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"Cold" colorectal cancer faces a Bottleneck in immunotherapy

Liu JL *et al.* "Cold" CRC faces a Bottleneck in immunotherapy

Abstract

The advent of immunotherapy and the development of immune checkpoint inhibitors (ICIs) are changing the way we think about cancer treatment. ICIs have shown clinical benefits in a variety of tumor types, and ICI-based immunotherapy has shown effective clinical outcomes in immunologically “hot” tumors. However, for immunologically “cold” tumors, such as colorectal cancer (CRC), only a limited number of patients are currently benefiting from ICIs owing to limitations such as individual differences and low response rates. In this review, we discuss the classification and differences between hot and cold CRC and the current status of research on cold CRC, and summarize the treatment strategies and challenges of immunotherapy for cold CRC. We also explain the mechanism, biology, and role of immunotherapy for cold CRC, which will help to clarify the future development of immunotherapy for cold CRC and discover more emerging strategies for the treatment of cold CRC.

Key Words: Immunotherapy; “Cold” colorectal cancer; Immune checkpoint inhibitors

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Core Tip: Immune checkpoint inhibitors (ICIs) are usually produced by antibodies, and their effectiveness relies on the antitumor effects of immune cells (especially T cells).

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Colorectal cancer (CRC) is one of the most common forms of cancer worldwide. only a limited number of patients are currently benefiting from ICIs owing to limitations such as individual differences and low response rates. In this review, we discuss the classification and differences between hot and cold CRC and the current status of research on cold CRC, and summarize the treatment strategies and challenges of immunotherapy for cold CRC.

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INTRODUCTION

Colorectal cancer (CRC) is one of the most common forms of cancer worldwide^[1]. Globally, CRC is the second most common cancer in women and the third most common in men^[2]. More than half of the development of CRC can be attributed to modifiable risk factors such as smoking, unhealthy diet, heavy alcohol consumption, lack of physical activity, and overweight; therefore, the disease is preventable^[3]. Despite some progress in the diagnosis and treatment of CRC, it remains a significant cause of cancer-related deaths^[2]. The global burden of CRC is expected to increase by 60% by 2030^[4]. Therefore, there is an urgent need to develop new preventive and treatment strategies for this disease^[1].

In contrast to traditional cancer therapies that affect the proliferation, survival, and metabolic activities of tumor cells^[5], immunotherapy mainly works by modulating the tumor microenvironment (TME), restoring anticancer immunity, and stimulating or suppressing the immune system to play an antitumor role^[6]. Immune checkpoint inhibitors (ICIs) are usually produced by antibodies, and their effectiveness relies on the antitumor effects of immune cells (especially T cells)^[7].

However, most solid tumors have little T-cell infiltration and are defined as non-T-cell inflammatory or “cold” tumors^[8]. In CRC, it has been shown that only patients with mismatch repair deficiency (dMMR) or microsatellite instability (MSI) high (dMMR/MSI-H) tumor subpopulations respond to treatment with ICIs^[9-11]. Clinical trials related to ICIs have been conducted for the treatment of CRC (Table 1). In these patients, there is an urgent need to improve the efficacy of tumor immunotherapy by improving intratumoral T-cell infiltration and converting cold tumors into “hot” or T-cell inflammatory tumors. In this review, we discuss the classification and differences between hot and cold CRC and the current state of research on cold CRC, the therapeutic strategies and challenges of immunotherapy, and the pathological mechanisms of cold CRC.

CLASSIFICATION AND DIFFERENCE BETWEEN COLD AND HOT CRC

Tumor-immune system interactions provide a basis for patient stratification and treatment strategies for various cancers, which can more accurately predict survival in CRC^[12]. An immune scoring system for tumor classification was developed based on the quantification of two lymphocyte populations (CD3 and CD8)^[13,14] at the tumor center and aggressive margins^[15-19]. The scoring system has four levels [immune score 0 (i0), i1, i2, i3, and i4]. The concepts of hot (highly invasive immune score i4) and cold (noninvasive immune score i0) tumors were introduced^[15]. In colon cancer, the consensus immune scoring system has a greater relative prognostic value than pathological T staging, pathological N staging, lymphovascular infiltration, tumor differentiation, and MSI status^[20].

