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Research Status of CAR-T Cell Immunotherapy in Tumor Treatment

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Abstract

In recent years, chimeric antigen receptor T-cell (CAR-T) therapy has made breakthroughs in the treatment of hematological tumors. However, due to the different characteristics of solid tumors from hematological tumors, CAR-T has not achieved good efficacy in the treatment of solid tumors. The key factors limiting the efficacy of CAR-T mainly include the solid tumor cells themselves and their special tumor microenvironment (TME), which damage CAR-T function in multiple processes such as CAR-T infiltration to tumor tissue sites, CAR-T maintaining anti-tumor activity in TME, and target recognition and killing of tumor cells by CAR-T. To solve these problems, more and more preclinical studies have proposed potentially effective solutions, and corresponding clinical studies have been carried out one after another. In this article, the existing challenges and corresponding optimization strategies of CAR-T cell therapy for solid tumors will be reviewed, to provide a reference for the future exploration of CAR-T therapy.

Keywords

CAR-T, Tumor, Cell Therapy, Immunotherapy

1. Introduction

Since 2017, a series of Chimeric antigen receptor T-cell (CAR-T) treatments represented by Kymriah (CTL019) and Yescarta (KTE-C19) have been approved for marketing, and CAR-T therapy has made breakthroughs in the treatment of hematological tumors [1]. In March 2021, Rebecca C Larson published a review article in the journal Nature, "Recent advances and discoveries in the mechanisms and functions of CAR T cells", which mainly introduced the efficacy and safety issues in the treatment of CAR-T in the past 3 years. As a new therapy for tumor immunotherapy, CAR-T can overcome the host immune tolerance state,

identify and target tumor cells in a restrictive way with the main histocompatibility complex (MHC), and has the advantages of strong targeting, a wide range of tumor-killing and long-lasting effect, and has been successfully applied to the clinical treatment of a variety of hematological tumors [2] [3]. Hematological malignancies originate from the uncontrolled growth of hematopoietic cells and lymphocytes at different stages of maturation and differentiation and currently account for 6.5% of all cancers worldwide [4]. As of August 2022, the Food and Drug Administration (FDA) has approved the listing of 6 CAR-T products, and the National Medical Products Administration (NMPA) has also approved two CAR-T products in 2021, indicating the arrival of the era of CAR-T treatment. Although CAR-T cell therapy has obvious efficacy for hematological tumors, its application in solid tumors still faces many challenges. A 2019 meta-comprehensive analysis (913 subjects) of 42 hematology and 18 solid tumors clinical trials showed that 54.4% of hematology patients were treated with CD 19 CAR-T while the complete response rate was only 4.1% in patients with solid tumors [5]. Given the urgent need to meet the therapeutic needs of solid tumors, the challenges and feasible solutions of CAR-T therapy for solid tumors are summarized and discussed, to provide ideas for subsequent research on CAR-T cell therapy for solid tumors.

2. CAR-T Cell Immunotherapy

2.1. Development of CAR

Compared with T cells, CAR-T cells carry CAR parts in addition to antigen receptor (TCR) complexes, and the CAR structure determines the specificity of CAR-T. CAT is a synthetic protein, and the CAR structure used in currently approved CAR-T cells includes two parts: extracellular region and intracellular active region that recognize tumor-associated antigens [6].

Since CAR-T technology was first proposed in 1989, CAR has developed into five generations, mainly in the intracellular signaling region changes [7]. The first-generation CAR design is to fuse the variable region of tri nitrophenyl (TNP) antibody with the constant region of TCR, and this structure can be stably expressed on the surface of T cells after transduction, and MHC unrestricted recognition of killer target cells and secretion of IL-2 [8] [9]. Although the first-generation CAR-T cells have the advantage of tumor killing *in vitro*, in clinical experiments, CAR-T cells have limited expansion ability and duration *in vivo*, and cannot completely remove tumor cells, resulting in tumor recurrence [10]. The second-generation CAR draws on the classical signal of T cell activation and adds a co-stimulatory molecule, such as 4-1BB (also known as CD137) or CD28, based on the first-generation CAR structure, which can significantly improve the activation level and proliferation ability of CAR-T cells, and clinical data show that the tumor burden of patients receiving second-generation CAR-T cell therapy is effectively controlled for a long time [11] [12]. At present, newly discovered co-stimulatory molecules include inducible costimulatory molecules (ICOS),

