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T-cell-associated immunotherapy: a promising strategy for the treatment of hepatocellular carcinoma

“Decades of improvement have accelerated the perfection of T-cell immunotherapy. Given the rapid developments in this field, T-cell immunotherapy could be more mature, safe and effective.”

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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]. The progress gained from knowledge of the association between HCC and the immune system has yielded significant treatment strategy breakthroughs. The loss of tumor-associated antigens (TAAs) or decreased MHC antigen expression, inactivation of T cells by reduced TCR signaling or IL-10 and TGF- β -mediated suppression, eventually cause a scene of immune tolerance and inactivate tumor-specific T cells that immune system cannot recognize TAAs and arm effective immune response against tumors [3]. T cells are good candidates for tumor immunotherapy to overcome these obstacles and rekindle the compromised immune response of the patient against the tumors. The frequencies of CD8⁺ T, CD4⁺ cytotoxic T cells, NK, Treg cells in the tumor tissue of HCC are significantly associated with survival. Among patients diagnosed with HCC, CD8⁺ T cells respond to HCC-specific TAAs such as AFP, GPC3, MAGE-A1 and NY-ESO-1, which can be applied to distinguishing tumor cells from normal tissues [4]. HCC is correlated with persistent HBV and HCV infections, viral proteins of HBV and HCV could also play a role of TAA in HCC [5]. Through RNA-sequencing and

immunohistochemistry data, HCC-specific genes have been identified and validated.

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. Various tumor immunotherapy techniques associated with T cells have been developed during the last two decades and brought excellent outcomes.

Lymphokine-activated killer (LAK) cells therapy could significantly prolong the survival time of HCC patients but failed to decrease the tumor mass [6]. Not only the limited efficacy, but also more seriously, the severe side effects simultaneously owing to the dose of rIL-2 required led to increasing doubts and fading enthusiasm for LAK cells. With the ability of recognizing the TAAs, autologous tumor-infiltrating lymphocytes in HCC patients following tumor resection could be successfully performed with low toxicity. However, these TAA-specific T cells naturally existed in HCC tissues with restricted antitumor ability [7]. The process of isolating tumor-infiltrating lymphocytes from HCC patients and the proliferation *in vitro* was challenging and only patients in good status could endure rigorous lymphodepletion- and IL-2-based treatments. Compared with LAK

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cells, cytokine-induced killer (CIK) cells have the feature of high-proliferation rate, potent antitumor effects and minimal cytotoxicity to normal cells. The combined treatment of transcatheter arterial chemoembolization and radiofrequency ablation with sequential CIK immunotherapy could lower the recurrence rate of HCC and prolong the recurrence-free survival and overall survival of HCC patients without severe adverse effects [8,9]. 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 [10].

Cytotoxic T lymphocytes (CTLs) are CD8⁺ $\alpha\beta$ T cells which could recognize MHC molecules, present TAAs and release granzyme B and perforin to lyse tumor cells. However, the lack of MHC, the existence of suppressor immune cells, like Tregs, myeloid-derived suppressor cells and the shortage of costimulatory molecules restrained the effect of reinfused CTLs. Peptide vaccines similar to TAAs have been attempted to stimulate T cells, causing the proliferation of effective CTLs to kill TAA-positive target cells. Besides, the immune checkpoint blockade is another way. The blocking antibodies of CTLA-4 and PD-1 that disrupt CTLA-4/PD-1 pathways aiming to reversing exhausted T cells have already been approved by US FDA for the treatment of malignancies and currently in development in HCC. CTLA-4 expressed on Tregs and suppressed effector T-cell activation and function [11]. CTLA-4 acts early for tolerance induction, while PD-1 acts late for long-term tolerance maintenance. The drastically increased expression of PD-1 was found on tumor infiltrating CD8⁺ T cells in HCC and correlated with poorer disease progression and postoperative relapse. Tremelimumab (CP-675,206), which blocks the binding of CTLA-4, has been applied on advanced HCC with HCV-related cirrhosis resulting in promising safety profile and antitumor and antiviral effect [12]. The combined treatment of tremelimumab and tumor ablation for advanced HCC patients brought supporting clinical activity and causes expansion of intratumoral CD8⁺ T cells [13]. PD-1/PD-L1 blockade could increase the immune response of vaccine-induced GPC3-specific CTLs, and therefore enhance the antitumor effects of a GPC3 peptide vaccine for HCC [14].

By genetic engineering, T cells could be endowed with the capacity to be reactive against tumors. When a tumor antigen peptide fragment presented on the tumor cell MHC was encountered by TCR engineered T cells, the phosphorylation of immunoreceptor tyrosine-based activation motifs happened, following an intracellular signaling cascade causing the release of cytokines and cytotoxic compounds from T cells. TCR

redirected HBV-specific T cells derived from peripheral blood mononuclear cells (PBMCs) of chronic HBV and HBV-related HCC patients had the capabilities of recognizing HBV-infected cells and HCC tumor cells expressing viral antigens [15]. These reinfused HBV-TCR-T cells obviously decreased the level of HBsAg in the patient, while, unfortunately, no detectable reduction of the volume of the HCC metastasis [16]. Unlike TCRs, chimeric antigen receptors (CARs) armed T cells could get rid of the restriction of MHC. Connected by single-chain variable regions (ScFv) of monoclonal antibody heavy and light chains that could identify the TAA and T-cell signal transduction zone, CAR-T cells could distinguish an overexpressed tumor cell surface antigen directly. The second- and third-generation CAR-T cells had the advantage of cytokine production and proliferation *in vitro*, and mediated tumor regression in xenograft models compared with the first-generation CAR-T cells, the main difference among the three generations was the amount of costimulatory molecules. The success of CAR-T cells in treating hematological malignancies spurred interest in their application to solid tumors. GPC3-specific CAR-T cells has been constructed for the treatment in HCC, which showed significant antitumor activity both *in vitro* and *in vivo* [17,18]. CAR-T cells specific for HBV envelope proteins localized to liver in mice, which reduced HBV replication with transient liver damage [19]. Unfortunately, most currently defined tumor antigens were TAAs that were not expressed in vital tissues or relatively higher expressed in tumor cells than normal cells. Therefore, CAR-modified T cells might attack the tumor tissues and cause damage to normal tissues or organs at the mean time. The inefficient homing of T cells to tumor sites, and the immunosuppressive microenvironment of solid tumors are still waiting to be solved.

Derived from the bispecific antibodies composed of two ScFvs, one that recognizes a TAA and another that binds CD3 on T cells, bispecific T-cell engagers (BiTEs) could recruit and activate T cells, which could be largely indispensable for conventional T-cell recognition molecules like MHC class I molecules and costimulatory proteins. The epithelial cell adhesion molecule (Ep-CAM)-specific BiTE (MT110) had the potential to be applied in various human carcinomas. The encouraging results ignited the passion of applying BiTE to treat HCC. Recently, anti-EpCAM BiTE 1H8/CD3 has been proved a promising therapeutic agent for HCC treatment [20].

Decades of improvement have accelerated the perfection of T-cell immunotherapy. Given the rapid developments in this field, T-cell immunotherapy could be more mature, safe and effective.

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