Progress of research on γδ T cells in colorectal cancer (Review)

LIJUAN PAN[1], YIRU ZHOU[2], YEYE KUANG[2], CHAN WANG[2], WEIMIN WANG[1], XIAOTONG HU[2,3] and XIABIN CHEN[1]

[1]School of Pharmacy, Hangzhou Normal University, Hangzhou, Zhejiang 311121, P.R. China; [2]Biomedical Research Center and Key Laboratory of Biotherapy of Zhejiang, Sir Run Run Shaw Hospital, Zhejiang University, Hangzhou, Zhejiang 310016, P.R. China; [3]Department of Pathology, Sir Run Run Shaw Hospital, Zhejiang University, Hangzhou, Zhejiang 310016, P.R. China

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Abstract. Colorectal cancer (CRC) ranks as the third most prevalent malignancy and second leading cause of cancer-related fatalities worldwide. Immunotherapy alone or in combination with chemotherapy has a favorable survival benefit for patients with CRC. Unlike αβ T cells, which are prone to drug resistance, γδ T cells do not exhibit major histocompatibility complex restriction and can target tumor cells through diverse mechanisms. Recent research has demonstrated the widespread involvement of Vδ1T, Vδ2T, and γδ T17 cells in tumorigenesis and progression. In the present review, the influence of different factors, including immune checkpoint molecules, the tumor microenvironment and microorganisms, was summarized on the antitumor/protumor effects of these cells, aiming to provide insights for the development of more efficient and less toxic immunotherapy-based anticancer drugs.

Correspondence to: Dr Xiaotong Hu, Department of Pathology, Sir Run Run Shaw Hospital, Zhejiang University, 3 Qingchun East Road, Hangzhou, Zhejiang 310016, P.R. China
E-mail: hxt_hz@zju.edu.cn

Dr Xiabin Chen, School of Pharmacy, Hangzhou Normal University, 2318 Yuhangtang Road, Hangzhou, Zhejiang 311121, P.R. China
E-mail: xch226@hznu.edu.cn

Abbreviations: CRC, colorectal cancer; MHC, major histocompatibility complex; TCR, T-cell receptor; TNF-α, tumor necrosis factor alpha; IFN-γ, interferon gamma; TME, tumor microenvironment; IEL, intraepithelial lymphocyte; pAg, phospho-antigen; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; IPP, isopentenyl pyrophosphate; IL-2, interleukin-2; CICs, cancer initiating stem cells; 5-FU, 5-fluorouracil; DXR, doxorubicin; CSCs, cancer stem cells; ADC, antibody-drug conjugates; inf-DCs, inflammatory dendritic cells; PMN-MDSCs, polymorphonuclear myeloid-derived suppressor cells; MSI, microsatellite instability; PD-1, programmed cell death protein 1; PD-L1, programmed cell death-Ligand 1; HSCs, hematopoietic stem cells; Tet1, Ten Eleven Translocation 1; NKT, natural killer T cell; Tregs, regulatory T cells; MSS, microsatellite-stable; BiTE, bispecific T-cell engager; CAR, chimeric antigen receptor

Key words: CRC, γδ T cells, immunotherapy, BTNL, microorganisms

Contents

1. Introduction

Colorectal cancer (CRC) ranks as the third most prevalent malignancy and the second leading cause of cancer-related mortality worldwide (1). Previous clinical studies have demonstrated that immunotherapy monotherapy or in combination with chemotherapy confers a favorable survival benefit for patients with CRC (2). Previously, CRC immunotherapy focused primarily on αβ T cells, which exert cytotoxicity by recognizing mutant antigens in tumor cells through the major histocompatibility complex (MHC) (3). However, cancer cells typically exhibit depletion of MHC molecules, which renders tumor cells immune to αβ T-cell-mediated cell mortality (4). Another T-cell type in humans, the γδ T cell, exhibits MHC-unrestricted lytic activity against different tumor cells *in vitro*, suggesting the possibility for application in cancer treatment (5).

In humans, γδ T cells associated with CRC can be generally categorized into three types according to the chains on the T-cell receptor (TCR) surface: Vδ1, Vδ2 and Vδ3 T cells (6). The thymus and mucosal epithelial tissues contain

the majority of Vδ1 T lymphocytes, which release various cytokines, including tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ), which have cytotoxic effects on tumor cells and are crucial in the development of numerous illnesses. Vδ2 T cells comprise 50-90% of all γδ T cells, mostly in the peripheral circulation. The TCR of Vδ2 T cells primarily utilizes Vγ9 and Vδ2, which may detect phosphorylated antigens for activation and release perforin and granzymes, resulting in cytotoxicity. Activated Vδ2 T cells can act as antigen-presenting cells (6-8). The proportion of Vδ3 T cells among the total γδ T cells is <1%, and Vδ3 T cells are predominantly localized in the liver and intestine (9). These cells exhibit cytotoxicity through the expression of genes encoding cytotoxic molecules such as granzyme B, perforin, granulysin, and also possess NKG2D receptors for tumor cell recognition and elimination (10). γδ T cells can be classified into regulatory γδ, γδ T17, IFN-γ^+ γδ, and other functional types. The main impediment to the therapeutic application of these cells lies in the immune evasion mechanisms employed by tumor cells (11). Tumor cells can alter the function of the host immune system and create a tumor microenvironment (TME) conducive to tumor development, allowing immune evasion (12). Additionally, several studies have demonstrated a correlation between the gut microbiota and γδ T cells (13), with an imbalance in the gut microbiota potentially promoting the progression of inflammation toward CRC (14). An understanding of γδ T-cell characteristics and the mechanism of action involving the TME and the gut microbiota with γδ T cells will facilitate the development of novel anti-CRC therapeutics and establish a foundation for clinical treatment combinations.

2. Vδ1 T cells

The predominant infiltrating γδ T cells in CRC tissues are Vδ1 T cells (15). Vδ1 T cells have been shown to exert anticancer effects in colon cancer through the secretion of enzymes and proteins (CD107a, granzyme B and perforin) and direct interactions with cytotoxicity-related receptors and ligands (Fas, MICA/B, death receptor 4/5 and ICAM-1) (16,17). NKp46 is one of the three natural cytotoxic receptors first identified as a germline-encoded protein. The percentages of total Vδ1 and NKp46$^+$/Vδ1 subgroups among intraepithelial lymphocytes (IELs) in CRC tumors are significantly lower than those in disease-free/healthy intestinal tissue samples. Additionally, there is a correlation between a decreased frequency of NKp46$^+$/Vδ1 IEL subgroups in healthy intestinal tissue samples from patients with CRC and faster tumor growth and the emergence of metastatic illness (18). The liver of patients with CRC liver metastasis is infiltrated by CD69 Vδ1 T cells, which play crucial roles in limiting metastasis. These cells can also be used as a reliable prognostic marker in 'liquid biopsy' (19). De Vries *et al* (10) revealed that PD1$^+$ Vδ1 T cells can eliminate tumor cells via the NKG2D/NKG2D-ligand interaction pathway (10). In addition to their potential as antitumor agents, Vδ1 T cells have demonstrated the ability to prevent tumor metastasis, effectively suppressing primary tumor growth and inhibiting the development of spontaneous liver and lung metastases in a xenograft model utilizing immunodeficient mice (20).

