Review

T Cell–Associated Immunotherapy for Hepatocellular Carcinoma

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Key Words

T cellular immunotherapies • Hepatocellular carcinoma • Immune checkpoint blockade • T cell receptor-engineered T cells • Chimeric antigen receptor-engineered T cells • Bispecific T cell engagers

Abstract

Hepatocellular carcinoma (HCC) is one of the most common malignant diseases worldwide with limited therapeutic options. Accumulating evidences suggest that immunotherapy could be a promising option for treating HCC. T cell-associated immunotherapy lights up the hope for the improvement of complementary approach to conventional HCC treatments, which needs further research to consummate the clinical consequences. The present work reviewed several T cells associated cellular immunotherapies for HCC, including immune checkpoint blockade, gene-engineered T cells, bispecific T cell engagers, and so on. We also analyzed how these immunotherapies can mediate tumor cell eradication and evaluated their superiority or insufficiency.

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Introduction

Hepatocellular carcinoma (HCC) ranks the sixth most common cancer and the third most common cause of cancer mortality worldwide; it has poor prognosis and limited therapeutic options [1, 2]. Therefore, HCC prevention and treatment are of great concern. The progress gained from knowledge of the association between HCC and the immune system has yielded significant treatment strategy breakthroughs. Owing to the exquisitely specific immune responses, immunotherapy is dramatic and promising for the treatment of HCC [3].

The immune response is responsible for controlling nascent cancer through immunosurveillance [4]. If the early-stage micro tumors are not eradicated thoroughly by the innate or adaptive ways of immune system, then a long period of equilibrium (expansion of transformed cells is held in check by immunity) exists [5]. Eventually, the loss of tumor-associated antigens (TAAs) or decreased major histocompatibility complex (MHC) antigen

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expression, inactivation of T cells by reduced T cell receptor (TCR) signalling or interleukin (IL)-10 and transforming growth factor (TGF)- β -mediated suppression are developed. Then, a scene of immune tolerance and inactivate tumor-specific T cells caused by the above immune evasion mechanisms lead to the loss of capabilities that immune system cannot recognize TAAs and arm effective immune response against tumors [6].

T cells are suitable candidates for tumor immunotherapy to overcome these barriers and rekindle the compromised immune response of the patient against the tumors. Reports have stated that the frequencies of CD8⁺ T [7], CD4⁺ cytotoxic T cells [8], natural killer (NK) [9], and regulatory T (Treg) cells [10] in the tumor tissue of HCC are significantly associated with survival. CD4⁺ T cells can also efficiently promote tumor regression through their ability to secrete interleukin-2 (IL-2) [11], enhancing tumor-specific CD8⁺ T cells [12]. Among patients who diagnosed with HCC, CD8⁺ T cells respond to HCC-specific TAAs such as α -fetoprotein (AFP), glypican-3 (GPC3), melanoma antigen gene A1 (MAGE-A1), and NY-ESO-1, which can be applied to distinguishing tumor cells from normal tissues [13]. In view of the fact that HCC is correlated with persistent hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, viral proteins of HBV and HCV could also play a role of TAA in HCC [14]. Overexpression of APOBEC3F in tumor tissues is potentially predictive for poor recurrence-free survival from HBV-HCC patients [15]. Through RNA-sequencing and immunohistochemistry data, HCC-specific genes have been identified and validated. Two of them (namely, AKR1B10 present in the "HCV-HCC-specific" signature; IGF2BP3 common to both "HBV- and HCV-HCC-specific" signatures) which are detected not being expressed in most of normal tissues, showed a strictly HCC-specific protein expression pattern, suggesting their highly potential for HCC immunotherapy [16].

However, there still are various mechanisms considered as the factors which lead to the weak and often inefficient antitumor immune responses in HCC, such as insufficient TAA processing and presentation, the existence of immunosuppressive cell, like regulatory T cells (Treg), shortage of CD4⁺ T helper cell responses and the negative regulation by PD-1/PD-L1 (programmed cell death-1/ ligand for programmed cell death-1) pathway [17]. Cancer-secreted TGF- β 1 may increase the quantity of Tregs and induce Treg cell polarization, therefore promoting the progression of HCC [18, 19].

