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TOPIC HIGHLIGHT

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Immunotherapy for colorectal cancer

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Abstract

The incidence of colorectal cancer (CRC) is on the rise, and the prognosis for patients with recurrent or metastatic disease is extremely poor. Although chemotherapy and radiation therapy can improve survival rates, it is imperative to integrate alternative strategies such as immunotherapy to improve outcomes for patients with advanced CRC. In this review, we will discuss the effect of immunotherapy for inducing cytotoxic T lymphocytes and the major immunotherapeutic approaches for CRC that are currently in clinical trials, including peptide vaccines, dendritic cell-based cancer vaccines, whole tumor cell vaccines, viral vector-based cancer vaccines, adoptive cell transfer therapy, antibody-based cancer immunotherapy, and cytokine therapy. The possibility of combination therapies will also be discussed along with the challenges presented by tumor escape mechanisms.

Key words: Colorectal cancer; Cytotoxic T lymphocyte; Dendritic cell; Immunotherapy; Vaccine

Core tip: The prognosis for patients with recurrent or metastatic colorectal cancer (CRC) is extremely poor. Immunotherapy may be effective for treating CRC patients and/or preventing relapse. The immunotherapeutic approaches for CRC, including peptide vaccines, dendritic cell-based cancer vaccines, whole tumor cell vaccines, viral vector-based cancer vaccines, adoptive cell transfer therapy, antibody-based cancer immunotherapy, and cytokine therapy have been demonstrated. The blockade of multiple immune regulatory checkpoints combined with immunotherapy and/or conventional chemotherapy may be effective in treating patients with advanced CRC.

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INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer in men (accounting for 10.0% of all cancers) and the second most common cancer in women (accounting for 9.4% of all cancers) worldwide. Additionally, CRC is the fourth most common cause of cancer-related death[1]. Optimization of surgical resection for patients with localized disease has dramatically improved 5 year and 10 year survival rates. The prognosis for patients with resectable tumors depends on the disease stage. CRC patients with distant metastasis have a 12% survival rate[2], and more than 20% of CRC patients have metastatic disease at the time of diagnosis[3,4]. Moreover, despite the fact that 80% of CRC patients with localized disease demonstrate complete macroscopic clearance of the tumor by surgery, 50% of CRC patients will relapse due to the presence of micro-metastasis at the time of surgery[5]. Chemotherapy is approved for the treatment of regionally metastatic CRC, but it shows only modest efficacy and is ineffective against distant metastases[6]. The prognosis for patients with advanced disease remains unfavorable due to the frequency of recurrence, distant metastasis, and resistance to chemotherapy. Thus, novel treatment modalities are needed. Interestingly, tumors that develop chemotherapy or radiation resistance are still suitable targets for immunotherapy[7-10]. Therefore, cancer immunotherapy may be effective for treating CRC patients and/or preventing relapse.

ANTITUMOR IMMUNE RESPONSES

T cells

Tumor cells degrade endogenous and exogenous tumor-associated antigens (TAAs) into short peptides (usually 8-10 amino acids) and present them on the cell surface in the context of major histocompatibility complex (MHC) class I molecules. T cell receptor (TCR) interaction with the complex of peptides and MHC class I molecules on tumor cells is a critical event in the T cell-mediated antitumor immune response. T cells that express the αβ TCR generally express CD4+ (helper T cells) or CD8+ (cytotoxic T cells) lineage markers[11]. In particular, CD8+ T cells recognize peptides (usually 8-10 amino acids) derived from TAAs bound by MHC class I molecules on tumor cells. Thus, immunotherapy may promote cancer cell killing by eliciting antitumor immune responses by recognizing specific TAAs on tumor cells.

To induce antigen-specific CD8+ cytotoxic T lymphocytes (CTLs), peptides derived from TAAs must be presented on the surface of antigen presenting cells (APCs) in the context of MHC class I molecules. In contrast, CD4+ T cells recognize peptides (usually 10-30 amino acids) in association with MHC class Ⅱ molecules on APCs and enhance the persistence of antigen-specific CD8+ CTLs through secretion of interleukin (IL)-2 and interferon (IFN)-γ[12]. Therefore, the interaction of the αβ TCR with complexes of peptides and MHC class I and class Ⅱ molecules on APCs is a central event in cancer immunotherapy. The αβ TCR expressed by CD8+ CTLs recognizes MHC class I -peptide complexes on tumor cells and leads to tumor cell killing through effector molecules such as perforin and granzyme B[13]. Moreover, there is increasing evidence that CD4+ T cells play a more direct role in generating efficient antitumor immunity beyond simply assisting[14]. Therefore, effective antitumor responses depend on the presence and function of T cells that recognize and eliminate tumor cells[14,15].

A unique subset of human T cells expresses the TCR-γδ. Human γδT cells include several subsets of cells defined by their TCR. The most common subset of TCR-γδT cells in circulating blood express the Vγ9Vδ2 receptor[16,17]. Although cancer immunotherapy strategies primarily focus on activation of these MHC-restricted T cells, γδT cells and αβT cells share certain effector functions such as cytokine production and potent cytotoxic activity. However, they recognize different sets of antigens, usually in a non-MHC-restricted fashion[16,18]. Thus, T cells can attack tumors in their HLA-unrestricted cytotoxic capacity, as well as by secreting cytokines. Indeed, tumor-infiltrating γδT cells have been detected in a broad range of cancers, including CRC[19]. Importantly, activated γδT cells can kill cells from metastatic renal cell carcinomas, mammary carcinomas, prostate carcinomas and colorectal carcinomas, while sparing normal, untransformed cells[18,19].