Currently, there is a paucity of research on Vδ1 T cells in CRC, likely because of the heterogeneous nature of Vδ1 T-cell populations in this malignancy (21), which poses challenges for investigation. The feasibility of categorizing Vδ1 T cells and selectively acquiring distinct subsets of Vδ1 T cells for targeted investigations may be explored in the future.

3. Vδ2 T cells

The reduced presence of Vδ2 T cells in patients with colitis-induced cancers can potentially be attributed to impaired recruitment of Vδ2 T cells from the peripheral circulation and sustained inflammatory processes resulting in the depletion of Vδ2 T cells (22). A potential strategy for treating tumors involves promoting the proliferation and augmenting the functionality of Vδ2 T cells. Currently, research efforts have focused predominantly on investigating the antitumor potential of Vγ9Vδ2 T cells.

Antitumor effects of Vγ9Vδ2 T cells. The recognition of tumor cells by Vγ9Vδ2 T lymphocytes is predominantly MHC-unrestricted, with CRC cell lines being recognized by ascites-derived Vγ9Vδ2 clones and regulated by both TCR-dependent and TCR-independent signals (23,24). It has been reported that Vγ9Vδ2 T cells recognize tumor cells through the CDR3δ region of the γδ-TCR (25). In a subsequent study, Zhao *et al* (26) engineered CDR3δ-transplanted Vγ9Vδ2 T cells capable of producing antitumor cytokines upon stimulation with tumor cell extracts. Furthermore, this antitumor effect was attenuated by the administration of anti-γδ-TCR monoclonal antibodies (26). Another study identified specific sequence and structure patterns in CDR3δ, including rearrangement within the J1 region, the presence of atypical T-cell receptor genes, the positioning of hydrophobic amino acids in CDR3δ, the distribution of CDR3δ lengths, and the number of N insertions. These factors may impact the affinity between T-cell receptors and antigens, consequently influencing T-cell activation and expansion (27).

The activation of Vγ9Vδ2 T cells can be induced by the overexpression of phospho-antigen (pAg) (18) and the interaction between NKG2D receptors and ligands in CRC (28,29). Once activated, Vγ9Vδ2 T cells can eliminate tumor cells through various mechanisms, including the engagement of death receptors/ligands with Fas ligands and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and the secretion of perforins, cytokines (such as TNF-α), or granzymes (30). These pAgs are mainly pyrophosphates produced in eukaryotes via the mevalonate pathway (31). Different phosphate antigens activate Vγ9Vδ2 T cells through different mechanisms. For example, bromo-hydro-pyrophosphate directly stimulates Vγ9Vδ2 T cells, whereas amino-bisphosphonates, such as pamidronate and zoledronate, indirectly activate Vγ9Vδ2 T cells by inhibiting the mevalonate pathway, thereby increasing the intracellular accumulation of isopentenyl pyrophosphate (IPP) (32,33). IPP accumulates in numerous types of cancer, and the resulting disordered metabolic processes render cancer cells susceptible to Vγ9Vδ2 T-cell-mediated mortality (6). Reportedly, interleukin-2 (IL-2) stimulates the production of the adaptor molecule

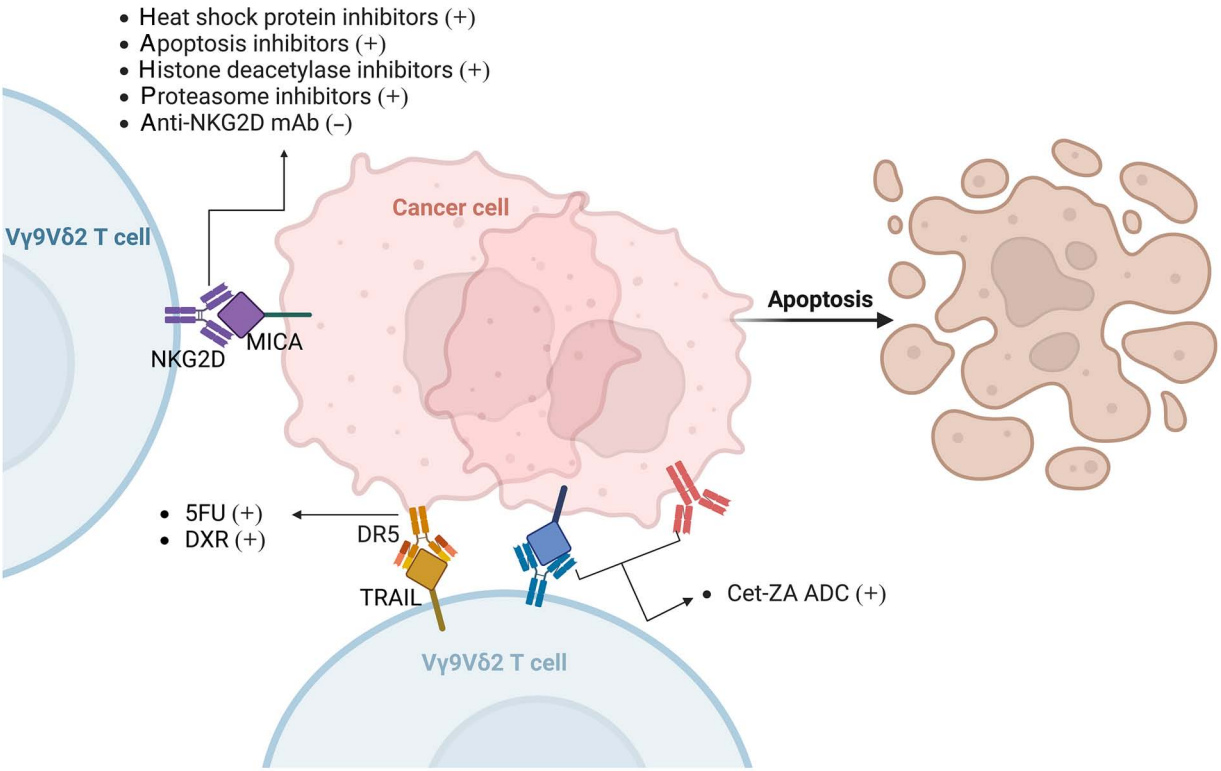

Figure 1. Dual effects of drug treatment on Vγ9Vδ2 T-cell cytotoxicity. Proteasome inhibitors, histone deacetylase inhibitors, apoptosis inhibitors, 5-FU and DXR can increase the cytotoxicity of Vγ9Vδ2 T cells against tumor cells by increasing the expression of NKG2D ligands and DR5 on tumor cells, whereas the use of anti-NKG2D mAbs can inhibit the cytotoxicity. Cet-ZA ADCs can activate T-cell receptor-induced tumor cell death. 5-FU, 5-fluorouracil; DXR, doxorubicin; ADC, antibody-drug conjugates; mAb, monoclonal antibody.