OX40 (also known as CD134), and CD40, and the second-generation CAR structure is also the most mature in clinical application [13] [14] [15] [16]. The third generation of CAR is to add 1 co-stimulatory molecule based on the second generation CAR structure, that is, the same CAR structure expresses a total of 2 co-stimulatory molecules. Compared with the second-generation CAR-T cells, the cytotoxicity of third-generation CAR-T cells was further improved, and the results of Cappell *et al.* [17] showed that the proliferation and cytokine release levels of CAR-T cells co-expressed by 4-1BB and CD28 were better than those of CAR-T cells containing only a single co-stimulatory molecule. At the same time, ICOS-BBz CAR-T cells constructed by Guedan *et al.* [13] [18] showed a longer duration *in vivo*. Fourth-generation CAR, also known as T cells redirected for universal cytokine killing (TRUCK T), is based on second- or third-generation CAR that co-expresses some other molecules, including IL-7, IL-15, and IL-21 [19] [20] [21] [22], which promote T cell proliferation, or IL-12 and IL-18, which enhance the effect ability of T cells [23] [24] [25], or chemotaxis of other immune cells or CAR-T cells to the C-C motif chemokine 19 (CCL-19) and CCL-21 around tumor cells [26].

T cell proliferation and cytokine secretion are enhanced by increasing co-stimulatory molecules (e.g., CD28, 4-1BB, or OX40) to the intracellular signaling domain [27], and can release pro-inflammatory factors in the tumor microenvironment [28]. However, CAR-T still has many problems in its application, such as CD19-specific CAR in the treatment of lymphoid leukemia, which has achieved great success, but CD19 escape variants have been found after treatment, and subsequent target loss patients show disease recurrence. To overcome the problem of antigen targets of escaped mutants, the treatment of double CAR and other means such as combined immune checkpoint antibodies has emerged, which have become the focus of related research.

2.2. Mechanism of CAR-T Therapy

CAR-T therapy is to activate T cells *in vitro* and modifies genes, so that CAR that specifically recognizes tumor cell surface molecules is expressed on the surface of T cells, and through CAR-specific binding to tumor surface antigens, it causes the activation of intracellular signaling molecules and further activates T cells to play a specific anti-tumor effect [29]. The CAR-T mechanism is shown in **Figure 1**. Normal T cell-mediated cytotoxicity relies on major histocompatibility complexes (MHCs), T cells undergo cascade reactions through signaling pathways, activating T cells into cytotoxic T cells (CLT), and when CLT encounters tumor cells containing the same MHC-antigen peptide, the two specifically bind to stimulate CLT cells to exert cytotoxic effects [30] [31], while the antigen recognition process of CAR-T does not depend on the single-chain variable region (scFv) on the MHC, CAR molecule It can directly recognize and bind tumor cell antigens, and has targeted specificity, which is a major advantage of CAR-T therapy.

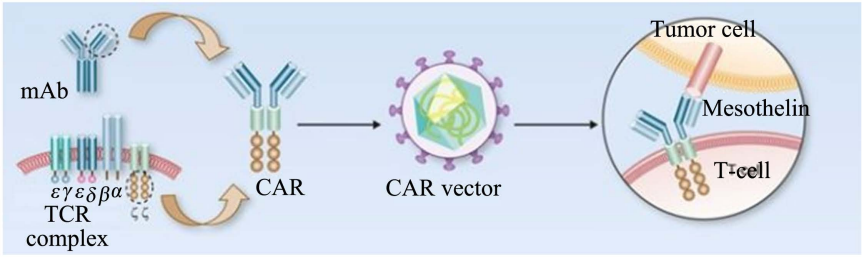

Figure 1. The CAR-T mechanism.

3. Challenges with CAR-T Therapy

3.1. Lack of Tumor-Specific Target Antigens

The chimeric antigen receptor (CAR) on CAR-T cells consists of an extracellular antigen-binding region, a transmembrane region, a hinge region, and an intracellular signal transduction region that recognizes tumor-associated antigens (TAAs). The effectiveness of CAR-T cell therapy depends mainly on the recognition of TAA. The commonly used targets and quantities of CAR-T therapy for hematological tumors are CD19 (140), CD22 (20), and BCMA (22). Unlike hematological tumors, specific TAAs are difficult to find in solid tumors, and most TAAs are not only highly expressed on the surface of cancer cells, but also in normal cells, so most CAR-T cells therapies face the dilemma of "on-target, off-tumor" that attacks normal cells [32]. As an example, HER2 targets are overexpressed on the surface of breast cancer cells but are also expressed in small amounts in normal cardiopulmonary tissue, and a colon cancer patient who received higher doses of HER2 CAR-T cells developed acute respiratory distress and died the day after treatment only within 15 minutes of infusion [33].