Natural killer cells

Natural killer (NK) cells are component of innate immunity responses to tumor cells[20]. NK cells can rapidly kill certain target cells, including tumor cells with down-regulated MHC class I molecules[21]. Thus, NK cells play a critical role in antitumor immunity. NK cells recognize tumor cells *via* cellular stress or DNA damage signals[22]. Activated NK cells directly kill target tumor cells through several mechanisms, including[23]: (1) cytoplasmic granules such as perforin and granzyme B[24]; (2) tumor necrosis factor-related apoptosis-inducing ligand and Fas ligand (FasL)[25,26]; (3) effector molecules such as IFN-γ and nitric oxide (NO)[24,27]; and (4) antibody-dependent cellular cytotoxicity (ADCC)[28]. NK cell activators (IL-2, IL-12, IL-15, and IL-18), have also been validated in preclinical cancer models[23].

Dendritic cells

Dendritic cells (DCs) are potent APCs that have been used in cancer vaccines due to their ability to initiate antitumor immune responses[12]. DCs are characterized by expression of MHC class Ⅰ, class Ⅱ, and costimulatory molecules (B7, ICAM-1, LFA-1, LFA-3, and CD40)[29-31].

These molecules function in concert to generate a network of secondary signals essential for reinforcing the primary antigen-specific signal in T-cell activation[29-31]. DCs process endogenously synthesized antigens into antigenic peptides, which are presented on the cell surface in MHC class I -peptide and recognized by the αβ TCR on naïve CD8+ T cells[12]. DCs can also capture and process exogenous antigens, which are then presented by MHC class I molecules through an endogenous pathway in a process known as "cross-presentation"[32]. Moreover, exogenous antigens from the extracellular environment are also captured by DCs and delivered to the endosomal/lysosomal compartment, where they are degraded to antigenic peptides by proteases and peptidases. These antigens then complex with MHC class Ⅱ for recognition by the αβ TCR of naïve CD4+ T cells[12]. Efficient antigen presentation by MHC class I and class Ⅱ on DCs is essential for evoking tumor-specific immune responses[33]. Mature DCs are significantly better at processing and presenting MHC-peptide to the TCR and inducing CTLs due to higher expression of MHC class I and class Ⅱ and costimulatory molecules[33].

Immature DCs reside in peripheral tissues where they take up and process antigens that are degraded to peptides. These peptides are then bound to MHC class I molecules for presentation to CD8+ CTLs or bound to MHC class Ⅱ molecules for presentation to CD4+ T helper (Th) cells. Differentiation of the immature DCs into mature DCs is triggered by molecular stimuli that are released in response to tissue disturbance and local inflammatory responses caused by pathogens[34]. After antigen uptake and stimulation by the inflammatory response, immature DCs in the peripheral tissues undergo a maturation process characterized by the up-regulation of MHC class I and class Ⅱ and costimulatory molecules, the up-regulation of chemokine receptors such as CCR7, and the secretion of cytokines such as IL-12[34,35]. The mature DCs migrate to secondary lymphoid organs, where they present antigens to CD4+ and CD8+ T cells through the MHC class I and class Ⅱ pathways, respectively[12,34]. Therefore, the aim of immunotherapy is to simultaneously activate CD8+ CTLs (which recognize TAA) and CD4+ Th cells.

Immune suppressive cells

CD4+ Th cells are critical for inducing and regulating immune responses. Immune homeostasis is primarily controlled by two distinct helper T cell subsets, Th1 and Th2 cells[36]. Th1 cells secrete IFN-γ, which can further sensitize tumor cells to CTLs by inducing the up-regulation of MHC class I molecule expression on tumor cells and antigen-processing machinery in DCs[12]. Th2 cells secrete type Ⅱ cytokines such as IL-4 and IL-10 that enhance humoral immunity (the antibody-based antitumor response)[12]. Importantly, tumor cell-derived soluble factors such as transforming growth factor-β (TGF-β) and IL-10 induce tolerance by promoting the expansion of the CD4+α-2R (CD25)+ forkhead box P3 (Foxp3)+ natural Treg subset[37]. Induced Tregs (CD4+CD25+Foxp3-) secrete TGF-β and IL-10 and suppress Th1 and Th2 phenotypes[38,39]. Therefore, Tregs play a pivotal role in tumor progression and the suppression of antitumor immunity.

The cancer microenvironment consists not only of cancer cells but also stromal cells such as cancer-associated fibroblasts, tolerogenic DCs, myeloid-derived suppressor cells, immunosuppressive tumor-associated macrophages (TAMs), and Tregs. These immune suppressive cells secrete vascular endothelial growth factor (VEGF), IL-6, IL-10, TGF-β, soluble FasL, and indolamine-2,3-dioxygenase (IDO)[40], which inhibit antitumor immunity by various mechanisms, including depletion of arginine and elaboration of reactive oxygen species (ROS) and NO. Moreover, the tumor microenvironment promotes the accumulation of Tregs that suppress CD8+ CTL function due to the secretion of IL-10 or TGF-β from Tregs and tumor cells[40] (Figure 1).