DAP10, increasing the surface expression of NKG2D (34). Similarly, Smyth *et al* (35) reported that the cytotoxicity of IL-12-induced cells toward tumor cells is contingent upon the interaction between NKG2D and its corresponding ligand. Pei *et al* (36) reported that CD137 co-stimulation can overcome the inhibitory effect of endogenous IL-10 (hIL-10 and vIL-10) on the antitumor activity of Vγ9Vδ2 T cells, thereby enhancing the efficacy of this specific subset in tumor therapy. However, according to Zhang *et al* (37), soluble NKG2DLs impair the cytotoxicity of γδ T cells to tumor cells. Therefore, increasing the expression of NKG2DLs within tumors or employing targeted delivery of synthetic adhesives to tumors may be an effective approach for enhancing the antitumor efficacy of γδ T cells.

Dual effects of drug treatment on Vγ9Vδ2 T-cell toxicity. Evidence from three lines of investigation demonstrated that chemotherapy enhances the susceptibility of colonic cancer initiating stem cells (CICs) to Vγ9Vδ2 T-cell toxicity. Pioneering work by Mattarollo *et al* (38) demonstrated that the combination of Vγ9Vδ2 T cells and chemotherapeutic agents yields a high level of cytotoxicity in cell lines derived from solid tumors. IL-17-producing γδ T cells play a decisive role in immune responses against cancer induced by chemotherapy in mice (39). Simultaneous or immediate *in vivo* activation of Vγ9Vδ2 T cells or adoptive transfer of *in vitro*-activated Vγ9Vδ2 T lymphocytes following treatment with the chemotherapeutic drugs 5-fluorouracil (5-FU) and doxorubicin (DXR) significantly increased antitumor activity (7).

Vγ9Vδ2 T-cell elimination post-chemotherapy in CICs is mediated through the activation of NKG2D and TRAIL (7). 5-FU and DXR significantly increase the expression of DR5 (TRAIL-R2) in colon cancer stem cells (CSCs). Additionally, the anti-NKG2D mAb effectively suppresses the cytotoxicity of Vγ9Vδ2 T cells against colon CSCs, whereas neither anti-CD3 nor anti-TCR antibodies nor mevastatin (a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor that prevents endogenous pAg accumulation) demonstrate significant inhibitory effects (34). The expression of NKG2D ligands on tumor cells can be induced by various drugs, including proteasomes, histone deacetylases, heat shock proteins and apoptosis inhibitors (40-44), thereby increasing the toxicity of Vγ9Vδ2 T cells and inhibiting tumor development. In addition, Benelli *et al* (45) developed a Cet-ZA antibody-drug conjugate (ADC) that targets CRC cells and enhances Vδ2 T-cell cytotoxicity through the TCR pathway (Fig. 1). Although numerous studies are underway, drug toxicity and targeting remain challenges.

4. γδ T17 cells

γδ T17 cells represent a prominent source of IL-17 within the TME. Activated inflammatory dendritic cells (inf-DCs) can induce γδ T17 cells to generate TNF-α, IL-8 and GM-CSF, while immunosuppressive polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) accumulate in tumors. The regulatory axis of inf-DC-γδT17-PMN-MDSCs in human CRC establishes a connection between MDSC-mediated

Table I. Classification of γδ T cells.

Classification basis	Cell type	Critical functions	Supplement	(Refs.)
δ chain	Vδ1 T cells	• Secrete cytokines: TNF-α, IFN-γ; • Secrete enzymes and proteins: CD107a, granzyme B, and perforin; • Express cytotoxicity-related receptors and ligands: Fas, MICA/B, death receptor 4/5 and ICAM-1, NKp46, NKG2D	• Are the mainly invasive γδ T cells in rectal cancer tissue; • Have heterogeneity in tumors, are less researched	(6,7,10, 15-18,21)
	Vδ2 T cells	• Release perforin, cytokines and granzyme; • Act as antigen present cells; • Recognize tumor cells through the CDR3δ region of γδ-TCR; • Bind to death ligands expressed by tumor cells: Fas ligands, TRAILs, NKG2DLs; • Express immunosuppression related genes: B7-H3, PD-1, Tim-3	• Comprise 50 to 90% of all γδ T cells; • Numerous studies have been conducted on Vγ9Vδ2 T cells	(6-8,25, 28-30, 63,76,79)
	Vδ3 T cells	• Secrete granzyme B, perforin, granulysin; Express NKG2D receptors	• Are the lowest, less than 1%	(9,10)
Function	IFN-γ-producing γδ T cells	• Produce IFN-γ	• Mainly include $V\gamma1^+$ and $V\gamma7^+$ cells; • Antitumor activity was dependent on Glut1 expression	(50)
	IL-17-producing γδ T cells	• Produce IL-17	• Mainly include $V\gamma4^+$ and $V\gamma6^+$ cells; • Protumor activity was dependent on lipid content	(52,53)

immunosuppression and tumor-induced inflammation, highlighting the potential role of γδ T17 cells in the progression of human CRC (46).

The percentage of tumor-infiltrating γδ T17 cells positively correlates with the progression of TNM stage and other clinicopathological characteristics, including tumor size, tumor invasion, lymphatic and vascular invasion, lymph node metastasis and the serum carcinoembryonic antigen level (46,47). Furthermore, inf-DC, PMN-MDSC, IL-23 and IL-17 levels in tumor tissue are significantly related to the proportion of tumor-infiltrating γδ T17 cells (46,48). Following acute intestinal injury, $IL\text{-}23R^+ROR\gamma T^+\gamma\delta$ T cells located in the colonic lamina propria serve as pivotal sources of initial protective IL-17 within the intestines, playing an indispensable role in preserving and enhancing the integrity of the intestinal mucosal epithelial barrier (49). The dual role of γδ T17 cells in tumors poses a challenge for developing immunotherapies targeting this specific cell subset. Further comprehensive investigations are warranted to elucidate their functional pathways within the TME and identify pivotal breakthroughs.

5. Discrimination of pro- and antitumor intestinal γδ T-cell subsets

Using human CRC samples and mouse CRC models, Reis *et al* (50) discovered that in premalignant or nontumor colons, most γδ T cells exhibit cytotoxic markers, whereas tumor-infiltrating γδ T cells display protumorigenic characteristics. The aforementioned observation is linked to distinct TCR-Vγδ gene expression patterns in both humans and mice.

