Journal of Medical Sciences. 24 Sep, 2024 - Volume 12 | Issue 4. Electronic - ISSN: 2345-0592
Medical Sciences 2024 Vol. 12 (4), p. 38-46, https://doi.org/10.53453/ms.2024.9.5

e-ISSN: 2345-0592
Online issue
Indexed in *Index Copernicus*

Medical Sciences

Official website:
www.medicsciences.com

Chimeric antigen receptor T–cell therapy for solid tumors: indications, progress, challenges and possible strategies

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Abstract

Background. Adoptive cell therapy using chimeric antigen receptor (CAR) technology is one of the most advanced engineering platforms for cancer immunotherapy. CAR–T cells have shown remarkable efficacy in the treatment of hematological malignancies. However, many challenges limit the therapeutic efficacy of CAR–T cells in solid tumors. Here, we review these challenges and discuss strategies to improve the effector function of CAR–T cells for the adoptive immunotherapy for solid tumors.

Aim. To select and analyze the material provided by experts about CAR–T cell therapy for solid tumors.

Materials and methods. The literature search was performed in the PubMed database using keywords "CAR–T cell", "solid tumors", "cancer immunotherapy", "tumor microenvironment". The literature review includes 53 articles in English, published between 2014 and 2024.

Results. Better antitumor effect of CAR–T cell therapy can be achieved by finding more specific and abundant surface antigens, promoting the expression of cytokines, choosing different injection methods, constructing tertiary lymphatic structures, and improving immune memory. Moreover, other immune cells, such as NK cells and macrophages, are becoming potential alternatives for CAR–T cells.

Conclusions. To improve the efficacy of CAR–T cell therapy, feasible solutions were proposed from the aspects of design, infiltration and working of CAR–T cells. Further in–depth investigations should be conducted to overcome the present restrictions of CAR–T cell therapy for it to be as effective in the treatment of solid tumors as it is in the treatment of hematological ones.

Keywords: CAR–T cell, solid tumors, cancer immunotherapy, tumor microenvironment.

1. Introduction

Cell immunotherapy using chimeric antigen receptor (CAR) technology is one of the most advanced methods of cancer immunotherapy engineering. CAR–T cells have demonstrated excellent efficacy in treating malignant hematological tumors, but there are many challenges limiting their effectiveness in treating solid tumors, including immunosuppressive tumor microenvironment (TME), insufficient tumor infiltration, and the absence of tumor–specific antigens [1]. Here we will review these challenges and discuss future prospects and potential strategies that could improve the effector function of CAR–T cells in the treatment of solid tumors.

2. Materials and methods

The literature review was conducted by searching for scientific publications in the PubMed database, using the following keywords in English: CAR–T cell, solid tumors, cancer immunotherapy, tumor microenvironment. The review includes articles written in English, published during the period 2014–2024.

3. Results

3.1 CAR–T cell therapy – what is it?

CAR–T cell therapy is an innovative cancer treatment approach in which genetically modified T cells are used to combat cancer cells. CARs are synthetic receptors that redirect lymphocytes, typically T cells, to recognize and eliminate cells expressing a specific target antigen [2, 3]. The binding of CARs to target antigens is independent of tissue compatibility complex receptors, thus activating T cells and inducing a potent anti-tumor response [4, 5].

3.2 Genetic modification of CAR–T cells

T cells are extracted from a patient's blood, genetically modified in a lab to target specific cancer antigens, and then reintroduced into the patient's body. This modification process spans several weeks. CAR–T cell therapy is designed to attack only specific types of cancer, meaning a therapy effective against one cancer type won't work against another [2]. The therapy involves three key stages: collecting T cells via apheresis, genetically engineering T cells to recognize cancer cells, and reinfusing the modified cells back into the patient to fight the cancer [6, 7].

3.3 CAR structure

3.3.1 Extracellular antigen–binding domain

The CAR antigen–binding domain grants specificity to target antigens, usually derived from the variable regions of monoclonal antibodies to form a single–chain variable fragment (scFv). This allows CARs to target cancer antigens on cell surfaces, activating T cells independently of the tissue compatibility complex [2]. However, there are also CARs that can recognize antigens associated with intracellular tumors using T cell receptors that depend on the tissue compatibility complex, mimicking CAR [7, 8]. The affinity and specificity of CARs depend on the interaction and positioning of scFv's heavy and light chains [9]. While a high affinity is crucial for effective antigen binding and T cell activation, excessively high affinity can lead to toxicity [10, 11]. Factors like epitope location and antigen density also play roles in optimizing CAR function, highlighting the need to consider these elements to enhance CAR–T cell efficacy [7].