IMMUNOTHERAPY

Immunotherapy is an active therapeutic approach designed to trigger the immune system to respond to tumor-specific antigens and attack tumor cells. Immunotherapy strategies include the use of peptides derived from TAAs, whole tumor cells, *in vitro*-generated DCs, or viral vector-based cancer vaccines (Table 1).

Peptide vaccines

A peptide vaccine is based on the identification and synthesis of epitopes, which can induce TAA-specific antitumor immune responses. CRC cells express TAAs such as carcinoembryonic antigen (CEA)[41,42], mucin 1[41-43], epidermal growth factor receptor (EGFR)[44], squamous cell carcinoma antigen recognized by T cells 3 (SART3)[45], β-human chorionic gonadotropin (β-hCG)[46], Wilms' Tumor antigen 1 (WT1)[47], Survivin-2B[48], MAGE3[49], p53[50], or mutated KRAS[51], which are potential targets for immunotherapy. Peptide vaccines targeting these TAAs may be a useful approach for immunotherapy in CRC patients.

Peptide vaccines are simple, safe, stable, and economical. Multiple MHC class I -binding peptides have been identified and tested for immunogenicity. Several peptide vaccines for CRC have been tested in phase I clinical trials. Fifteen patients with advanced or recurrent CRC expressing survivin were vaccinated with a peptide derived from HLA-A*2402-restricted epitopes[48]. In 6 patients, tumor marker levels (CEA and CA19-9) decreased transiently during the survivin-2B peptide vaccination. Moreover, in phase I trial of peptide-cocktail vaccines derived from ring finger protein 43 (RNF43) and translocase of the outer mitochondrial membrane 34 (TOMM34), 8 of 21 patients exhibited antigen-specific CTL responses to both RNF43 and TOMM34, and 12 patients exhibited CTL responses to one of the peptides only[52]. The patients who did not demonstrate any CTL responses had the lowest survival rates. By vaccination with a 13-mer

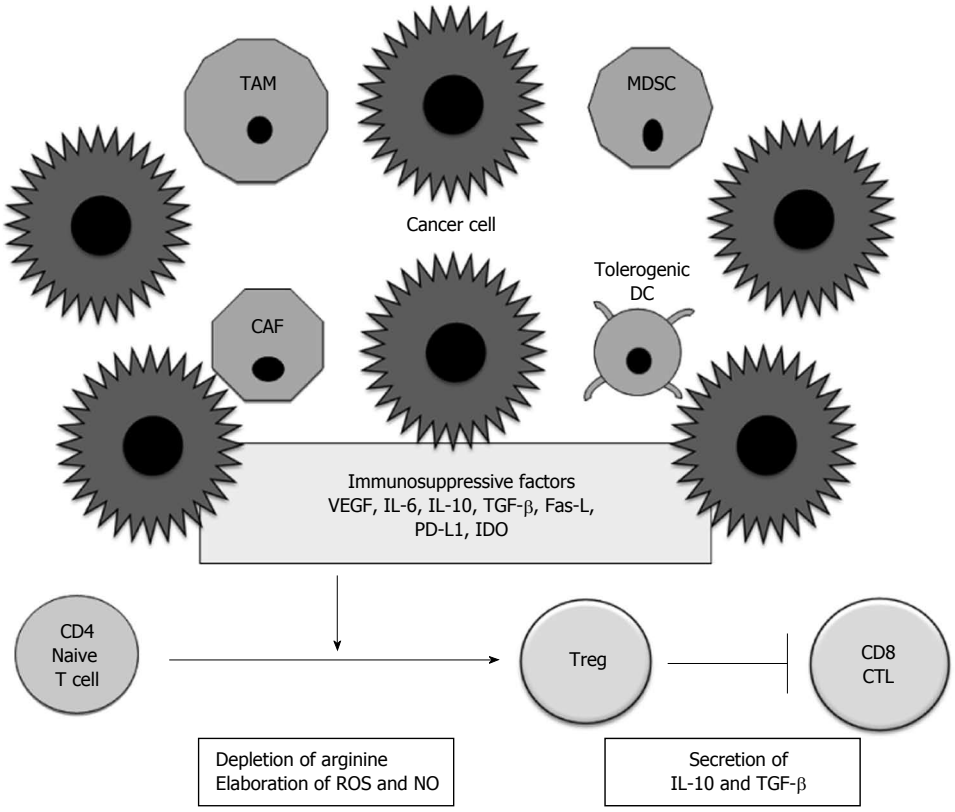

Figure 1 Immunosuppression in the tumor microenvironment. Cancer cells secrete various factors such as vascular endothelial growth factor (VEGF), interleukin (IL)-6, IL-10, transforming growth factor-β (TGF-β), Fas ligand (FasL), PD1 ligand 1 (PD-L1), and indolamine-2,3-dioxygenase (IDO), all of which promote the accumulation of heterogeneous populations of cancer-associated fibroblasts (CAFs), tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), and tolerogenic dendritic cells (DCs). These immunosuppressive cells inhibit antitumor immunity by various mechanisms, including depletion of arginine and elaboration of reactive oxygen species (ROS) and nitric oxide (NO). The tumor microenvironment promotes the accumulation of Tregs that suppress CD8+ cytotoxic T lymphocyte (CTL) function through secretion of IL-10 and TGF-β.