The γδ T cells that produce IFN-γ, particularly the $V\gamma1^+$ and $V\gamma7^+$ cells, exhibit antitumor activity that is dependent on Glut1 expression (50). These findings suggest a link between diabetes and cancer. A study conducted by Mu *et al* (51) on tumor immune monitoring in diabetic patients via γδ T cells also demonstrated that elevated glucose levels can impair the antitumor activity of Vγ9Vδ2 T cells through lactate-induced inhibition of AMPK activation, resulting in a reduced ability to secrete lytic granules and increased susceptibility to cancer in individuals with type 2 diabetes. IL-17-producing γδ T17 cells express Vγ6 (according to the Vγ nomenclature of Heilig and Tonegawa) and Vγ4 TCR chains (52), which rely on oxidative phosphorylation, continuously proliferate in lipid-rich environments, such as tumors, and promote tumor progression, indicating that this may be another mechanism connecting cancer and obesity (53). In addition, the balance of tissue recovery mechanisms may involve cytokines or molecules other than IL-17 produced by $V\gamma4^+$ or $V\gamma6^+$ cells, which can also promote the formation of tumors (50). This effect is because the generation of IL-17 by γδ T cells in the gut is also related to tissue healing (49,50) (Table I).

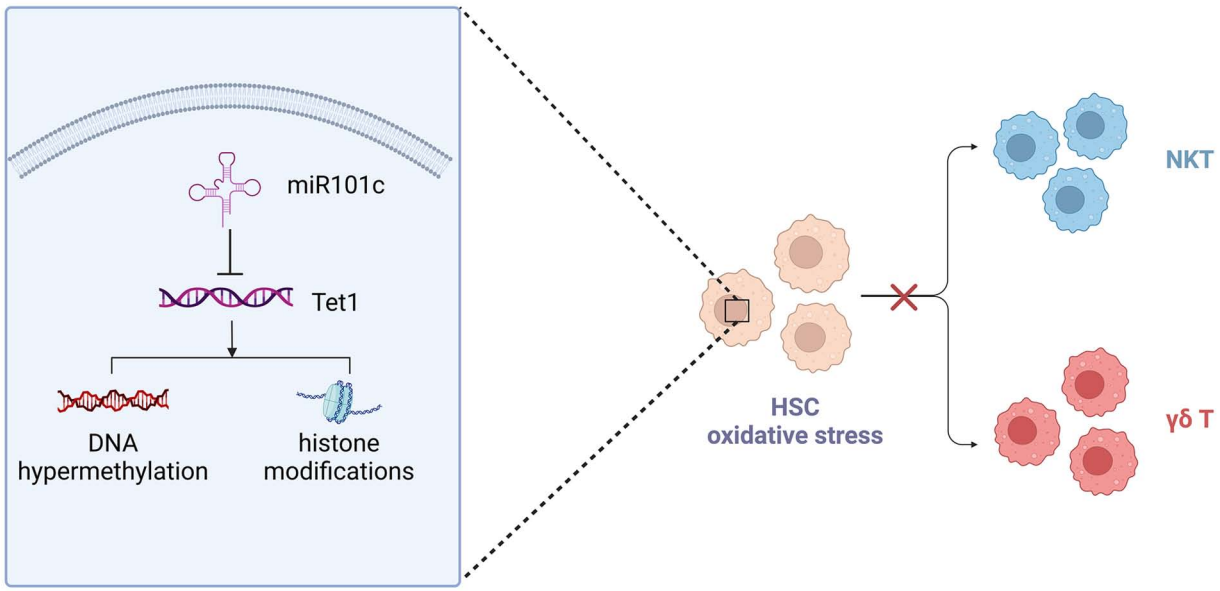

Figure 2. Inhibitory effect of the downregulation of Tet1 on γδ T cells. The presence of hypercholesterolemia can induce miR-101c-mediated oxidative stress, resulting in the downregulation of Tet1 in HSCs. This leads to increased DNA hypermethylation and histone modifications in genes crucial for the differentiation of NKT and γδ T cells. Tet1, Ten Eleven Translocation 1; miR, microRNA; HSCs, hematopoietic stem cells; NKT, natural killer T.

TCR sequencing research has shown that γδ T cells with antitumor characteristics include polyclonal Vγ7$^+$ and Vγ1$^+$ cells, and a minority of tumor-promoting cells that produce IL-17 are Vγ4$^+$ cells; most are clonally expanded Vγ6Vδ1$^+$ cells (54). Although Vγ6$^+$ cells are the predominant progenitor subset in tumors, Vγ4$^+$ cells appear to be able to compensate when Vγ6$^+$ cells are damaged, similar to Vγ7$^+$ cells (mostly gut-specific γδ T cells) and Vγ1$^+$ cells (with broad tissue distribution); elimination of Vγ1$^+$ cells from the tumor is required when performing antitumor functional analyses of Vγ7$^+$ cells (54,55).

6. Immune checkpoint genes that act on γδ T cells

The utilization of synthetic immune checkpoint inhibitors has emerged as a prominent area of research in the field of CRC immunotherapy and has demonstrated remarkable efficacy, especially in patients with microsatellite instability (MSI)-high CRC (56). These agents target immune checkpoints, such as the programmed cell death protein 1 (PD-1)/programmed cell death-Ligand 1 (PD-L1) pathway, which tumors utilize to evade detection by the immune system. By obstructing this interaction, these inhibitors can augment the immune response against cancer cells (57). Despite the potential for adverse effects, immune checkpoint inhibitors have been shown to have a greater safety profile than chemotherapy (58-60). Several studies on γδ T cells have identified immune checkpoint genes, which are expected to be used to screen drugs for the treatment of CRC.

Inhibitory effect of the downregulation of Ten Eleven Translocation 1 (Tet1) on γδ T cells. Tie *et al* (61) reported that hypercholesterolemia leads to oxidative stress in hematopoietic stem cells (HSCs), accelerating HSC senescence and impairing the regenerative capacity of HSCs (61). Tet1 is a direct target of miR101c, and mechanistic studies have revealed that hypercholesterolemia induces oxidative stress that is mediated by miR101c, which causes Tet1 to be downregulated in HSCs. This effect causes genes essential for natural killer T (NKT) and γδ T-cell development to undergo an increase in DNA hypermethylation and histone alterations (Fig. 2). Consequently, the quantity and functionality of terminally differentiated NKT and γδ T cells within the thymus, colonic submucosa, and early stages of tumorigenesis are reduced. This impairment compromises immune surveillance against colonic tumors, which can be ameliorated by restoring Tet 1 expression (62).

Inhibition of Vδ2 T-cell cytotoxicity by B7-H3. Despite a significant reduction in the proportion of γδ T cells in both peripheral blood mononuclear cells and tumor areas among patients with colon cancer, there is an increase in the proportion of B7-H3$^+$γδ T lymphocytes. It is postulated that B7-H3 functions as a negative immune checkpoint molecule, modulating the activity and biological function of γδ T cells in colon cancer. It has been revealed that blocking or reducing B7-H3 leads to enhanced proliferation, inhibition of apoptosis, and upregulation of activation markers (CD25 and CD69) in Vδ2 T cells. Conversely, the B7-H3 agonist 4H7 exerts the opposite effect. In the presence of IL-2 and zoledronic acid, Vδ2 T cells treated with MIH35 (a specific inhibitory antibody against B7-H3) or B7-H3 siRNA presented increased cell viability, a reduced rate of apoptosis, and increased expression of the signaling molecules CD25 and CD69 (63).