Currently, hot and cold tumors are typically referred to as T-cell infiltrated, inflammatory but noninfiltrating, and noninflammatory tumors^[15]. This immune classification has been validated in melanoma and breast cancer^[21,22]. In addition to the presence of tumor-infiltrating lymphocytes (TILs), other features are the consensus molecular subtype (CMS) classification developed through a comprehensive reassessment and comparison of CRC molecular gene expression profiles: CMS1 and CMS4 are hot tumors (Figure 1); they are considered immunoreactive and highly infiltrated by immune cells. These tumors are immunoreactive and highly infiltrated by immune cells, as opposed to CMS2 and CMS3, which are cold tumors^[23]. A small group of CRCs with dMMR/MSI-H benefits from immunotherapy. dMMR/MSI-H in solid tumors, including CRC, suggests a good tumor response to immunotherapy^[7]; however, the majority of patients with skilled MMR (pMMR) or microsatellite stable (MSS) CRC do not respond well to this treatment^[24]. However, immune scoring is a better predictor of prognosis in CRC patients than MSI testing alone^[25], and MSI has been used to predict the response to anti-programmed cell death protein 1 therapy in cancer^[26]. The expression of anti-programmed cell death ligand 1 (PD-L1) on tumor-associated immune cells, possible genomic instability, and the pre-existing antitumor immune response are characteristics of hot tumors^[27].

Currently, the most comprehensive approach to define hot and cold tumors remains the immune scoring system, but there are still some tumors with characteristics intermediate between hot and cold tumors, and the four main categories of tumor classification^[32], namely hot, altered exclusion, altered immunosuppression, and cold, provide classification of the four major tumor categories^[28]. This system provides a more comprehensive approach to classification and helps to suggest new ideas for immunotherapy strategies.

With the development of immunotherapy and its achievements, it is important to determine how to use immune scoring to classify tumors and help and guide the choice of treatment. A blanket use of parameters to score may produce bias, which reinforces the need to incorporate the details of each individual case and to adequately integrate clinical practice to develop a rational, standardized, and coordinated scoring approach to guide treatment decisions. For immunotherapy to overcome the bottleneck of cold CRC, a general consensus is still required.

Immunotherapy has made significant progress in cancer treatment^[29]. In particular, hot tumors with an immune microenvironment of highly TILs are highly responsive to most immunotherapies; a property that plays a key role in obtaining good antitumor responses to immunotherapy^[30-33]. The discovery and development of ICs and IC-related drugs are of importance in cancer immunotherapy. This immunotherapeutic approach has excellent long-term regression efficacy in hot tumors; however, hot tumors have a low response rate to immunocooled tumors lacking predominant infiltration of tumor immune cells^[34-40]. Therefore, the absence or low number of lymphocytes in the TME also serves as a biomarker for cold tumor unresponsiveness to ICIs^[41].

Therefore, it is important to consider a proper treatment plan for cold CRC. Classifying tumors according to their immunophenotypes is too homogeneous; an emphasis on tumor heterogeneity can enable us to have a better understanding of individualized cancer treatment^[6]. Most solid tumors are non-T-cell inflammatory or cold tumors^[8,42], and CRC is no exception. Therefore, there is an urgent need to improve

the TME to convert cold tumors into hot or T-cell inflammatory tumors to improve the efficacy of tumor immunotherapy.

It is important to elucidate the mechanisms involved in cold CRC that do not respond to immunotherapy to provide additional insights into the therapeutic strategies that can be developed. In this section, we outline the mechanisms and approaches related to the possible modulation of non-immune-responsive cold CRC to improve the efficacy of treatment approaches against non-immune-responsive tumors.

Increasing tumor inflammation

Establishing an inflammatory response in the TME is a key goal of immunomodulatory approaches for all cold tumors, including CRC. Infection by pathogens can activate the immune system, thus stimulating a series of immune attacks^[43]. The involvement of such pattern recognition receptors can activate immune cells and lead to an immune-system-mediated antitumor response^[44]. Interventional radiology has enabled the precision treatment of local tumors, and a variety of therapeutic substances, including pattern recognition receptor agonists, such as tumor lysing ²peptides or lysing viruses, cytokines, encoded nucleic acid sequences, bispecific T-cell participants, nanoparticles or particles, and immune cells, can be delivered locally^[45]. The immunogenic cell death pathway induced by precise radiotherapy and cryoablation or radiofrequency ablation that produces massive tumor antigen release can convert tumors into *in situ* vaccines, which provides us with new insights and options^[45,46].

Although a growing number of studies has demonstrated the effectiveness of radiotherapy^[47-50], the benefits obtained in these trials cannot be attributed exclusively to radiotherapy. Explicit demonstration of the contribution of radiation to the immunotherapeutic response is challenging but crucial. The optimal integration of radiobiology and tumor immunology may lead to potentially significant clinical benefits.

Precision therapy is limited by several operational, clinical, and biological factors, in addition to the numerous complications that may arise from injecting drugs or biologics

directly into the tumor. Therefore, a systemic approach to tumor-specific therapy remains attractive. Chemotherapeutic regimens as systemic treatments can induce immunogenic cell death by releasing damage-associated molecular patterns^[51,52] and activating necrotic or apoptotic pathways^[53]. Some studies have indicated that drugs such as 5-fluorouracil can induce apoptosis of myeloid-derived suppressor cells (MDSCs) and increase CD8 cell function to enhance inflammatory immunity^[54].