mutant ras peptide, 2 of 7 CRC patients showed antitumor immune responses that were significantly associated with prolonged overall survival[53]. Moreover, in a phase Ⅱ trial, vaccination with the β-hCG peptide induced anti-β-hCG antibody production in 56 of 77 CRC patients[46]. Interestingly, anti-β-hCG antibody induction was associated with longer overall survival[46]. However, some clinical trials report a discrepancy between clinical and immunological responses. In SART3 peptide vaccine therapy, IgE-type anti-peptide antibodies were detected after vaccination; however, immunological responses were not detected in the patients[45]. Peptide vaccines for CRC patients are generally well-tolerated, with no patients requiring cessation due to toxicity; however, a high frequency of reactions were observed at the injection site due to the use of adjuvants such as incomplete Freund's adjuvant, IL-2, granulocyte-macrophage colony-stimulating factor (GM-CSF), and bacillus Calmette-Guerin (BCG). Importantly, peptide vaccines have shown only limited success in clinical trials. There are several drawbacks to the peptide vaccination strategy, including: (1) limitations due to the patient's HLA type[54]; (2) ineffectiveness of CD8+ CTLs due to the down-regulation of certain antigens and MHC class I molecules; (3) impaired DC function in patients with advanced cancer[55]; and (4) tumor microenvironments, where immune suppressive cells such as Tregs exist[13]. Synthetic long peptides may be more attractive candidates for peptide-based vaccines. In a phase I/Ⅱ trial, 10 CRC patients were vaccinated twice with a set of 10 overlapping p53 synthetic long peptides[50]. P53-specific CD4+ and CD8+ T-cell responses were observed in 9 of 10 CRC patients, and 6 of 9 tested patients maintained p53-specific T-cell reactivity for at least 6 mo. New trials may focus on improving the long peptide vaccine-induced antitumor immune responses.

DC vaccines

Three signals were required for induction of efficient CTL responses: (1) simultaneous presentation of multiple TAAs by both MHC class I and class Ⅱ molecules; (2) costimulation by membrane-bound receptor-ligand pairs; and (3) cytokines to direct polarization of the resultant antitumor immune responses. DCs can provide all three of these signals that are essential for the induction of antitumor immunity[33]. Therefore, many clinical trials of antigen-pulsed DCs have been conducted in patients with various types of tumors, including CRC.

To date, various strategies for delivering TAAs to DCs have been developed to generate potent CTL responses against tumor cells. DCs have been pulsed with synthetic peptides derived from the known TAAs[56], tumor cell lysates[57], apoptotic tumor cells[32], and tumor RNA[58], or

Table 1 Immunotherapy strategies for colorectal cancer

Vaccine	Clinical response	Immunological response	Ref.
Peptide			
Survivin-2B	PR (1/15) SD (3/15) PD (11/15) Temporary decrease of CEA level 40% (6/15)	Increase of Survivin-2B-specific CTL frequency DTH 40% (6/15)	[48]
Combination chemotherapy with peptide vaccine against RNF43 and TOMM34	SD (16/19) PD (3/19)	8 of 21 patients exhibited antigen-specific CTL responses to both RNF43 and TOMM34, and 12 patients exhibited CTL responses to one of the peptides only	[52]
13-mer mutant ras	Of nine patients who completed all six vaccinations, seven patients showed no remaining evidence of disease	Two CRC patients showed immunological responses, and the antitumor immune response was significantly associated with prolonged overall survival	[53]
β-hCG	Prolongation of survival in patients with a high level of antipeptide antibodies	Induction of serum antipeptide antibody (56/77)	[46]
SART3	Diagnosis at 5 mo after first vaccination: SD (1/19) PD (10/19)	Increased CTL activity (2/11), induction of serum antipeptide IgG (2/12), IgE (5/12), DTH (0/12)	[45]
A set of 10 overlapping p53 synthetic long peptides		Induction of p53-specific CD4+ and CD8+ T-cell responses (9/10), maintained p53-specific CTL reactivity for at least 6 mo (6/9)	[50]
DC			
DC pulsed with CEA peptide or CEA mRNA	Disease stabilization was observed in several patients	The majority of CRC patients demonstrated induction of CEA-specific T cell responses	[60-65]
DCs pulsed with CEA-derived altered peptides combined with the adjuvant Flt3 ligand	2 of 12 patients exhibited SD for 3 and 6 mo; 2 patients exhibited CR for more than 10 mo; 1 patient had a mixed response with some regression of liver metastases	Expansion of CD8+ T cells that recognize both the native and altered epitopes and possess an effector CTL phenotype	[64]
Whole tumor cell			
Autologous tumor cells combined with BCG	No significant clinical benefit was seen with whole tumor cell vaccines in surgically resected patients with stage Ⅱ or Ⅲ CRC	When treatment compliance was evaluated, the trend indicated benefits from vaccination in terms of disease-free survival (P = 0.078) and overall survival (*P* = 0.12)	[68]
NDV-infected irradiated autologous tumor cells	A randomized phase Ⅲ study of 50 patients with resectable CRC liver metastases demonstrated that vaccination with NDV-infected whole tumor cell did not significantly improve overall survival.	DTH (21/31)	[74,75]
Viral vector			
Replication-defective recombinant fowlpox and vaccinia viruses encoding the CEA antigen and TRICOM (B7.1, ICAM-1, and LFA-3)	SD (3/9)	Induction of CEA-specific CTL (3/9)	[79]
Combination chemotherapy and vaccination with a nonreplicating canarypox virus (ALVAC) expressing the CEA and T-cell costimulatory molecule B7.1 (ALVAC-CEA/B7.1)	Objective response (42/118)	Increases in CEA-specific T cells were detected in patients treated with chemotherapy and booster vaccination	[80]