The inhibition of Vδ2 T cells by B7-H3 is mediated mainly by the suppression of T-bet and a decrease in IFN-γ and perforin/granzyme B expression, which involves STAT3 activation and a reduction in ULBP2 expression (11,63). Cryptotanshinone, an inhibitor of STAT3 phosphorylation, can reverse the decrease in ULBP2 expression and attenuate the B7-H3 overexpression-induced elimination of colon cancer cells by Vδ2 T cells (11). The B7-H3-mediated STAT3/ULBP2

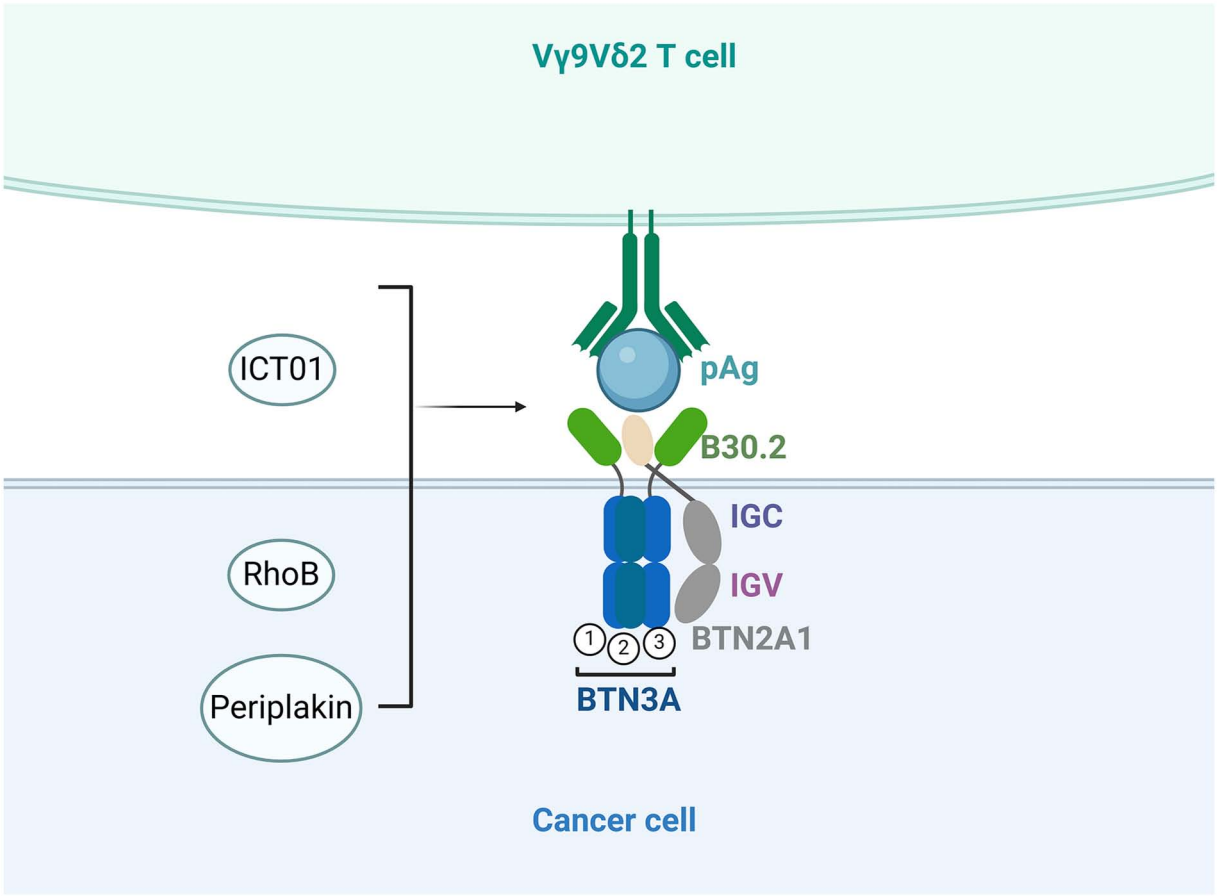

Figure 3. The structure of BTN3A and its ability to activate Vγ9Vδ2 T cells. The activation of Vγ9Vδ2 T cells by BTN3A1 requires the presence of BTN3A2 or BTN3A3, and the cytotoxicity of Vγ9Vδ2 T cells mediated by BTN3A must involve BTN2A1. ICT01, Periplakin and RhoB play important roles in this activation process.

axis may be a potential target for enhancing the efficiency of γδ T-cell-based colon cancer immunotherapy (11,63).

Diverse impacts of BTN/BTNL on γδ T cells. In mice, Btnl proteins are predominantly expressed on the epithelial cells lining the intestinal villi (64). Previously, the expression of Btnl1 in intestinal villi in the early stage of life was shown to selectively promote the maturation and proliferation of $V\gamma7^+$ IELs in tissues (65), revealing its antitumor potential, whereas the expression of Btnl2 in tumor cells specifically recruits IL-17-producing γδ T cells that promote tumorigenesis (66).

The Butinophil-3A (BTN3A, also known as CD277) protein subfamily plays a crucial role in the antitumor process of γδ T cells by serving as a pivotal mediator of pAg signal transduction (67). The BTN3A molecular subfamily is part of the B7 costimulatory molecular family and includes the BTN3A1, BTN3A2 and BTN3A3 subtypes (68). The three subtypes can stimulate Vγ9Vδ2 T cells following treatment with the 20.1 agonist mAb, activating the cells through mechanisms involving mobility reduction (67) and BTN3A molecular polymerization (69). However, BTN3A1 cannot mediate the activation of Vγ9Vδ2 T cells without BTN3A2 or BTN3A3; Cano *et al* (70) also demonstrated that BTN3A-mediated cytotoxicity of Vγ9Vδ2 T cells toward cancer cells must involve BTN2A1.

De Gassart *et al* (71) developed a humanized monoclonal antibody, ICT01, which has sub-nanomolar affinity for all three subtypes of BTN3A. Its activity depends on BTN3A and BTN2A (Fig. 3). The activation of Vγ9Vδ2 T cells by ICT01 eliminates multiple tumor cell lines and primary tumor cells (71). It has been reported that periplakin and RhoB are pivotal in activating Vγ9Vδ2 T cells, mediated by BTN3A (72). Additionally, Vγ9Vδ2 T cells exhibit cytotoxicity against CRC cell lines upon exposure to zoledronate, which is also related to the expression of BTN3A1 in the membrane and cytoskeleton and its redistribution in cells (32). Due to the absence of a B30.2 intracellular domain, the BTN3A2 subtype fails to activate Vγ9Vδ2 T cells when pAgs accumulate. Consequently, it can be considered a decoy receptor, and its increased expression in acute myeloid leukemia primitive cells or other tumors may constitute an immune escape mechanism recognized by Vγ9Vδ2 T cells (72).