Immune microenvironment analysis of patients with liver metastases from CRC revealed that cytotoxicity and memory T-cell density were significantly higher in patients who received preoperative chemotherapy than in patients with untreated metastasis^[55-57], suggesting that the use of chemotherapy can induce tumor inflammation to some extent, providing insight into the transformation of cold to hot CRC. Epidermal growth factor receptor (EGFR), vascular EGFR kinase, and MEK inhibitors are widely used in clinical solid tumor routine, and the clinical effects and related mechanisms of EGFR (cetuximab and panitumumab) and angiogenesis (bevacizumab, aflximab, or ramucizumab) as first- and second-line targeted agents for metastatic CRC are being actively investigated^[58-61]. Although there is a lack of knowledge regarding the detailed molecular mechanisms of action between targeted drugs and immunity, *in vivo* studies have shown that the activated mitogen-activated protein kinase (MAPK) signaling pathway can inhibit major histocompatibility class I components and antigen-presentation mechanisms. Use of MAPK inhibitors enhances T-cell-mediated killing of tumor cells^[62].

MEK inhibitors may also be involved in the immune effects of tumors^[63,64]. This effect promotes antigen presentation on the surface of tumor cells to activate the recognition of CD8 T lymphocytes, which then kills tumor cells. Additionally, it has been found that inhibition of the phosphoinositide 3-kinase-AKT-mammalian target of rapamycin pathway can prevent the activation of immunosuppressive pathways^[65], thereby affecting the immune microenvironment.

There is a large body of literature that strongly supports the idea that drugs targeting oncogenes and non-oncogenes can beneficially affect the TME in a wide range of

tumors and thus enhance tumor immune responses. However, many therapeutic methods have not yet been used in the routine management of cancer patients, as their preclinical and clinical development has only recently begun.

For example, toll-like receptors (TLRs) are highly expressed in immune cells in the TME, and in clinical development, the involvement of TLR agonists can activate antitumor immune responses^[66]. However, the complexity of the TLR system challenges the selection of agonists. Different agonists may cause different types of inflammatory responses. TLR agonists are currently being used as monotherapy and in combination with ICIs^[66,67].

Stimulator of interferon (IFN) genes is an endoplasmic reticulum transmembrane protein, whose mechanism of action is to sense cytosolic DNA, induce type I IFN gene transcription, and promote antigen cross-presentation^[68]. IFN gene-stimulating factor agonists increase CD8 T cells in the TME^[69] and, when combined with anti-PD-L1, reduce local immunosuppression to mediate a systemic antitumor response^[70]. This combination may be used as a method of local immunosuppression, and the study of its agonist is in the clinical development stage.

Cytokines in the TME

Cytokines and chemokines are molecular messengers of the immune system, and many cytokines [e.g., interleukin (IL)-2, IL-7, IL-15, IL-21, granulocyte macrophage colony-stimulating factor, and IFN α] in the immune TME^[71-76] regulate the function of T cells, and studies have reported their use as single agents or in combination with other drugs^[77]. Accumulation of MDSCs as a group of immature myeloid cells with immunosuppressive functions in tumors attenuates the regulation of immune responses. T regulatory (Treg) cells can promote tumor growth by suppressing cytotoxic immune responses. Therefore, therapeutic strategies that specifically eliminate MDSCs or Treg cells have been proposed^[78]. It has been suggested that monoclonal antibodies against immune checkpoints or agonists of the tumor necrosis factor receptor superfamily may have a modulatory effect on Treg cells^[79].

Immunosuppressive soluble ligands play an important role in the immune microenvironment. For example, prostaglandin E2 promotes tumor growth and exerts immunosuppressive effects^[80]. The TME is also rich in adenosine. The TME is also enriched in adenosine, which is released owing to the death of tumor cells through CD73 and CD39 ectonucleases^[81]. The increase in adenosine, a substance enriched in the TME, impairs the implantation and activation of immune cells in the TME. Antagonizing adenosine or its pathway can block CD39 to enhance T cell proliferation and induce proinflammatory cytokines, which can control tumor growth^[82].

Cell therapy

Since 1993, engineered T cells targeting artificial chimeric antigen receptors (CARs) on the surface molecules of tumor cells were first proposed^[83], and in 2010, their nature as anticancer drugs was revealed^[84]. CAR-T-cell-based therapies have shown specific, rapid, high success rates, and long-lasting effects^[85]. The principle of action is mainly through T cells with novel properties to induce a tumor rejection response. Patient-derived TILs that can be expanded *in vitro* with recombinant IL-2 have been demonstrated and have made important advances in the treatment of metastatic melanoma and other types of tumors^[86-88].