3.2. There Are Disorders of Cell Transport and Tumor Infiltration

The key to eliminating solid tumors is to transport CAR-T cells to the surface of tumor cells to bind to target antigens and exert a killing effect. Although CAR-T cells are in easy contact with hematological tumor cells that are dispersed and circulating in the blood and lymphatic system, their transport to solid tumor lesions still has many obstacles. On the one hand, the extracellular matrix (ECM) and tumor blood vessels with cancer-associated fibroblasts (CAFs) as the main components form a natural physical barrier for CAR-T cells, making it difficult to effectively homing and infiltrate tumors [34]. On the other hand, stromal cells, tumor cells, and tumor-associated immune cells of solid tumors secrete a special cytokine, chemokines, which can bind to their corresponding chemokine receptors and recruit immune cells into the tumor microenvironment (TME), thereby promoting or inhibiting tumor growth. The lack of chemokine receptors on the surface of CAR-T cells that match the chemokines secreted by solid tumors results in the poor homing ability of CAR-T to tumor sites [35]. The combination of the above two factors makes it difficult for CAR-T cells to accurately reach the solid tumor site and exert immune effects.

3.3. Immunosuppression of the Tumor Microenvironment

However, even if a portion of CAR-T cells can successfully infiltrate into solid tumors, their efficacy will be greatly reduced by the immunosuppressive effect of TME. First of all, there are soluble inhibitors such as prostaglandin E2 (PGE2), transforming growth factor-$\beta\beta$ (TGF-β), interleukin 6 (IL-6) and IL-10 in TME, which can inhibit tumor cell apoptosis and inhibit the activity of CAR-T cells entering the tumor. At the same time, the inhibitory immune cells in TME, namely myeloid-derived suppressor cells (MDSCs), regulatory cells (Tregs), etc., can also be generated by arginase-1 (Arg-1), reactive oxygen species (ROS), and other molecules. In addition, tumor cells can also use the intrinsic negative regulatory mechanism to reduce CAR-T cell activity by upregulating surface inhibitory receptors, such as programmed death-ligand 1 (PD-L1), resulting in immune tolerance in TME. Finally, tumor cell glycolysis makes the tumor microenvironment hypoxia, acidic, and nutrient-deficient, making it more difficult for CAR-T cells to survive [36] [37].

4. Possible Strategies for CAR-T Therapy

4.1. Enhances Antigen-Specific Recognition of CAR-T

Ideal-specific targets are fundamental methods to enhance CAR-T antigen recognition. The epidermal growth factor receptor variant III (EGFRvIII.) is one of the more widely studied targets, which is formed by the fusion of the first and eighth exons of EGFR under the linkage of a neo glycine. Because of its high tumor tissue specificity, it has been used as a target for CAR-T therapy in preclinical studies and has achieved good results [38]. More clinical trials have further confirmed the feasibility of EGFRvIII. as a clinical therapeutic target for CAR-T. The results of the phase I clinical study conducted by O'Rourke *et al.* [39] in nine patients with recurrent glioblastoma (GBM) showed that EGFRvIII. CAR-T treatment had a good anti-tumor effect, safety (no off-target toxicity), and tolerability. Oncolytic virus (OV)-mediated antigen delivery and labeling of tumor cells is another effective way to provide CAR-T-specific targets. An oncolytic virus antigen delivery labeling system encoding truncated CD19 protein (CD19t) is typical. Park *et al.* [40] infected a variety of solid tumor cells with oncolytic vaccinia virus (OV19t) encoding CD19t, which significantly promoted CAR-T cell infiltration and tumor killing. At the same time, CAR-T-mediated tumor lysis further leads to the release of OV19t, promotes the expression of CD19t on solid tumor cells, and forms positive feedback for tumor clearance.

By distinguishing the difference in TAA density between tumor tissue and normal tissue, it is also possible to increase the specificity and sensitivity of CAR-T antigen recognition. Hernandez-Lopez *et al.* [41] used the synNotch (synthetic Notch) receptor system to construct an ultrasensitive antigen density-sensing CAR-T, that is, a low-affinity synNotch receptor against HER2 controls the expression of a high-affinity CAR against HER2. When the syn-Notch receptor is fully activated by high-antigen density HER2, it induces the expression

of high-affinity CAR, followed by the specific killing of tumor cells by CAR-T. *In vitro* and *in vivo* studies have confirmed that CAR-T cells expressing this system have significant differences in the killing of non-tumor cells expressing normal amounts of HER2 and tumor cells expressing 100-fold HER2. In addition, the regulation of antigen density by a variety of traditional epigenetic modulators has also been shown to increase the sensitivity of CAR-T antigen recognition. For example, decitabine, as a DNA methyltransferase inhibitor, has been found to upregulate the expression of antigen MUC1 on pancreatic cancer cells through DNA demethylation, increasing the specific recognition and killing of MUC1 CAR-T [42].