Human intestinal epithelial cells express BTNL3 and BTNL8, and the concurrent expression of $BTNL3^+BTNL8$ induces a selective TCR-dependent response in $V\gamma4^+$ cells of the human colon (65). According to the analysis by Blazquez *et al* (72), the homing and maintenance of BTNL3 and BTNL8 in the semi-activated state in human intestinal $V\gamma4^+$ γδ T cells may be relevant to the pathogenesis of intestinal autoimmune disorders, such as ulcerative colitis and

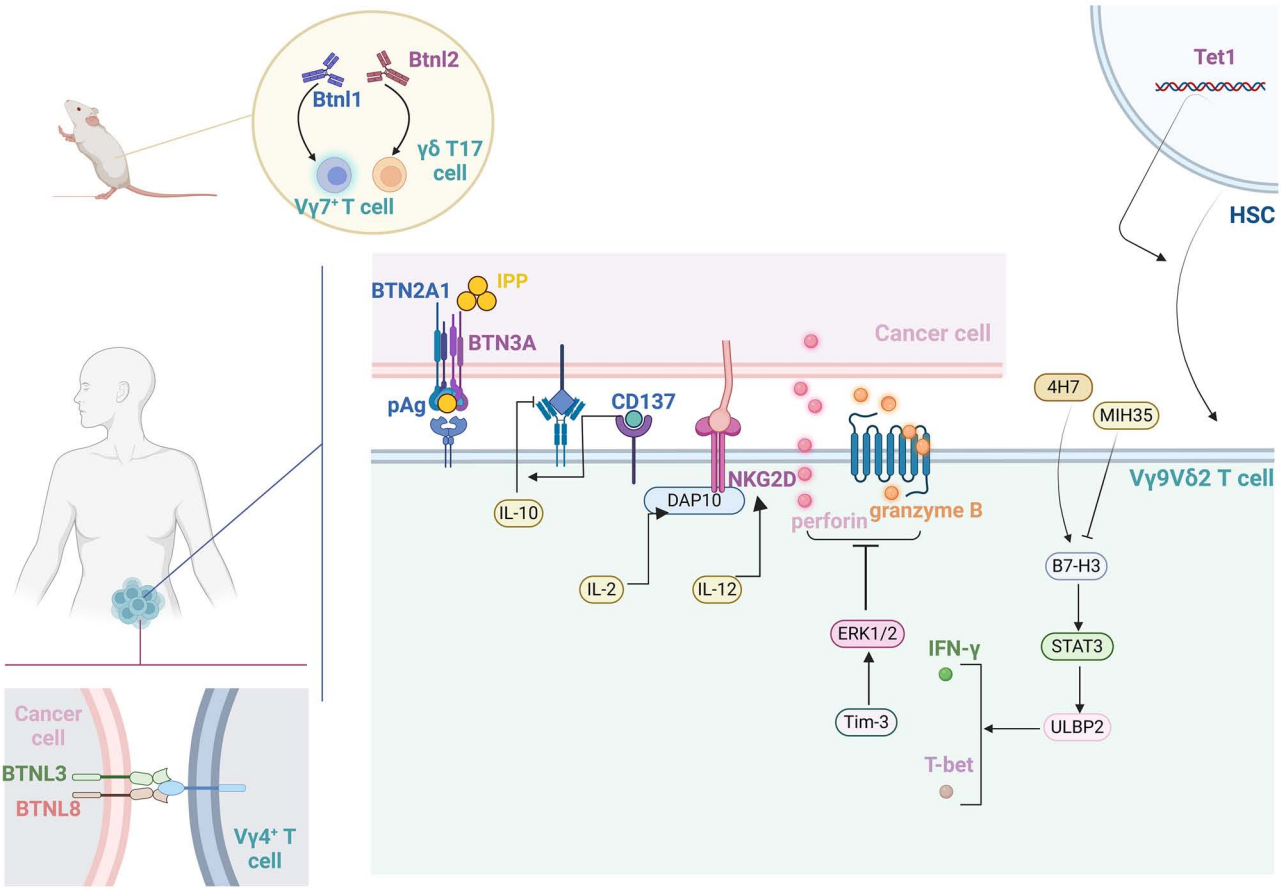

Figure 4. Effects of different factors on γδ T-cell activity. In mice, Btnl1 promotes the maturation and proliferation of Vγ7+ intraepithelial lymphocytes, whereas Btnl2 recruits IL-17-producing γδ T cells. In humans, the co-expression of BTNL3 and BTNL8 results in a selective T-cell receptor-dependent response in human colon Vγ4+ cells. The downregulation of Tet1 results in a decrease in the quantity and functionality of terminally differentiated NKT and γδ T cells. Isopentenyl pyrophosphate accumulation increases the vulnerability of cancer cells to Vγ9Vδ2 T-cell-mediated elimination. IL-2 enhances the expression of NKG2D by inducing DAP10. CD137 co-stimulation can overcome the inhibitory effect of endogenous IL-10 on the antitumor activity of Vγ9Vδ2 T cells. Tim-3 downregulates the expression of perforin and granzyme B in Vγ9Vδ2 T cells. The inhibition of Vδ2 T cells by B7-H3 is mediated mainly by the suppression of T-bet and the downregulation of IFN-γ and perforin/granzyme B expression, which involves STAT3 activation and a reduction in ULBP2 expression. 4H7 and MIH35 can participate in regulating the aforementioned process involving B7-H3. BTN3A plays a role in the antitumor process of γδ T cells as a key mediator of pAg signal transduction. Tet1, Ten Eleven Translocation 1; HSC, hematopoietic stem cell.

inflammatory bowel disease. Chronic intestinal inflammation can promote the formation of colorectal tumors (73,74), revealing the correlation between BTNL3 and BTNL8 and CRC. Lebrero-Fernández *et al* (75) reported significantly lower levels of BTNL3 and BTNL8 expression in colon cancer tissues than in adjacent normal tissues, providing further support for this notion.

Other immune checkpoint genes. The expression of PD-1 can serve as a partial indicator of γδ T-cell function and impact patient prognosis. However, the upregulation of PD-1 alone is insufficient to fully characterize the functional phenotype of γδ T cells in cancer, necessitating comprehensive evaluation of other markers and indicators (76). In academic research, PD-1 is frequently investigated in conjunction with Tim-3 (77), whereas in clinical practice, the combination of PD-1 and CTLA-4 antibodies has demonstrated successful outcomes in the treatment of CRC (78). As a crucial negative regulator of Vγ9Vδ2 T-cell activation, Tim-3 was found to downregulate the expression of perforin and granzyme B in Vγ9Vδ2 T cells via an ERK1/2 signaling pathway-dependent mechanism, thereby attenuating the cytotoxicity of Vγ9Vδ2 T cells to colon cancer cells (79) (Fig. 4). The inhibitory receptors CTLA-4, LAG-3 and TIGIT have been demonstrated to be present on the surface of T cells (80-82). However, the specific mechanism underlying their interaction with γδ T cells remains unclear, particularly in treating CRC. Further investigations into the mechanisms underlying these immune checkpoint genes and the development of diverse immune checkpoint inhibitors for combination therapy may represent promising approaches to enhance the current landscape of CRC treatment.