3.3.2 Intracellular region

In CAR–T cells, there is an intracellular structural region that connects the binding units to the transmembrane domain. This allows the antigen–binding domain to reach the target epitope. The length and composition of the hinge region can affect CAR functionality, including flexibility, CAR

expression, signaling, epitope recognition, and activation strength [12, 13]. The spacer length is also important for the formation of the immunological synapse. The optimal spacer length depends on the position of the target epitope and steric hindrances in the target cell [7]. Short spacers are better for binding distal membrane epitopes, while long spacers provide flexibility to access proximal membrane or complex glycosylated antigens [12]. The choice of hinge region often relies on amino acid sequences from CD8, CD28, IgG1, or IgG4, but spacers derived from IgG can interact with Fcγ receptors and lead to CAR–T cell depletion [7]. To avoid this effect, different spacer regions or additional engineering can be used.

3.3.3 Transmembrane domain

The transmembrane domain is a crucial yet understudied component of CAR structures, mainly anchoring the CAR to the T cell membrane and impacting CAR–T cell function, including expression, stability, signaling, and synapse formation [14]. Commonly sourced from proteins like CD3ζ, CD4, CD8α, or CD28, the specific effects of different transmembrane domains on CAR functionality are not well understood due to frequent modifications. For instance, the CD3ζ domain can enhance T cell activation but might reduce CAR stability compared to CD28. Furthermore, the combination of transmembrane and hinge domains influences cytokine production and activation–induced cell death [15]. Studies indicate that optimal CAR–T cell signaling and stability are achieved by matching the intracellular with the appropriate transmembrane domain, with CD8α or CD28 domains potentially enhancing performance [7].

3.3.4 Intracellular signaling domain

In CAR engineering, significant focus has been placed on the stimulatory effects to develop CARs with optimal intracellular domains [7]. First–generation CARs from the late 1990s, which included CD3ζ or FcRγ signaling domains, were found inadequate alone for effective T cell response, showing limited clinical effectiveness [7, 16]. Second–generation CARs incorporate an additional co–stimulatory domain, such as FDA–approved CD28 or 4–1BB, enhancing T cell differentiation and metabolic functions. These have shown strong therapeutic responses in hematological malignancies and are being tested in solid tumors [4, 17, 18]. Alternative co–stimulatory domains like ICOS, CD27, MYD88, CD40, and OX40 have demonstrated preclinical efficacy. Third–generation CARs, featuring two co–stimulatory domains along with CD3ζ, vary in effectiveness across preclinical models, with some showing superior outcomes compared to second–generation CARs [19].

3.4 Indications and adverse effects of CAR–T cell therapy

3.4.1 Indications

Currently, CAR–T cell therapy is approved and used to treat B–cell lymphomas and B–cell leukemias, including acute lymphoblastic leukemia and chronic lymphocytic leukemia. CAR–T cell therapy has shown excellent clinical efficacy in treating these diseases, however, the occurrence of adverse reactions limits further therapeutic effectiveness of CAR–T cells in treating other hematological or solid tumors [18].

3.4.2 Adverse effects

CAR–T cell therapy can have serious side effects that vary depending on the patient and their health condition. Adverse reactions include cytokine release syndrome (CRS), immune cell–related neurotoxicity, off–target effects, anaphylaxis, infections from infusion, tumor lysis syndrome, B–cell aplasia, hemophagocytic lymphohistiocytosis,

macrophage activation syndrome, and coagulation disorders [6]. Early identification and effective treatment of these reactions are crucial as they can be life–threatening. Ongoing research aims to develop CAR–T cells with greater anti–tumor activity and less toxicity to the human body [20, 21].