Immunotherapy strategies including peptides derived from tumor-associated antigens, whole tumor cells, *in vitro*-generated dendritic cells (DCs), or viral vector-based cancer vaccine. PD: Programmed cell death protein; CTL: Cytotoxic T lymphocytes; CEA: Carcinoembryonic antigen; CRC: Colorectal cancer; RNF43: Ring finger protein 43; TOMM34: Translocase of outer mitochondrial membrane 34; β-hCG: β-human chorionic gonadotropin; SART3: Squamous cell carcinoma antigen recognized by T cells 3; NDV: Newcastle disease virus.

physically fused with whole tumor cells[59] to induce efficient antitumor immune responses (Figure 2). Because CEA is a tumor-associated antigen expressed by most CRCs, DCs have been pulsed with CEA peptides[60-64] or CEA mRNA[63,65]. In these phase I clinical trials, the majority of vaccinated CRC patients demonstrated the induction of CEA-specific T cell responses. Moreover, disease progression stabilized in several patients, and the vaccine was safe and well-tolerated. As CEA is a self-antigen and poorly immunogenic, Fong *et al*[64] generated altered peptide ligands that were derived from native T cell epitopes and contained amino acid substitutions that either increased the peptide affinity for the MHC peptide-binding groove or modified interactions with the T cell receptor. In this trial, 12 patients were immunized with DCs loaded with altered peptides derived from CEA and the Flt3 adjuvant ligand. Two of 12 patients showed disease stabilization for 3 mo and 6 mo, 2 patients showed complete responses for more than 10 mo, and one patient had a mixed response with some regression of liver metastases. To improve the clinical efficacy of DC-based cancer vaccines, it is crucial to design novel strategies that boost

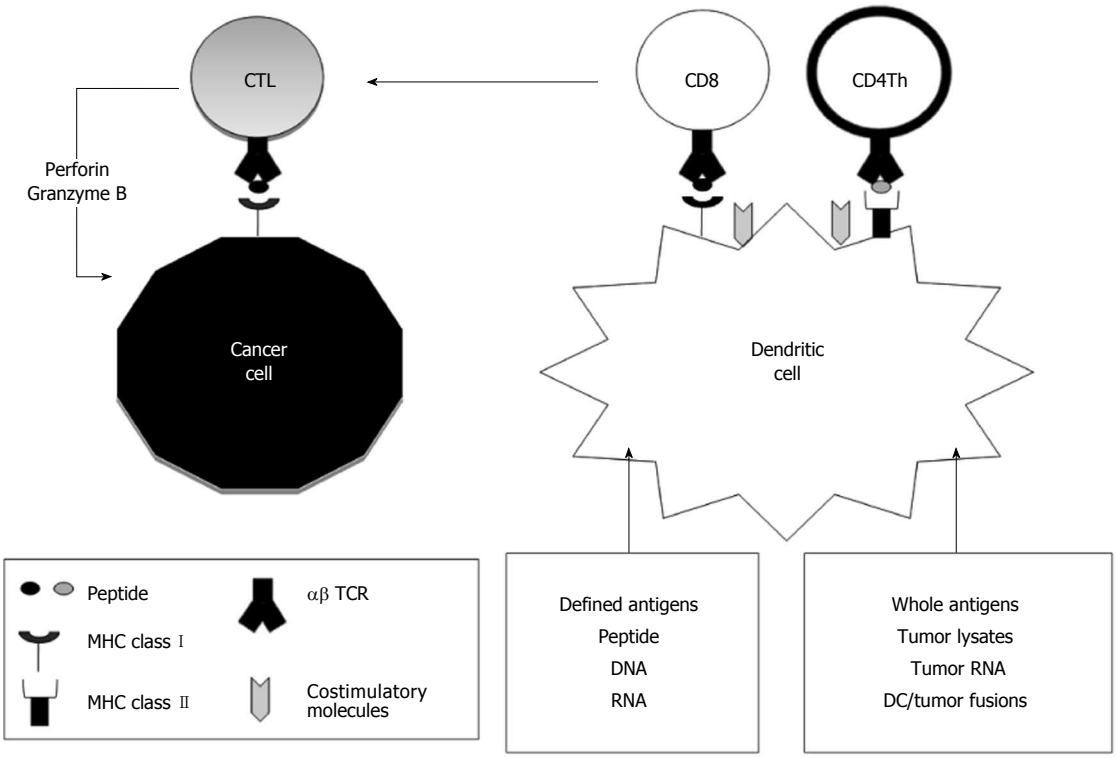

Figure 2 Dendritic cell vaccines. Dendritic cells (DCs) are loaded with antigens, which are taken up and degraded into peptide fragments by antigen-presenting cells such as immature DCs. DCs process tumor-derived peptides and major histocompatibility complex (MHC) class Ⅰ peptides derived from DCs. They form MHC class Ⅰ-peptide complexes in the endoplasmic reticulum, which are then transported to the surface of the DCs and presented to CD8+ T cells. DCs also synthesize MHC class Ⅱ peptides in the endoplasmic reticulum, which are transported to the cytoplasm where MHC class Ⅱ-peptide complexes are assembled with tumor-derived peptides and presented to CD4+ T cells. CD8+ T cells become cytotoxic T lymphocytes (CTLs), which destroy cancer cells through effector molecules such as perforin and granzyme B. TCR: T cell receptor.

adaptive antitumor immunity to overcome tolerance.