7. Obstruction of the antitumor process of γδ T cells via the TME

The CRC TME is a complex communication system comprising cancer cells and various other cell types (including endothelial cells, immune cells and cancer-associated fibroblasts). This intricate communication relies on a dysregulated regulatory network comprising chemokines, cytokines, growth factors and their corresponding receptors. Consequently, this dynamic interaction gives rise to an inflammatory TME that facilitates tumorigenesis and progression (83).

In the TME, CRC can be divided into 'hot' and 'cold' subtypes. Hot tumors are characterized by the presence of activated immune cells that exhibit proinflammatory cytokine signaling, and immune checkpoint inhibitors have shown promising efficacy in inhibiting the growth of such tumors (84). By contrast, cold tumors typically express receptors and ligands associated with immunosuppression and are encompassed by populations of immunosuppressive cells, including regulatory T cells (Tregs), MDSCs and tumor-associated macrophages (85). T Immunosuppressive cells can express IL-10 and TGF-β, thereby impeding the infiltration and functionality of effector T cells, including γδ T cells, while facilitating immune evasion (33,85,86). Modification of the immune microenvironment is essential for treating this type of tumor, including converting a cold tumor into a hot tumor or enhancing effector cell function to achieve effective immunotherapy (84).

Hu *et al* (87) reported that TGF-β1 derived from human CRC could induce CD39γδ T cells from paired normal colon tissue to differentiate into CD39γδ Tregs and that differentiated CD39γδ Tregs could exert adenosine-mediated immunosuppressive activity (87). Another study revealed that the polarization of CD39γδ Tregs is also related to arachidonic acid. Owing to the abnormal activation of the phospholipase a2-IVa/arachidonic acid metabolic pathway, the content of tumor-infiltrating CD39γδ Tregs in right-sided CRC is markedly greater than that in left-sided CRC, indicating a poor prognosis (88). Inhibiting the production and function of CD39γδ Tregs may represent a promising strategy to improve the prognosis of patients with CRC. In addition, hypoxia is a characteristic shared by numerous solid tumors (89). Exosomes undergo alterations in the hypoxic TME and can enhance the inhibitory impact of MDSCs on γδ T cells through a regulatory axis involving miR-21/PTEN/PD-L1 (90). Combining immunotherapy with strategies to increase the tumor oxygen content may improve the treatment outcome for patients with CRC.

8. The microbiota is involved in the antitumor process of γδ T cells

Various studies have demonstrated the profound impact of intestinal microbes on DNA damage, DNA methylation, chromatin structure, and noncoding RNA expression in colon epithelial cells (91). Furthermore, alterations in certain genes and pathways induced by intestinal microbes are closely associated with the CRC development and influence the functionality of γδ T cells in this context. According to previous reports, certain bacteria and their metabolites, including *Bacteroides fragilis*, *Lactobacillus acidophilus*, desulfurizing *Vibrio* and *Citrobacter*, have been found to assist γδ T cells in combating tumors. Conversely, specific gut bacteria, such as *Clostridia* and enterotoxigenic *Bacteroides fragilis*, may accelerate the development of CRC by activating γδ T cells that promote tumor growth (13). Li *et al* (92) reported that phosphatidylethanolamine and phosphatidylcholine, metabolites of *Desulfovibrio*, induced the proliferation of IL-17A-producing γδ T cells, which aggravated intestinal injury. Some probiotics can protect the normal intestinal mucosa in CRC by producing short-chain fatty acids, such as acetate and propionate (93). Propionate can directly act on γδ T17 cells and inhibit IL-17 production in a histone deacetylase-dependent manner (94), whereas *Akkermansia* can reduce the number of IL-17-producing γδ T cells in mice (95), thereby improving intestinal inflammation. Hydroxymethyl-butyl pyrophosphate produced by microorganisms can act as a pAg to activate γδ T cells (96). A study conducted by Roselli *et al* (97) demonstrated that the combination of *L. acidophilus* and *B. longum* effectively impeded the progression of colitis through the modulation of the γδ T-cell population. The α-GalCer produced by *Bacteroides fragilis*, *Bacteroides vulgatus*, *Prevotella copri* and other unidentified bacteria can exert antitumor effects by indirectly inducing the production of IFN-γ by γδ T cells through the activation of invariant NKT cells (98) (Fig. 5). These studies suggested that the gut microbiota actively participates in the antitumor process of γδ T cells, assuming distinct roles. However, most of these studies have focused primarily on the cellular level. Consequently, whether clinical intervention targeting specific gut microbiota components can effectively decelerate tumor progression remains uncertain. In addition, several studies have demonstrated that the gut microbiota can serve as a reliable biomarker for the non-invasive diagnosis of CRC (99-101). However, the selection of appropriate biomarkers and the development of highly sensitive detection methods pose limitations for its clinical application, and further research is needed to achieve breakthroughs.

9. The challenges and prospects of translating research on γδ T cells in CRC into clinical application

In recent years, clinical studies on the application of γδ T cells in CRC immunotherapy have focused primarily on their role in MSI-high CRC and microsatellite-stable (MSS) CRC. MSI CRCs can be categorized into MSI-H and MSI-L groups according to the level of instability, with MSI-L and MSS often grouped together in clinical studies. Given their greater mutation load and neoantigen exposure, MSI-H tumors are more readily recognized and targeted by the immune system, making conventional immune checkpoint inhibitors more effective for treating MSI-H CRC than MSS CRC (56). However, MSS tumors account for the majority of CRC cases, underscoring the pressing need for the development of innovative immunotherapies targeting patients with CRC with MSS tumors (102,103).

Recently, Stary *et al* (104) reported that the dysfunctional cytotoxic potential of Vδ1$^+$ T cells can be restored by *in vitro* activation in MSS CRC, suggesting the possibility of reactivating these cells to exert potent antitumor effects. This discovery offers potential for the advancement of immunotherapies targeting γδ T cells in MSS CRC and holds promise for future development. Additionally, numerous studies have provided further evidence supporting the clinical investigation of γδ T cells in CRC. Stary *et al* (104) conducted single-cell RNA sequencing and TCR sequencing on γδ T cells from human CRC specimens and revealed that Vδ1$^+$ cells derived from MSS CRC contribute to tumor immune evasion by upregulating exhaustion-associated genes while downregulating effector genes. Furthermore, it was