As an emerging method of tumor clearance aiming to afford more effective and selective targeting of tumor cells by recruiting or facilitating the existing tumor-specific immune response, T cell immunotherapy circumvents many immune evasion mechanisms. T cell immunotherapy for HCC has been maturing through decades' worth of effort. Various tumor immunotherapy techniques associated with T cells (Fig. 1) have been developed during the last two decades and brought excellent outcomes. The present work reviews several methods of immune modulation (Table 1) that have been explored and shown effective in suppressing HCC growth.

Lymphokine-activated killer cells

Peripheral blood leukocytes (PBL) isolated from patients with cancer and activated *in vitro* by IL-2, leading to the enhancement of effector cells cytotoxic to autologous fresh solid tumor cells in a manner unrestricted by MHC, were reported in the early 1980s [20]. These lymphocytes are called lymphokine-activated killer (LAK) cells. It has been reported that LAK therapy could significantly prolong the survival time of HCC patients, but failed to decrease the tumor mass [21]. Although LAK cell therapy for HCC has induced objective responses in a minority of patients [22], it produces considerable side effects simultaneously because the dose of recombinant IL-2 (rIL-2) required is usually toxic [23]. In view of the limited efficacy and increasing doubts whether LAK cells indeed cause the observed responses, the initial enthusiasm for LAK cells has waned and has been transferred to other immunotherapies.

Fig. 1. The process of T cellular immunotherapy CD4⁺/CD8⁺ T cells are isolated through patient's peripheral blood or tumor tissues, and expanded and activated with suitable doses of interleukins, cytokines or peptides *ex vivo*. Or, by genetic modification, these T cells are endowed the specific anti-tumor activity. After expansion and alteration, certain amount of these T cells re-infused to patients to exert the function of cytotoxic for autologous tumor cell. Besides, the infusion of immune checkpoint blockade antibody or BiTE is not only working on immune cells, but also tumor cells.

Tumor-infiltrating lymphocytes

Reported in 1986, tumor-infiltrating lymphocytes (TIL) isolated from tumor samples could recognize tumors *in vitro*; the adoptive transfer of these syngeneic TIL, expanded in IL-2 affected the regression of established lung and liver tumors on murine tumor models [24]. TILs which play a positive role in the survival of HCC patients [25] could recognize TAAs on tumor cells and then trigger antitumor responses [26]. So, isolating, stimulating and expanding TILs *in vitro* from HCC patients, then infusing into the hosts could theoretically kill the tumor cells. Stefan M. Brunner, *et al* indicated that tumor-infiltrating, IL-33-producing effector-memory CD8⁺ T cells were independently related to prolonged HCC patient survival [27]. Clinical trials utilizing autologous TIL in patients with HCC following tumor resection have indicated that re-infusion of autologous TIL after activation and expansion *in vitro* can be successfully performed with low toxicity [28].

However, these therapies have limitations. These TAA specific T cells naturally exist in HCC tissues, but the antitumor ability is restricted and exhausted [29]. Let alone the enormous difficulties in the isolation TIL from HCC patients and the proliferation *in vitro*, only patients with good performance status who can endure the rigorous lymphodepletion- and IL-2-based treatments currently used which could cause associated adverse effects are appropriate for such treatments.

Cytokine-induced killer cells

Cytokine-induced killer cells (CIK cells) are a mixture of non-MHC-restricted T lymphocytes comprising CD3⁺/CD56⁺ cells, CD3⁺/CD56⁺ NK cells, and CD3⁺/CD56⁻ cytotoxic

Table 1. Ongoing studies for T cell associated immunotherapy for Hepatocellular carcinoma