Whole tumor cell vaccines

Because autologous tumor cells express a whole TAAs including those known and unidentified, using whole tumor cells could greatly diminish the chance of tumor escape compared to using a single epitope peptide[41,42]. A significant disadvantage to this approach is the difficulty in generating a "universal" vaccine that could be applicable to all patients with a given cancer. Autologous whole tumor cells have been used as cancer vaccines to induce polyclonal CTL induction in several cancer types[66,67], including CRC[68]. A randomized phase Ⅲ clinical trial of a combined autologous whole tumor cell plus BCG vaccine was conducted to determine whether surgical resection plus vaccination was more beneficial than resection alone in 412 stage Ⅱ and Ⅲ CRC patients. This study showed no significant clinical benefit from whole tumor cell vaccination in surgically resected patients with stage Ⅱ or Ⅲ CRC. However, effective immune responses were associated with the improved disease-free and overall survival. Only a small proportion of the proteins in an autologous whole tumor cell vaccine are specific to tumor cells, while a vast majority of antigens in the vaccine are shared with normal cells. Moreover, whole tumor cell vaccines are likely to be poorly immunogenic. Therefore, the immune response generated by whole tumor cell vaccines is generally insufficient to provide benefit to patients. To improve the immunogenicity of whole tumor cell vaccines, autologous tumor cells have been genetically modified to secrete GM-CSF and then re-administered to the patient[69]. The trials have shown promising results in patients with prostate carcinoma[70], renal cell carcinoma[71], metastatic non-small-cell lung carcinoma[72], and melanoma[73]. This approach is based on the fact that GM-CSF is required at the site of the tumor to effectively prime TAA-specific immunity[69]. Another tumor cell vaccine approach utilizes Newcastle disease virus (NDV)-infected irradiated tumor cells as autologous CRC vaccines[74]. This approach resulted in a 97.9% two-year survival rate in patients with resected CRC, compared to 66.7% when treated with autologous tumor cells combined with BCG. However, a randomized phase Ⅲ study of 50 patients with resectable CRC liver metastases demonstrated that vaccination with NDV-infected whole tumor cells did not significantly improve overall survival, disease-free survival or metastases-free survival, although subgroup analyses suggested that the vaccines were somewhat beneficial[75]. The immunogenicity of whole tumor cells needs to be improved for this vaccination strategy to be effective. However, it is unclear which specific agents (such as cytotoxic chemotherapeutics, ionizing irradiation, and chemical agents) are best suited for killing tumor cells to generate highly immunogenic whole tumor cell vaccines.

Viral vector vaccines

Recombinant viral vectors are potentially useful vaccine vehicles for cancer therapy. Many types of recombinant viruses are naturally immunogenic, infect APCs (specifically DCs), and express TAAs[76]. The natural immunogenicity of viral vectors acts as an adjuvant to help boost TAA-specific immune responses. In one study, CRC patients were immunized with vaccinia virus or a replication-defective avian poxvirus encoding CEA. In this phase I study, viral-based vaccination with replication-defective recombinant fowlpox and vaccinia viruses encoding the CEA antigen and TRICOM (B7.1, ICAM-1, and LFA-3) induced CEA-specific T cell responses[77] and disease stabilization in 40% of patients with metastatic cancer (including CRC) for at least 4 mo[78]. A phase Ⅱ clinical trial in patients with metastatic CRC examined the efficacy of chemotherapy combined with vaccination with a nonreplicating canarypox virus (ALVAC) expressing the CEA and T-cell costimulatory molecule, B7.1 (ALVAC-CEA/B7.1). Anti-CEA-specific T cell responses were successfully generated in 50% of patients undergoing chemotherapy and booster vaccination. Objective clinical responses were observed in 40% of the patients[79,80]. Interestingly, chemotherapy does not appear to inhibit vaccine-mediated immunity.

ADOPTIVE CELL TRANSFER THERAPY

Cancer immunotherapy can be either active or passive. In passive cellular immunotherapy, specific effector cells are directly infused and are not induced or expanded within the cancer patient. Because most tumor cells express MHC class I -peptide, which can be recognized by antigen-specific CD8+ CTLs. Therefore, adoptive transfer of activated CTLs successfully used in patients with advanced cancer[81]. In adoptive cell transfer therapy (ACT), autologous T cells are removed from patients, activated, expanded to large numbers *in vitro* and transferred back into the patients. ACT overcomes tolerogenic mechanisms by enabling the selection and activation of highly reactive T cell subpopulations and manipulating the host environment into which the T cells are introduced. Indeed, upon the successful induction of specific CTLs *in vitro*, a clinical response to adoptive immunotherapy can be expected even in patients with advanced CRC[82]. Moreover, injection of IFN promotes the MHC class I -peptide on the cell surface, thus antitumor immune responses are augmented. However, there are several drawbacks to ACT that should be considered, including a potential lack of immune memory, poor persistence of activated effector cells in patients, the prohibitive expense, and the time required to expand the cells.