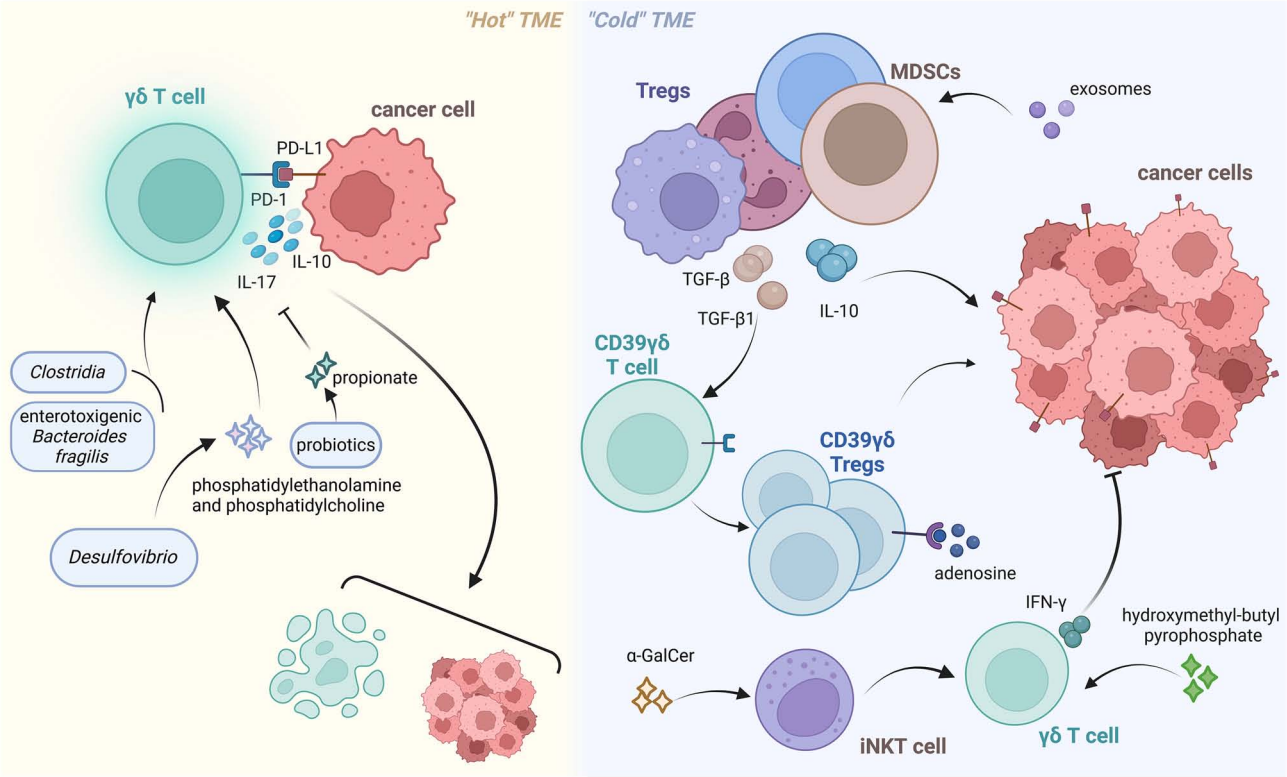

Figure 5. Effects of TME and gut microbiota on antitumor function of γδ T cells. The activated γδ T cells surrounding the hot tumor can secrete immunosuppressive cytokines and express receptors involved in immunosuppression, which can bind to antibodies present on the surface of tumor cells. This leads to depletion of γδ T cells within the TME and promotes tumor progression. Cold tumors are typically surrounded by immunosuppressive cells, such as Tregs and MDSCs, which express IL-10 and TGF-β to suppress the antitumor effect of γδ T cells. TGF-β1 induces differentiation of CD39γδ T cells into CD39γδ Tregs, contributing to adenosine-mediated immunosuppression. Microbes and their metabolites play various roles in this regulatory network. *Clostridia* and enterotoxigenic *Bacteroides fragilis* activate tumor-promoting γδ T cells. Phosphatidylethanolamine and phosphatidylcholine, metabolites of *Desulfovibrio*, induce proliferation of γδ T17 cells. Propionate, a probiotic metabolite, inhibits IL-17 production. Hydroxymethyl-butyl pyrophosphate is used as a phospho-antigen to activate γδ T cells. α-GalCer activates iNKT cells and indirectly induces IFN-γ production by γδ T cells against tumors. TME, tumor microenvironment; Tregs, regulatory T cells; MDSCs, myeloid-derived suppressor cells; iNKT, invariant natural killer T.

discovered that this dysfunction can be reversed through modulation of the TIGIT-NECTIN axis (104). Wu *et al* (105) found that knocking out QPCTL in cancer cells could promote their escape from Vγ9Vδ2 T-cell elimination through genetic screening and experimental validation, suggesting that QPCTL may be a new entry point to solve CRC immune evasion. A study conducted by Xu *et al* (106) demonstrated the safety and efficacy of allogeneic Vγ9Vδ2 T-cell immunotherapy in prolonging the survival of patients with advanced lung or liver cancer, which could also have implications for the treatment of CRC. Additionally, ongoing investigations are exploring the utilization of bispecific T-cell engager (BiTE) technology, chimeric antigen receptor (CAR) modification and synthetic phosphorylated antigens (107-110). However, the clinical application of BiTE and CAR modification technologies in CRC is hindered by off-target effects, cytokine release syndrome and neurotoxicity (111,112). The optimization of targeting technology and the development of combination drugs will significantly expedite the implementation process of these two novel therapies for CRC. Although there are significant obstacles to overcome in harnessing the potential of γδ T cells for CRC treatment, ongoing research and clinical development efforts are paving the way for potentially transformative immunotherapies that could provide new hope for patients with this prevalent cancer.

10. Conclusions

γδ T cells have emerged as pivotal players in the immunotherapy landscape of CRC, exerting their antitumor effects independently of MHC restrictions and exhibiting the ability to recognize and respond to tumor cells that may have evaded conventional αβ T-cell surveillance. With the discovery that Vδ2 T cells can recognize pAgs and be activated by them to exert antitumor activity, Vδ2 T cells have emerged as a prominent research focus in recent years. Numerous researchers have dedicated efforts to investigating pAgs and their associated activation pathways, enhancing our understanding of Vδ2 T cells. Several immune checkpoint genes have been identified during investigations into the mechanism of action between Vδ2 T cells and tumor cells. The combination of PD-1 and CTLA-4 inhibitors has demonstrated efficacy in treating CRC. Moreover, ongoing research is exploring additional immune checkpoint genes as promising therapeutic targets for CRC in the future. The finding that the cytotoxic potential of dysfunctional Vδ1 T cells in tumors can be reactivated *in vitro* holds promise for the treatment of MSS CRC unresponsive to immune checkpoint inhibitors. In addition, utilizing BiTE and CAR modification technology, along with integrating multiple approaches, significantly enhances CRC therapy efficacy. However, given the intricate regulatory

network of the TME, research on the specific regulatory mechanism is lacking. In the future, combining drugs that target diverse cellular components within the TME to impede the progression of CRC may be possible. Recent studies have shown that gut microbes and their metabolites also interact with γδ T cells and tumors. However, the diversity of gut microbes and the lack of methods for sample collection, storage and analysis pose obstacles to related research. Therefore, the mode of interaction between the gut microbiota and γδ T cells in CRC and the microbial flora involved in this process remains unclear. In the future, analyses of the gut microbial species involved in the antitumor process of γδ T cells can be initiated to discover new methods for treating or diagnosing CRC.

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Authors' contributions

XH and XC conceived and designed the study. LP, YZ and WW prepared and wrote the manuscript. YK and CW edited the manuscript. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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