Strategy	Clinicaltrials.gov identifier	Phase	Intervention	Status
TIL	NCT01462903	1	Biological: tumor infiltrating lymphocytes, IL-2	recruiting
	NCT01174121	2	Biological: Young TIL; Drug: Aldesleukin Drug: Cyclophosphamide; Drug: Fludarabine	recruiting
	NCT02315066	1	Drug: PF-04518600	recruiting
	NCT01821482	2	Biological: Dendritic and Cytokine-induced Killer Cells	Not yet recruiting
CIK	NCT02568748	3	Biological: CIK Procedure: TACE	recruiting
	NCT02487017	2	Procedure: Transcatheter Arterial Chemoembolization(TACE) Biological: DC-CIK	recruiting
	NCT01914263	1	Biological: cytokine induced killer cell	recruiting
	NCT01868490	1, 2	Drug: cytokine induced killer cells	recruiting
CTL	NCT02239861	1	Biological: TAA-Specific CTLs	recruiting
TCR	NCT02686372	1	Biological: HBV antigen specific TCR redirected T cell	recruiting
	NCT02719782	1, 2	Biological: HBV antigen specific TCR redirected T cell	recruiting
	NCT02395250	1	Biological: anti-GPC3 CAR T	recruiting
	NCT02587689	1, 2	Biological: anti-MUC1 CAR T Cell	recruiting
CAR	NCT02723942	1, 2	Biological: CAR-T cell immunotherapy for GPC3 positive HCC	recruiting
	NCT02715362	1, 2	Drug: TAI-GPC3-CAR cells	recruiting
	NCT02905188	1, 2	Genetic: GLYCAR T cells; Drug: CytoxinDrug: Fludarabine	Not yet recruiting
	NCT02959151	1, 2	Drug: CAR-T cell	recruiting

T cells, among which the main effector cells are CD3⁺/CD56⁺ T cells which are rare in uncultured peripheral blood [30]. Capitalizing on patients' instinct to eradicate tumor cells via stimulation with cytokines like OKT3 monoclonal antibodies, IL-2, and interferon- γ (IFN- γ), CIK cells could be converted from peripheral blood mononuclear cells (PBMCs) [31]. Compared with LAK cells, CIK cells have the characteristics of high proliferation rate, potent anti-tumor effects and minimal cytotoxicity to normal cells [32-35], these advantageous aspects render CIK cells a favorable option in cancer immunotherapy [36].

Preclinical trials have shown that CIK cells have higher anti-tumor cytotoxic activity *in vitro* and stronger effects on tumor growth inhibition in tumor-bearing nude mice models than LAK cells and PBMC for treating HCC [37]. In addition, co-culture with dendritic cells enhances the anti-tumor effects of CIK cells [38, 39]. Moreover, several clinical studies chose CIK cell immunotherapy as adjuvant treatment following hepatectomy for HCC patients, indicating a significant increase in overall survival (OS) and recurrence-free survival (RFS) [34]. The median overall survival and RFS for patients who received hepatectomy and postoperative CIK cell immunotherapy were 41 and 16 months, respectively, while 28 and 12 months for the hepatectomy alone patients [40]. Besides the similar results that increased OS and RFS due to the adjuvant CIK immunotherapy, another phase 3 clinical trial has tested its efficacy and safety. Although adverse reactions, like pyrexia, chills, myalgia and fatigue were correlated to CIK cell agents in 17% of patients, they were not severe enough to delay or cease the therapy [41]. And, the combined treatment of transcatheter arterial chemoembolization (TACE) and radiofrequency ablation (RFA) with sequential CIK immunotherapy could lower the recurrence rate of HCC and prolong the RFS and OS of HCC patients without severe adverse effects [42, 43]. However, a golden prognostic system is still needed to be established which is relevant to weighing the benefit from the adjuvant CIK cells immunotherapy [40].

Cytotoxic T lymphocytes

When TAAs are processed and presented to the MHC by antigen-presenting cells (APCs), T cell activation is triggered involving two signals, one of which is from the binding of TCR to the MHC-bound antigen. Then the signals are magnified or counteracted by costimulatory molecules [4]. Cytotoxic T lymphocytes (CTLs) are CD8⁺ $\alpha\beta$ T cells that could recognize MHC molecules, present TAAs and release granzyme B and perforin to lyse tumor cells. However, the downregulation of MHC, the existence of suppressor immune cells, like Tregs, myeloid-derived suppressor cells, and the lack of costimulatory molecules restrained the effect of re-infused CTLs.