3.5 Challenges and potential strategies of CAR–T cell therapy

3.5.1 Lack of antigen specificity and heterogeneity

Unlike hematological cancers, solid tumors are characterized by heterogeneity. One consequence of tumor heterogeneity is the expression of heterogeneous antigens, leading to the growth of tumor cell subpopulations when a single target antigen is attacked. While CAR–T cells targeting a single antigen initially induce a high response rate, in some patients treated with these cells, the malignant tumor cells partially or completely lose the expression of the target antigen, a phenomenon known as "antigen escape" [22]. Overcoming antigen specificity and heterogeneity loss in solid tumors can be achieved by modifying CAR–T cells to target two or more antigens, for example, using dual CAR constructs with different individual antigen recognition domains or tandem CAR–T cells with two individual antigen recognition domains in one CAR [23]. Multiple tandem CARs have been tested in preclinical models, including HER2 and IL13Ra2 for glioblastoma and HER2 and MUC1 for breast cancer, achieving better anti–tumor responses compared to single targeted therapy [24, 25]. Efforts are also being made to develop CAR–T cells expressing a bispecific T–cell engager (BiTE), which would activate bystander T cells to recognize tumor cells – this includes the expression of transgenic cytokines (such as IL–18, IL–36g) or CD40L [26]. Future optimization of target antigen selection could lead to not only improved anti–tumor responses but also reduced incidence of antigen escape.

3.5.2 Physical barriers

CAR–T cell therapy for solid tumors faces challenges due to physical barriers that hinder cell penetration, such as the dense extracellular matrix and uneven tumor vasculature, which cause tissue hypoxia and limit T cell movement [27]. This hypoxia increases CTLA–4 and PD–L1 expression and reduces necessary adhesion molecules, complicating T cell infiltration into the tumor microenvironment [28]. To improve infiltration and minimize systemic toxicity, therapies are administered directly to the tumor area. Various delivery methods, like intravenous and intratumoral injections, are explored to optimize efficacy [29]. Intravenous delivery can lead to cytokine release syndrome, a potentially fatal condition, while intratumoral injections reduce systemic side effects and prevent the loss of CAR–T cells due to migration [29]. Some tumors can be injected via the third space, e.g., CNS tumors via cerebrospinal fluid or lung tumors via the pleural cavity [31, 32]. Additionally, engineering CAR–T cells to express heparanase might help them break through the extracellular matrix, facilitating deeper tumor penetration [33].

3.5.3 Immunosuppressive tumor microenvironment

The solid tumor microenvironment is a significant barrier to CAR–T cell therapy due to the immunosuppressive conditions that hinder T cell proliferation and survival. Key suppressive factors include cytokines, immune checkpoints like PD–1 and CTLA–4, and a challenging metabolic environment [34]. Additionally, the tumor stroma contains myeloid–derived suppressor cells (MDSCs), tumor–associated macrophages (TAMs),

and regulatory T cells (Tregs) that promote tumor growth and metastasis [35]. To overcome these issues, strategies include enhancing the tumor's metabolic environment with essential amino acids and neutralizing suppressive factors by promoting IL–15 expression in T cells [37]. Moreover, simply infiltrating the tumor with CAR–T cells is insufficient; these cells must also produce cytokines to attract more immune cells (Figure 1).

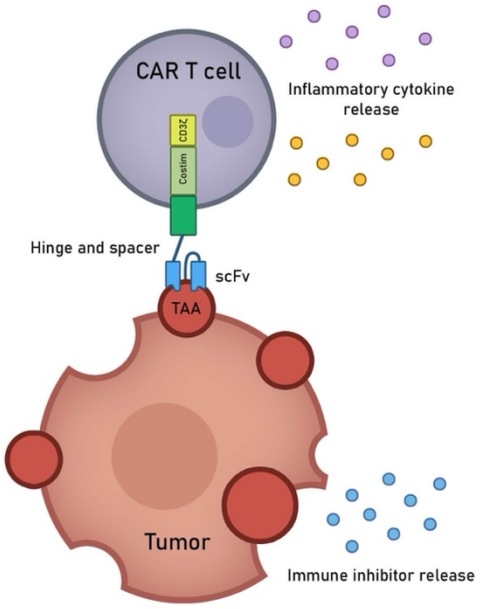

Figure 1: *CAR–T cell interaction with a cancer cell. CD3ζ, Cluster of differentiation 3 zeta, Costim, Costimulatory domain, scFv, Single–chain variable fragment, TAA, Tumor associated antigen.*