To some extent, the efficacy of T-cell therapy depends on the potency of the T cells themselves; however, T cells can be influenced by the dose (absolute number of T cells injected) as well as the characteristics of the tumor-specific T cells administered. Controlling the dose of tumor-specific T cells is crucial for activation of the endothelial complement by IFN- γ to overcome the vascular endothelial barrier^[89]. Therefore, the effect of pericyte therapy alone may be limited, and patients may benefit more from its combination with tumor-targeted interventions aimed at reprogramming the TME. A combination of many factors (intervention of tumor-intrinsic pathways, local inflammatory response, or intercellular messaging) to ensure proper engraftment and function of relayed metastatic T cells may be beneficial for cold tumors. Repeated stimulation of tumor-specific T cell expansion by cell lines established in lesions from

patients with resected melanoma has been shown to be effective^[90]. Melanoma is the best solid cancer type to respond to adoptive cell therapy^[91].

In summary, peritoneal cellular immunotherapy is a promising treatment modality for CRC. The approach is based on the collection T cells from patients, which are expanded *in vitro* and then transfused back into the patient. These T cells are designed to express CARs, which ¹ can be designed to recognize not only tumor antigens but also to produce anticancer cytokines or ICIs. However, despite the results of CAR-T therapy in the treatment of hematological tumors such as B-cell malignancies, the effectiveness and applicability of the approach to convert cold tumors into hot tumors, and whether it can be successful in CRC and other solid tumors remains to be elucidated^[7,92].

Other possible methods that can be used to make the tumor hotter

As a branch of hyperthermia, modulated electro-hyperthermia (mEHT) has been gradually applied in the treatment of various cancers in recent years^[93,94]. The principle of mEHT is delivering locoregional clinical hyperthermia generated by 13.56 MHz amplitude-modulated radiofrequency^[95]. A series of studies have shown the effect of mEHT in the treatment of CRC^[96-99], and related mechanism studies have also shown its relevance in immunity^[100,101]. This treatment may be a good candidate for transforming cold CRC.

In addition, ³ some experiments are being conducted to make tumors hotter, such as combining oncolytic bacteria or viruses or peptides, tumor, virus or dendritic cell antigens with various adjuvants, with the goal of improving CRC immunogenicity. Tumor-associated macrophages, as a key driver of inflammation that facilitates tumor progression, are attractive targets to complement current immunotherapy^[102].

As a key target of tumor-associated macrophages, colony-stimulating factor 1 receptor (CSF1R) can bind to ³ CSF1 or IL-34 to activate macrophage proliferation and function^[103,104]. CSF1R-specific inhibitors and other macrophage modulators are currently being studied in clinical trials in solid tumors^[105].

THE CHALLENGES FOR IMMUNOTHERAPY TARGETING COLD CRC

Despite the widespread use of immunotherapy, poor clinical response to cold tumors is a current challenge^[22]. Prior to immunotherapy, the resected tumor (primary or metastatic) should be classified as hot, altered, or cold. Although the tumor sample is valuable in providing information about the disease, it is limited in that it is not representative of the entire tumor landscape^[28]. Recently, it was noted that, in addition to the CMS of CRC, the underlying epithelial cell diversity of CRC was summarized in a large transcriptome into two intrinsic subtypes, iCMS2 and iCMS3. This finding refines the CMS^[106]. Because of genomic and immune heterogeneity, each sample can be considered an individual tumor^[56]. Moreover, immune parameters change over the course of the disease^[107].

The concept of personalized cancer immunotherapy is being increasingly promoted. A major challenge for immunotherapy of cold tumors is the need to identify key immune- or tumor-related features at the time of diagnosis to establish a reliable classification strategy to support immunotherapy for maximum efficiency^[108].

CONCLUSION

With an increasing number of studies conducted on cold tumors, personalized cancer immunotherapy for individual patients is gaining ground. A major challenge hindering the development of therapeutic strategies may be the lack of mastery of the relationship between cancer and immunity. Even though a great deal of technological innovation and related research has been conducted to achieve some progress, the variability of cancer among individual patients cannot be generalized^[108]. Identifying key phenotypic features is of interest when developing treatment strategies, and considerable progress has been made with ICIs approved by the United States Food and Drug Administration for the treatment of patients with dMMR/MSI-HmCRC. Notably, subtype dMMR/MSI-H CRC represents only a small fraction of all CRCs, and most pMMR/MSS mCRC patients do not benefit from ICI treatment alone. Therefore, further tumor states need to be identified, which has led to the continued reporting of new biomarkers, such as

comprehensive immune scoring and complete CMS classification, and these results have led to a better understanding of the immune mechanisms of CRC and their relationship to tumor treatment strategies.

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