The re-activation of the immune system is considered as a crucial treatment strategy for cancers. New immunotherapy strategies such as peptide vaccines similar to TAAs have been

attempted, and were able to stimulate T cells, causing the proliferation of effective CTLs to eliminate TAA-positive target cells. Preclinical and clinical trials have demonstrated the anti-tumor activity of HCC vaccines that induce peptide-specific CTL responses [44-46].

Besides that, the immune checkpoint blockade is another way. The activated immune checkpoints caused by chronic exposure to antigens could lead to T cells exhaustion and facilitate tumor evasion from host immune system [47]. The most studied immune checkpoint receptors are CTLA-4, PD-1, TIM-3, BTLA, VISTA, LAG-3 and OX40 which reduce the antigen specific immune response [48]. The blocking antibodies of CTLA-4 and PD-1 which disrupt CTLA-4/PD-1 pathways aiming to reversing exhausted T cells have already been approved by FDA for the treatment of malignancies and currently in development in HCC. The process of T cell activation needs amplified signals mediated by CD28/B7 interactions. On the contrary, CTLA-4 shares two ligands (CD80 and CD86) with CD28 and obtains stronger affinity with B7-1 and B7-2 on activated B cells and monocytes, then inhibits further costimulation [49, 50]. It has also been reported that CTLA-4 expressed on Tregs and suppressed effector T-cell activation and function [51, 52]. The safety profile and antitumor and antiviral effect, have been tested by the usage of antibody, Tremelimumab (CP-675,206) which blocks the binding of CTLA-4, on advanced HCC with HCV-related cirrhosis [53]. The combined treatment of tremelimumab and tumor ablation for advanced HCC patients brings supporting clinical activity and causes expansion of intratumoral CD8⁺ T cells [54].

Similarly, PD-1 interactions with PD-L1 and PD-L2 inhibits the proliferation of T cells and restrains the release of cytokines [48]. PD-1 acts late for long-term tolerance maintenance, while CTLA-4 acts early for tolerance induction [55]. Tumor cells expressed PD-L1 or PD-L2 could evade the elimination of immune system by PD-L1/PD-1 signal pathway which stimulates PD-1 in TILs [48]. The drastically increased expression of PD-1 was found on tumor infiltrating CD8⁺ T cells in HCC and related with poorer disease progression and postoperative relapse. Moreover, by blocking PD-L1, CD8⁺ T cells apoptosis was reversed [56]. Therefore, inhibiting PD-1/PD-L1 pathway may enhance the efficacy of therapy. Phase I clinical trials blocking PD-1/PD-L1 in cancer patients with various malignancies have shown promising results [57, 58]. Blocking PD-1/PD-L1 which is expressed at high level on T cells from tumor and liver than peripheral blood from HCC patients increased the frequency of tumor-specific T cells in HCC patients [59]. PD-1/PD-L1 blockade could increase the immune response of vaccine-induced GPC3-specific CTLs, therefore enhance the antitumor effects of a GPC3 peptide vaccine for HCC [60]. Several clinical trials with PD-1 blockade for advanced HCC patients is under recruiting. Recently, Calderaro, *et al.* detected that PD-L1 expression by either neoplastic or intratumoral inflammatory cells was associated with tumor aggressiveness which suggested that PD-L1/PD-1 immune checkpoint blockade might be efficacious only in a subgroup of HCC patients and limited to particular HCC variants [61]. Therefore, the various tumor subtypes should be taken into account in the subsequent clinical trials, which could better illustrate the anti-tumor efficacy of PD-L1/PD-1 immune checkpoint blockade.

Realizing the great effect of immune checkpoint blockade, more and more researchers believe that anti-tumor effectiveness could be enhanced through combinatorial approaches. Recently, a clinical trial is under recruiting to evaluate the effectiveness, safety and tolerability of Nivolumab and the combination Nivolumab and Ipilimumab in patients with advanced HCC (NCT01658878).