4.2. Enhanced TME Infiltration of CAR-T

A variety of traditional therapies have an advantage in promoting tumor tissue infiltration of CAR-T because of their remodeling effect on TME. Chemotherapy pretreatment combined with CAR-T has been widely used in clinical studies. In a study (NCT01869166), albumin-bound paclitaxel plus cyclophosphamide before anti-EGFR CAR-T therapy promoted CAR-T cell infiltration and enhanced antitumor efficacy by depleting tumor stroma by binding to secreted protein acidic and rich in cysteine (SPARC) [43]. Local radiation therapy can also significantly enhance tumor infiltration of CAR-T cells. For example, in the GBM mouse model, it was found that local radiotherapy after intravenous injection of anti-GD2CAR-T can effectively promote vascular extravasation and tumor infiltration of CAR-T cells, and enhance their anti-tumor effect [44]. Because of the remodeling effect of hyperthermia on TME, including the destruction of the stroma and dilation of vascular structure, CAR-T combined with photothermal therapy can also significantly promote CAR-T accumulation and tumor control. In a mouse model of solid tumors under laser irradiation, the combined application of nano enzymes with dual photothermal-nano catalytic properties was found to enhance the infiltration of anti-B7-H3 CAR-T cells [45].

Tumor antiangiogenic therapy and matrix degradation therapy promoted CAR-T cell infiltration more directly. In a mouse model of human neuroblastoma in situ, Bocca *et al.* [46] demonstrated that bevacizumab treatment can enhance massive infiltration of anti-GD2 CAR-T cells by promoting tumor vascular normalization. The degradation of heparan sulfate proteoglycans (HSPG) components in the tumor matrix are considered to be the first step for CAR-T cells to cross the tumor matrix barrier. CAR-T (ANTI-HPSE CAR-T) expressing heparinase based on this design has been shown to enhance auto infiltration by degrading HSPG [47]. In addition, the fibrous structure composed of tumor-associated stromal cells is thought to be the main cause of the formation of the tumor stromal barrier, and fibroblast activation protein (FAP) as a marker of tumor-associated fibroblasts becomes a potential therapeutic target. For example, anti-FAP CAR-T is effective in reducing tumor stroma and inhibiting tumor growth in mouse models of lung cancer and pancreatic cancer [48].

4.3. Overcoming Inhibition of the Tumor Immune Microenvironment

Functional inhibition or clearance of immunosuppressive cells is one of the effective ways to maintain the function of tumor-infiltrating CAR-T cells. Low-dose chemotherapy, as one of the main pretreatment methods for clinical CAR-T therapy, can reshape the tumor immune microenvironment by inducing immunosuppressive cell clearance to enhance the efficacy of CAR-T. Guo *et al.* [43] pretreated albumin-bound paclitaxel and cyclophosphamide for anti-EGFR CAR-T therapy in patients with advanced cholangiocarcinoma, which significantly improved the therapeutic effect of CAR-T. In addition, more and more preclinical studies suggest that the combination of CAR-T with existing therapies or the modification of CAR-T itself can effectively remove immunosuppressive cells and enhance the efficacy of CAR-T. Watanabe *et al.* [49] combined oncolytic adenovirus (OAd) expressing TNF-α and IL-2 with CAR-T and found that it could promote the polarization of tumor-associated macrophages to M1 type and increase DC cell maturation, thereby significantly improving CAR-T infiltration and anti-tumor effects. A recent study proposed that the use of CAR-T cells to deliver the pattern recognition receptor agonist RN7SL1, an endogenous RNA that activates the RIG-I/MDA5 signaling pathway, significantly inhibits the development of MDSCs and promotes the growth of subsets of DC cells with co-stimulatory characteristics, thereby promoting CAR-T function [50].