A new approach using T cells genetically modified to express receptors that recognize TAAs with high specificity to tumor cells may provide significant clinical benefits, especially for large solid tumors[83]. Recently, several clinical trials have demonstrated the therapeutic potential of this approach, which lead to impressive tumor regression in cancer patients[84]. A phase I trial in CRC patients examined human T cells engineered to express a high-avidity CEA-specific TCRs[85]. In this study, autologous T cells genetically engineered to express a murine TCR against human CEA were administered to three patients with metastatic colorectal cancer that was refractory to standard treatments. All patients experienced profound decreases in serum CEA levels (74%-99%), and one patient had an objective regression of cancer metastatic to the lung and liver. However, all three patients developed severe transient inflammatory colitis.

ANTIBODY-BASED CANCER IMMUNOTHERAPY

Monoclonal antibodies (mAbs) that target surface antigens expressed on tumor cells are clinically effective as cancer therapeutics[86]. Three mAbs (Cetuximab, Bevacizumab and Panitumumab) are approved for the treatment of CRC in the United States, and many other mAbs are being tested in clinical trials[87]. Treatment with mAbs can induce tumor cell death by several mechanisms, including interference with vital signaling pathways. Moreover, it is becoming apparent that innate immune effector mechanisms that engage the Fc portion of the antibody *via* Fc receptors are equally important[88]. The immune cytotoxicity includes ADCC, complement-mediated cytotoxicity, and antibody-dependent cellular phagocytosis. Bevacizumab, a recombinant humanized monoclonal antibody that selectively binds to human VEGF, is effective in KRAS wild-type CRC patients[89]. Recent evidence has also shown clinical benefits from treatment with anti-EGFR, Cetuximab and Panitumumab in KRAS wild-type CRC patients[90].

CYTOKINE THERAPY

Cytokines are substances proteins and glycoproteins that are secreted by immune cells. They have autocrine and paracrine functions and function locally or at a distance to enhance or suppress antitumor immunity. To date, IL-2 and IFN-α are two cytokines approved by the FDA for cancer therapy. Cytokines may be useful for treating hematologic malignancies (hairy cell leukemia and chronic myelogenous leukemia) or immunogenic tumors (melanoma and renal cell carcinoma). The major cytokines currently in use or under evaluation for cancer therapy are IFN-α, IL-2, GM-CSF, and IL-12.

COMBINED IMMUNOTHERAPY

It is well known that even if CRC appears to have been eradicated by chemotherapy and radiation, a small cancer stem cell (CSC) fraction that can self-propagate and sustain tumor growth frequently persists, leading to relapse and therapeutic failure. Although CSC is often resistant to a variety of treatments, including chemotherapy and radiotherapy, immunotherapy may still be effective[8-10]. A combined approach that uses conventional chemotherapy