T cell receptor-engineered T cell immunotherapy

Via their T cell receptor, T cells can respond to TAAs occasionally. A commonly observed mechanism of tumor immune tolerance caused by reduction of major MHC antigens [62] usually impairs the ability of T cells to respond to tumor antigens throughout the lifetime of an individual, therefore presenting a major challenge to isolating tumor-specific T cells from most patients with cancer.

Fig. 2. The comparison of TCR, CAR and BiTE Genetic engineering techniques can be applied to introduce antitumor receptors into normal T cells. (a) The above image shows that insertion of a conventional TCR and CAR into a patient's T lymphocytes, followed by the expansion and infusion back into the patient. TCR is composed of one α chain and one β chain, and they recognize antigens that have been processed and presented by the patient's own MHC molecules. The CAR is composed of linked V chains from a TAA-specific monoclonal antibody, bound to a spacer arm from the hinge region of IgG or from the CD8 receptor. This then attaches to a CD28 transmembrane domain (TM), costimulation molecule (such as OX-40, 4-1BB) and cytoplasmic domain which is composed of the CD3 ζ signaling element. The gene modified CAR is inserted into CD4⁺/CD8⁺ T cells isolated from PBMC of patients to obtain the tumor specific CAR-T cells which can recognize TAA presented on the tumor cell surface. (b) BiTEs consist of two ScFvs originating from two separate mAbs; one ScFv recognizes a tumor antigen, while the other is specified for CD3 ϵ on T cells. A small fusion linker connects both variable fragments. Both arms of BiTE must be bound to mediate tumor cytotoxicity.

Fortunately, through genetic engineering, T cells could be endowed with the capacity to be reactive against tumors (Fig. 2). TCR genes which consist of α - and β -chains united with the γ -, δ -, ϵ -, and ζ -chains of the CD3 complex are derived from tumor-specific T cells, and they could encode cell surface receptors which can recognize TAAs. These TCR genes obtained from humans or the immunization of human leukocyte antigen (HLA) transgenic mice that express human MHC, alternatively, can be generated in bacteriophages that bind to tumor peptide antigens [63]. When a tumor antigen peptide fragment presented on the tumor cell MHC is encountered by TCR, the phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) happens, following an intracellular signaling cascade causing the release of cytokines and cytotoxic compounds from T cells [64].

There are many applications of TCRs in HCC (see more details in Table 2) [65-71]. TCR genes endow T cells with tumor-specific responses [66]. TCR therapy had been applied on the HBV-related HCC, as the close relationship between HCC and HBV infection and high level of HBV integrations in HBV-related HCC tumors [72]. Adam J. Gehring, *et al* demonstrated that TCR re-directed HBV-specific T cells derived from PBMC of chronic HBV and HBV-related HCC patients through retroviral transduction, had the capabilities of recognizing HBV-infected cells and HCC tumor cells expressing viral antigens from naturally integrated HBV DNA [65]. To overcome the deficiencies of the viral vectors, like underlying risk of oncogene

Table 2. Several studies of TCR immunotherapy

Antigens targeted	Study	Restriction	Construction of TCR	Source of T cells	T cell type	transduction	Trials
HBV	Gehring et al. [65]	HLA-A2	HBc18-27 specific TCR; HBs183-91-specific TCR (s183-TCR); HBsAg370-79, (s370-TCR)	5 healthy controls and 10 chronic HBV patients, 5 HBV-related HCC patients	CD8+ T cells	Retrovirus	<i>in vitro</i>
	Koh et al. [66]	HLA-A2	HBV s183-91-specific TCR (s183-TCR)	5 healthy donors	CD8+ T cells	Electroporation	<i>in vitro</i> & <i>in vivo</i>
	Qasim et al. [67]	HLA-A2	HBV s183-91-specific TCR (s183-TCR)	1 patient with HCC relapse 10 years following liver transplantation for HBV+ HCC	autologous T cells	Retrovirus	Clinical
	Bertoletti et al. [71]	HLA-A0201	HLA-A0201/HBs183-91-specific TCR	1 HBV related HCC patient with extrahepatic HCC metastasis	autologous T cells	Electroporation	Clinical
HCV	Zhang et al. [68]	HLA-A2	HCV NS3:1073-1081 TCR	1 patient with a chronic HCV infection	CD4+ and CD8+ T cells	Retrovirus	<i>in vitro</i>
GPC3	Dargel et al. [69]	HLA-A2	Glypican-3367 Specific T-Cell Receptor P1-1	1 healthy HLA-A2 negative donor	CD8+ T cells	Retrovirus	<i>in vitro</i> & <i>in vivo</i>
AFP	Sun et al. [70]	HLA-A2	AFP158-166-specific TCR transgenic CTLs	HLA-A*02:01 positive healthy donors	CD8+ T cells	Lentivirus	<i>in vitro</i> & <i>in vivo</i>