Immune checkpoints are another option to help CAR-T overcome the immunosuppressive microenvironment of solid tumors. CAR-T combined with immune checkpoint inhibitors has been widely demonstrated to be effective in enhancing the control of CAR-T on solid tumors. In a recent study, patients with clinically malignant pleural mesothelioma demonstrated a good safety profile and tolerability after receiving pembrolizumab in combination with anti-MSLN CAR-T, with median overall survival of 23.9 months [51]. CAR-T combined with OA-encoding immune checkpoint antibodies can also effectively help CAR-T overcome immunosuppression. OAd-encoding PD-L1 mini-antibodies designed by Tanoue *et al.* [52] have been shown to enhance anti-HER2 CAR-T function in prostate cancer models. In addition, by modifying CAR-T, including inhibiting the expression of immune checkpoints or blocking its signaling, it can also effectively enhance the efficacy of CAR-T. Zou *et al.* [53] found that CAR-T cells that simultaneously downregulated the expression of inhibitory immune checkpoint receptors PD-1, Tim-3, and Lag-3 had better anti-tumor effects. In addition to knocking out PD-1 by CRISPR/Cas9 technology, modifying the glycosylated residues of PD-1 using adenine-base editors can also reduce PD-1 expression, thereby enhancing CAR-T cytotoxicity [54]. By modifying the extracellular region of PD-1, its signaling can be blocked. For example, Pan *et al.* [55] cut PD-1/PD-L1 signaling by introducing a soluble PD1-CH3 fusion protein composed of the extracellular domain of PD-1 and CH3 of IgG4 in anti-GPC3

CAR-T to cut PD-1/PD-L1 signaling, and confirmed its significant anti-tumor effect in mouse models of hepatocellular carcinoma.

4.4. Improves the Persistence of CAR-T Cells in the Body

Even for hematological tumors with remarkable efficacy, the high recurrence rate of tumors is still a major obstacle limiting the development of CAR-T cell therapy, and improving the persistence of CAR-T cells *in vivo* is another key strategy for the treatment of solid tumors. BioNTech has developed and designed a BNT211 product consisting of an autologous CAR-T cell targeting the CLDN6 antigen and an mRNA vaccine (CARVac) encoding the CLDN6 antigen. After intravenous injection, CARVac is absorbed by DCs and translated into antigenic peptides, and then presented on the surface of DCs to continuously activate CAR-T cells, greatly improving the survival and expansion efficiency of CAR-T cells *in vivo*. *In vivo* experiments in mice confirmed that the CARVac strategy did show a better tumor-killing effect and significantly prolonged survival time *in vivo*, and repeated stimulation of CARVac could optimize the treatment window under low-dose CAR-T infusion so that the tumors of mice improved from delayed growth to complete regression. This year's American Association for Cancer Research (AACR) annual meeting disclosed the latest progress in phase I/II clinical trial of BNT211 (NCT04503278), preliminary results show that the combination of CLDN6 CAR-T cells and CARVac can significantly increase ORR and DCR in tumor patients, and 4 out of 5 evaluable patients have partial remissions. The new treatment is still well tolerated and safe. In addition, the lack of persistence of CAR-T cells in patients is directly related to the depletion of infusion T cells, and the study found that the framework regions of scFv are prone to mutual aggregation, which causes phosphorylation of the CD3ζ region in the CAR molecule, resulting in low-level, tonic signaling in the basal state, which eventually leads to T cell depletion. On the one hand, single-domain antibodies are only a single antigen-binding domain, which can effectively avoid the above-mentioned T cell depletion problems caused by scFv aggregation, which may be a feasible idea to solve the problem of recurrence of CAR-T therapy. On the other hand, the co-stimulatory signaling domain is also an important part of CAR-T cells, and a large number of clinical research examples have confirmed that CAR-T cells integrating CD28 co-stimulation molecules can quickly kill tumors but have poor persistence, while CAR-T cells containing 4-1BB co-stimulatory molecules have stronger persistence. A recent study shows that the design of two signaling domains (CD28 and CD3ζ) and 4-1BB ligands can combine the advantages of two co-stimulatory molecules, presenting balanced tumor-killing ability and enhanced T cell persistence, which undoubtedly provides more ideas for CAR-T cells to conquer solid tumors.

5. Summary and Outlook

Although CAR-T faces many difficulties in the treatment of solid tumors, with

the deepening of the understanding of solid tumors and the characteristics of CAR-T cells, innovative solutions have brought new hope for this therapy. Whether it is the optimization and modification of CAR-T's structure or the combination of CAR-T with other tumor treatment methods, it presents a good application prospect. CAR-T cell therapy is expected to achieve results in the field of solid tumors in hematological tumors, ultimately maximizing the benefits for clinical cancer patients.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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