have even been investigated as monotherapy in MSI-H mCRC.[149] Even in MSS CRCs, mutational/neoantigen load has demonstrated some association with immune infiltration and survival, showing promise for successful exploration of immunotherapies.[150]

The median PFS in patients with advanced CRC treated with pembrolizumab combined with chemotherapy was 16.9 months, and the median OS was 18.8 months (median follow-up 7.9 months).[151] In phase Ib/II trials of RAS wild-type mCRC patients treated with pembrolizumab plus cetuximab, seven of nine patients were stable and had good tolerance.[151]

Adoptive cell therapy (ACT) using TILs has shown significant efficacy in patients with metastatic melanoma. Striking responses have been observed in individual patients with CRC after ACT.[152] In order to improve NK cell tumor response, CAR was designed to fuse the extracellular domain of NK cell receptor NKG2D with DAP12. NKG2D ED and DAP12 make up the CAR. A study confirmed that NKG2Dp CAR-NK cells could indeed recognize tumor cells and exhibit anti-tumor effects in CLM patients.[153] This is an achievable way to combat liver metastasis by using new techniques developed in recent years. Because it can be harvested aseptically and does not contaminate the intestinal flora, liver metastasis may be an ideal source of TILs for the treatment of ACT in CRC patients.[154] TILs have been isolated from CRC patients in several studies and their potential as an immunotherapy modality has been evaluated. TILs expanded in vitro mainly contain effector memory T cells, and have been found to trigger antitumor responses.[155]

Cancer vaccines have been used to prevent relapse in patients suffering from CRC.[156] Approximately 61.3% of patients with advanced CRC who received vaccination developed cell-mediated immunity. However, whether vaccinated or not, the same overall survival rate of 48% in CRC patients was observed in all groups.[41]

Table 1 Summary of Immunotherapy Approaches for Colorectal Cancer with Liver Metastasis

Type	Immunotherapy	Specific Drug	Reference
Checkpoint inhibitors	Anti-PD-1 or PD-L1 antibodies	Pembrolizumab nivolumab	140
	Anti-CTLA-4 antibodies	Ipilimumab	140
ACT	CAR T cells	NKG2D	153
	TILs		152,154
Vaccines			41,156

Abbreviations: PD-1, programmed cell death 1; PD-L1, programmed cell death-ligand 1; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; ACT, adoptive T cell therapy; CAR, chimeric antigen receptor; TILs, tumor-infiltrating lymphocytes.

Overall, immunotherapy might be a feasible option for most CRC patients with liver metastases. Standard treatment of CRC also includes antiangiogenic drugs. When combined with immunotherapy, anti-angiogenic drugs may also show synergistic effects.[157] As monotherapy and in combination therapy, the role of immunotherapy will undoubtedly evolve.

A variety of current immunotherapies for CLM are shown in (Table 1).

Conclusions

Treatment of mCRC, especially CLM, is challenging because of its anatomical location and the immunosuppressive microenvironment. Immunotherapy is a novel effective therapeutic strategy. Despite increasing clinical data regarding the therapeutic role of ICIs among dMMR or MSI-H mCRC, most patients harboring pMMR or MSS tumors still do not benefit from immunotherapeutic agents.[158] This may partly be explained by the inhibitory impact of multiple suppressive networks on effector immune cells to enable CRC to develop and form metastases within the TME.[122] This necessitates further research to develop novel therapeutic approaches or identify biomarkers for personalized modulation of the TME to reverse immunosuppression, thus improving clinical outcomes.[106] Specifically, the immune cells in the TME, together with the soluble factors, might also be potential targets to treat CLM. Still, immunotherapy holds promise as a rational treatment option, either as

a monotherapy or as combinational therapy.[159] This is especially true when immunotherapy is combined with other therapies to address the problem of low mutational load by increasing tumor immunogenicity, thus overcoming the immunosuppressive microenvironment. This is also the case for the treatment of CLM. Outside of the discovery of new immunotherapy targets, further directions should be focused on relieving CLM from the inhibitory networks and activation hurdles constituting the TME by combining immunotherapies with other therapies.

Abbreviations

ACT, adoptive T cell therapy; APM, antigen processing machinery; BMDCs, bone marrow-derived cells; CAR, chimeric antigen receptor; CAR-T, chimeric antigen receptor T cell; CLM, colorectal liver metastasis; CRC, colorectal cancer; CTLA-4, cytotoxic T-lymphocyte-associated antigen 4; DCs, dendritic cells; dMMR, deficient DNA mismatch repair; EMT, epithelial-mesenchymal transition; EVs, extracellular vesicles; FDA, Food and Drug Administration; ICIs, immune checkpoint inhibitors; IFN, interferon; HGPs, histological growth patterns; KCs, Kupffer cells; LAG-3, Lymphocyte activation gene-3; LSECs, liver sinusoidal endothelial cells; mCRC, metastatic colorectal cancer; MDSCs, myeloid-derived suppressor cells; MMR, DNA mismatch repair; MSI, microsatellite instability; MSI-H, microsatellite instability-high; MSS, microsatellite-stable; M2-TAMs, M2 tumor-associated macrophages; NK, natural killer; ORRs, objective response rates; PD-1, programmed cell death 1; PD-L1, programmed death-ligand 1; pMMR, mismatch repair-proficient; TDSFs, tumor-derived secreted factors; TIGIT, T cell immunoreceptors with Ig and ITIM domains; TILs, tumor-infiltrating lymphocytes; TIM-3, T cell immunoglobulin mucin-3; TME, tumor immune microenvironment; TNF, tumor necrosis factor; Tregs, T regulatory cells; TCRs, T cell receptors.

Acknowledgments

This work was funded by the National Natural Science Foundation of China (grant number 31301141) and the Science and Technology Planning Fund of Guizhou Province (Guizhou Science and Technology Cooperation LH [2014] No. 7594).

Disclosure

The authors report no conflicts of interest for this work.

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