activation [73, 74], Sarene Koh, *et al* endowed T cells with an antigen-specific functionality by mRNA electroporation, these transiently expressing anti-HBV TCR could have efficient anti-HCC activity *in vitro* and a xenograft model of HCC, despite the transient functionality [66]. Furthermore, Antonio Bertoletti, *et al* applied the engineered HBV-TCR-T cells on a HBV-related HCC patient who had a liver transplant and then with extrahepatic HCC metastasis with HBV-DNA integration [71]. These re-infused HBV-TCR-T cells dramatically decreased the level of HBsAg in the patient, while, unfortunately, no detectable reduction of the volume of the HCC metastasis [71]. While, the HBV antigen might be expressed on the non-tumor hepatocytes, not exclusively on the tumor cells and this adoptive TCR modified HBV-specific T cells may trigger severe liver damage [66]. Now, limited knowledge on HBV integrations and antigen expression in HBV-related HCC and whether HBV integrations preferentially occur in HBV-infected hepatocytes or transformed/cancerous hepatocytes. Thus, the potential efficacy of TCR immunotherapy specific for HBV-related HCC patients should be considered in the future studies. Besides, the restriction of a specific HLA molecule for TCR-T cell to recognize the target antigen processed within the cytoplasm and presented on the surface of cancer cells, TCR immunotherapy should be improved in a wide range [75].

Chimeric antigen receptor-engineered T cell immunotherapy

Chimeric antigen receptors (CARs) arm T cells with ability of recognizing defined antigen specifically and permitting T cell targeting of specified tumor cell. Connected by single-chain variable regions (ScFv) of monoclonal antibody heavy and light chains that can identify the TAA and T cell signal transduction zone, CAR-T cells can distinguish an overexpressed tumor cell surface antigen directly.

The extracellular antigen-binding domain, the transmembrane domain, and the cytoplasmic signaling domain are the three main domains of a CAR. The signal sequence of costimulatory molecules may facilitate the proliferation of T cells and prevent activation-induced cell death (AICD) and T cell depletion [76]. In the application of CD20-CAR-T cell treatment of relapsed indolent B cell lymphoma or mantle cell lymphoma clinical trials, first-generation CAR-T cells which only fused CD3 ζ or Fc receptors as the signal domain did not obtain significant anti-tumor abilities, *in vivo* proliferation, or persistence [77]. To make improvements, one or more costimulatory molecules, like CD28, CD134 (OX-40), and CD137 (4-1BB) are fused to CD3 ζ [78]. The so-called second generation CAR contains two signaling domains and the third-generation contains additional ones. Both of them have the advantage of cytokine production and proliferation *in vitro*, and mediate tumor regression in xenograft models in NOD/SCID/ZC (NSG) mice in comparison with the first-generation CAR-T cells [79].

Another key point of CARs is the selection of tumor-specific antigen (TSA), which is not significantly expressed in normal tissues. Unfortunately, most currently defined tumor antigens are TAAs that are not expressed in vital tissues or have relatively higher expression in tumor cells than normal cells. However, TAA expression in normal tissues varies; therefore,

CAR-modified immune cells that attack the tumor tissues will also cause damage to normal tissues or organs. In one case, a female patient who received human epidermal growth factor receptor 2 (HER2)/neu-specific CAR-T cell immunotherapy died from off-target toxicity that caused cytokine storm and subsequent organ failure [80].