Enhancements in cytokine production such as IL–7, CCL19, CCL21, IL–12, CXCL11, and CCR2b have shown promise [38–41]. Improving CAR–T cell survival also involves targeting immunosuppressive cells like M2 macrophages, MDSCs, and Tregs. Combining CAR–T cell therapy with immune checkpoint inhibitors ensures both a robust T cell infiltrate and prolonged T cell function [42]. Pretreatments like oxaliplatin and cyclophosphamide can boost CAR–T cell accumulation in tumors and improve response to therapy [43, 44]. Special attention has been given to tertiary lymphoid structures (TLS), which enhance antigen–specific immune responses and help CAR–T cells survive in the tumor microenvironment [43, 44]. TLS also promote continuous immune cell influx to sustain immunity. Techniques such as vascular network normalization in melanoma treatments and the use of biomaterials like collagen matrices and hydrogels further support TLS formation and immune cell penetration [45].

3.6 CAR–based immunotherapy

3.6.1 CAR–NK cell therapy

CAR–NK cell therapy involves genetically modifying natural killer (NK) cells so they express CARs targeted at specific cancer cell antigens. NK cells are part of the innate immune system and regulate the death of cancer cells by recognizing target ligands. Unlike CAR–T cells, which attack tumor cells using specific antibodies against scFv, NK cells can be activated through their activating and inhibitory receptors [47]. Additionally, NK cells can induce the death of target cells via the death receptor pathway: death ligands expressed on the surface of NK cells (e.g., FasL, TRAIL) bind to death receptors on target cancer cells and promote their apoptosis [48]. CAR–NK cells can be generated from various sources, including peripheral and cord blood, induced pluripotent stem cells, and cell lines. A major advantage of CAR–NK therapy over CAR–T cell therapy is its safety and lower risk of side effects such as cytokine release syndrome (CRS), neurotoxicity, and graft–versus–host disease [49]. Despite the advantages of CAR–NK cells, they face significant challenges in combating solid tumors due to the immunosuppressive tumor microenvironment (TME) and tumor heterogeneity, which, like CAR–T cells, limit their penetration into the tumor and survival within it. CAR–NK therapy is still in early development but has the potential to be safe and effective for cancer patients – further research is

needed to determine its long–term safety and efficacy [50].

3.6.2 CAR–M therapy

CAR–M therapy is a promising treatment method that involves modifying macrophages to express CARs for the recognition and destruction of cancer cells. It also modulates the function of CAR–M cells in the tumor environment to enhance the anti–cancer response [51]. This therapy is promising for treating solid tumors due to the macrophages' ability to penetrate the tumor microenvironment: Tumor–associated macrophages (TAMs) are the dominant immune cells in the TME, attracted by chemokines and growth factors released by cancer cells [52]. TAMs consist of M1 macrophages, which exhibit anti–tumor activity, and M2 macrophages, which promote tumor growth. The cytotoxicity of CAR–M is activated by antigen–expressing tumor cells: CAR–M in the M0 state transitions to the M1 phenotype, which has specific phagocytic activity against solid tumor cells and can modulate the immunosuppressive tumor microenvironment by releasing cytokines and chemokines. CAR–M also has the potential to enhance the adaptive immune system by presenting antigens to T cells and activating their cytotoxicity. Several ongoing preclinical studies are investigating the efficacy of CAR–M therapy in treating various types of solid tumors. Current clinical trials are evaluating its safety and effectiveness in treating various types of cancer [53].

4. Discussion

Although CAR–T cell therapy has shown clinical success in treating malignant hematological tumors, there remain many challenges and obstacles in treating solid tumors. To improve the efficacy of CAR–T cell therapy, several solutions have been proposed concerning CAR–T cell engineering, infiltration, and function. By optimizing the selection of target antigens, enhancing cytokine expression, choosing different injection methods, developing tertiary lymphoid structures, and improving immune memory, a better anti–tumor response can be expected. Additionally, other immune cells such as NK cells and macrophages, due to their properties, have become potential alternatives to CAR–T cells. Thus, a better understanding of the challenges posed by solid tumors and ongoing clinical trials encourage the development of CAR–T cells that could show similar efficacy as in the treatment of malignant hematological tumors.

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