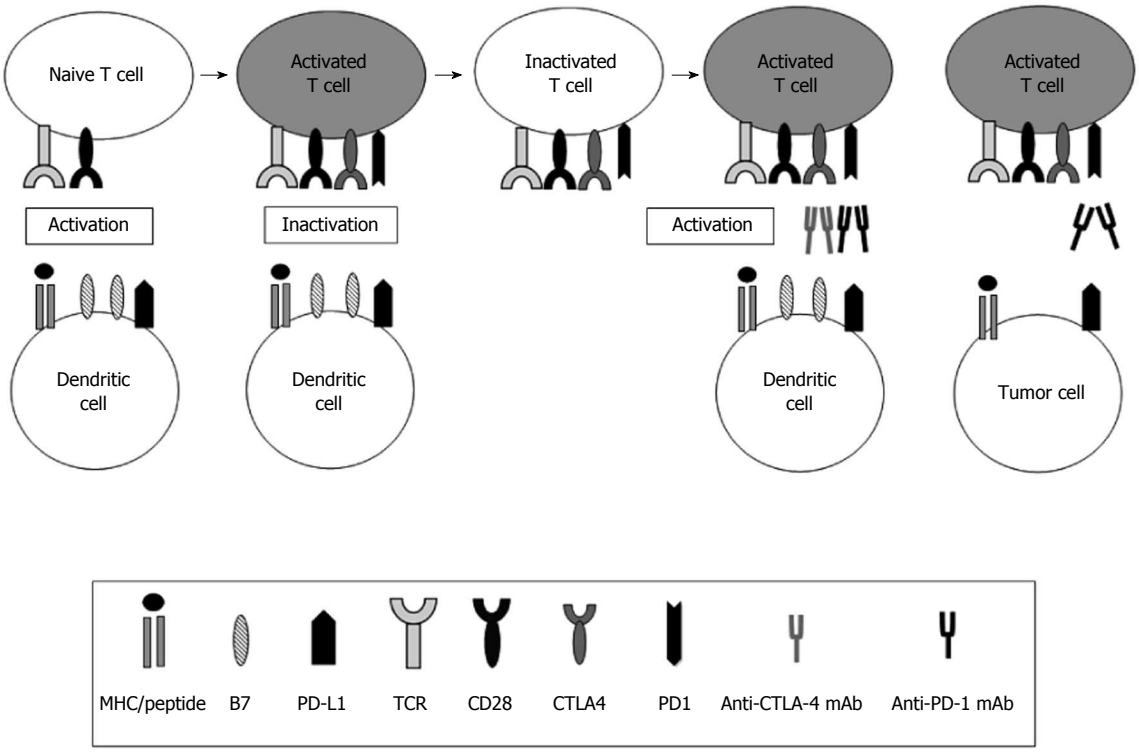

Figure 3 Immune regulatory checkpoints in cancer immunotherapy. Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death protein 1 (PD1) are two well-described co-inhibitory molecules that are expressed on naïve or memory T cells and decrease antitumor immune responses. The CTLA-4-mediated immune checkpoint is induced in T cells at the initial response to antigen (early activation phase). After the T cell receptor (TCR) is triggered by antigen encounter, CTLA-4 is transported to the surface of naïve or memory T cells. In contrast, the major role of the PD1 pathway is not at the initial T cell activation stage but rather the regulation of inflammatory responses by effector T cells that recognize antigen in peripheral tissue cells. Thus, PD-1 is highly expressed by antigen-specific cytotoxic T lymphocytes (CTLs) in malignancies and is associated with impaired T-cell function. The best-characterized signal for PD1 ligand 1 (PD-L1) induction is interferon-γ (IFN-γ), which is predominantly produced by Th1 cells. Although PD-L2 expression is limited to dendritic cells (DCs) and macrophages, PD-L1 is broadly expressed in tissues and is considered a molecular shield that protects cells from auto-reactive attack. In some tumors, PDL1 is not constitutively expressed but is induced in response to inflammatory signals that are produced by an active antitumor immune response. Loading DCs with soluble PD1 decreases their function. Therefore, antibodies can be used to block inhibitory ligand:receptor interactions by acting on tumor cells, DCs (*e.g.*, anti-PD-L1) or T cells (*e.g.*, anti-CTLA-4 or anti-PD1). Combining the blockade of multiple inhibitory pathways synergistically decreases T cell anergy and improves T cell responsiveness against tumors.

or radiation to kill the bulk of cancer cells and immunotherapy to keep residual CSCs and differentiated cancer cells in check may abrogate the replenishing pool of CRC cells[91]. In addition, treatment with chemotherapy such as cyclophosphamide or gemcitabine can augment the antitumor effects of cancer immunotherapy by depleting Treg, potentially enhancing antitumor immune responses[92]. Therefore, chemotherapy can kill cancer cells and boost antitumor immune responses all at the same time[93,94]. A recent study reported that immune checkpoint blockade with monoclonal antibodies targeting the inhibitory immune receptors cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), PD-1, and PD-L1 can be used to successfully treat patients with advanced melanoma (Figure 3)[95-98]. Combined, these approaches have the potential to significantly improve patient outcomes compared to treatment with conventional cancer therapies such as chemotherapy, radiation, monoclonal antibodies, hormonal therapy, and photodynamic therapy.

FUTURE PERSPECTIVE

Improved treatment options that selectively target cancer-dependent pathways with little or no toxicity to normal tissues are urgently needed. Work in our laboratory focuses on these key aspects by combining the use of DCs pulsed with MHC class I and II peptides and conventional chemotherapy. Immunotherapy may be combined with conventional therapy to reduce Tregs and enhance CTL responses. Knockdown of PD-L1 and PD-L2 on monocyte-derived DCs and tumor cells may help decrease tolerance (Figure 3). DCs electroporated with PD-L small-interfering RNAs are highly effective in enhancing T cell proliferation and cytokine production and are therefore attractive candidates for improving the efficacy of DC vaccines in cancer patients[99]. Moreover, combined blockade of PD1 and CTLA-4, which play key roles in inhibiting T-cell activation, enhances antitumor immune responses compared to either agent alone (Figure 3)[100]. Combining immunotherapies, particularly agents that target different immune checkpoints, may be a promising approach. Preliminary clinical findings indicate that combined targeted therapies and simultaneous blockade of multiple immune checkpoints could promote therapeutic synergy and long-term antitumor immunity to improve clinical outcomes for melanoma patients[101]. In the metastatic CT26 CRC mouse model, simultaneous blockade of CTLA-4 and PD-L1 enhanced antitumor

activity in an interleukin-15-dependent manner[102].

CONCLUSION

The limitations of surgery and adjuvant chemo/radio/antibody therapies in treating CRC patients necessitate the development of novel approaches, including immunotherapy. Despite tremendous progress in basic immunological research, effective immunotherapies for most types of cancer, including CRC, are still lacking. Immunotherapy alone may be insufficient for treating advanced CRC patients. The most promising therapeutic approach for activating antitumor immunity in cancer patients may be blockade of inhibitory immune regulatory proteins such as immune checkpoint ligands and receptors. Therefore, it is important to develop cancer vaccines that do not express inhibitory molecules such as PD-L1, but do express high levels of molecules that enhance CTL priming, such as CD80 and 4-1BBL. The blockade of multiple immune regulatory checkpoints combined with immunotherapy and/or conventional therapy may be effective in treating patients with advanced CRC.

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