The success of CAR-T cells in treating CD19-positive hematological malignancies have spurred interest in their application to solid tumors [81-83]. Recently, several studies selected GPC3 as a HCC TAA [69, 84, 85]. Gao H, *et al* and Li W, *et al* constructed CAR-T cells that could specifically target GPC3 on the surface of HCC cells, as the attempts to apply CAR-T cell immunotherapy for the treatment in HCC, which showed significant anti-tumor activity both *in vitro* and *in vivo* [84, 85]. CAR-T cells specific for HBV envelope proteins localized to liver in mice which reduced HBV replication with transient liver damage [86]. These ignited the hope for treating HBV related HCC patients in a non-HLA limited way.

Unlike TCRs, CARs can overcome the restriction of MHC, but still face the problem of safety and insertional mutagenesis. The shortage of appropriate TAA as described above, the inefficient homing of T cells to tumor sites, and the immunosuppressive microenvironment of solid tumors has largely impeded the success of CARs in the context of solid tumors [87].

Bispecific T cell engagers

Derived from the bispecific antibodies composed of two ScFvs, one that recognizes a TAA and another that binds CD3 on T cells [88], bispecific T cell engagers (BiTEs) have been proven as highly efficient T cell recruiters for tumor immunotherapy. BiTE-mediated cytotoxicity occurs only when CD3 and the TAA are both bound by BiTE, theoretically eliciting a polyclonal T cell response towards target tumor cells [89] which could be largely indispensable for conventional T cell recognition molecules like MHC class I molecules and costimulatory proteins [90]. BiTE could recruit and activate T cells resulting in the raising of the characteristic activation markers CD69 and CD25 and the secretion of cytokines [91, 92]. Moreover, the perforin-mediated delivery of granzyme B causes the calcium-dependent proteolytic activation of intracellular caspases leading to death of tumor cells [93, 94].

Blinatumomab (MT103), a CD19/CD3-targeting BiTE, has been approved in the US by the FDA for treating acute lymphocytic leukemia (ALL) recently. As the first tested BiTE in humans, clinical trials have demonstrated its safety and efficacy in treating hematological malignancy [95]. Targeting epithelial cell adhesion molecule (Ep-CAM), frequently overexpressed in most human carcinomas while having limited accessibility to normal epithelial tissues [96], the Ep-CAM-specific BiTE (MT110) has the potential to be applied in various human carcinomas. The great results of use of BiTE in hematologic malignancies and solid tumors, like pancreatic tumor [97, 98]. These good jobs explored the potential of BiTE and encouraged the passion of applying BiTE to treat HCC. Recently, an assay has detected the efficacy of anti-EpCAM BiTE 1H8/CD3 in HCC treatment *in vitro* and *in vivo* experiments [99].

Other than the similarity to CAR-T cells, namely independence of MHC and tumor specificity, BiTEs can not only reactivate T cells within tumor microenvironments [100], but can also convert Treg cell immunosuppression into tumor-directed cytotoxicity [101]. However, considerable clinical trials are needed to confirm the practical anti-tumor efficacy of BiTE in solid tumors including HCC.

Conclusions

T cell associated immunotherapies combined with cytokines, vaccines, and antibodies has achieved significant anti-tumor activity. Although much work have been carried out, the clinical results in solid tumors remain unsatisfactory, as previously reported [87]. Owing to the immune escape and the dearth of specific tumor antigens, only a proportion of T cells can

exert the full anti-tumor function. Decades of improvement have aided the development of T cell immunotherapy. The distinct advances of each strategy could be utilized to exploit better T cell immunotherapies in a wide range of carcinomas, including HCC. T cell immunotherapy could act as an effective therapy which would reduce the rate of relapse, improve the quality of life, and prolong survival in the near future.

Disclosure Statement

No conflict of interest.

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