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Structural and Research Advances in CAR-T Cell Therapy

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Abstract. The treatment of a variety of recurrent or resistant hematologic malignancies with chimeric antigen receptor T cell (CAR- T) cell therapy has seen significant success in recent years. The current CAR- T cell therapy approach is not without flaws, nevertheless, and there are still several issues with clinical treatment, including antigen escape, significant toxicity, and susceptibility to drug-resistant recurrence. This paper introduces the structural development and characteristics of CAR-T cells, reviews the limitations of CAR- T cell therapy, including antigen escape, toxicity, CAR-T cell depletion and drug-resistant relapse after treatment, and summarizes the related improvement and optimization strategies. The paper concludes that CAR-T cell immunotherapy has brought new hope to patients with hematologic malignancies, making a cure for refractory and recurrent hematologic malignancies possible. Although CAR-T cell therapy still has many challenges at present, such as immunogenicity, drug resistance, and toxicity.

Keywords: Chimeric antigen receptor, T cell, Structure, Toxicity, Drug resistance, T cell exhaustion

1. Introduction

The term "chimeric antigen receptor T cells" (CAR- T) refers to cells that have been genetically modified to express binding sites for particular antibodies in T lymphocytes as well as to precisely recognize and eradicate tumor cells without regard to the MHC (major histocompatibility complex). Cellular immunotherapy for refractory cancers now belongs to this promising class. Currently, CAR-T cell therapies are primarily used to treat hematologic tumors. In 2017, the FDA approved the marketing of Kymriah and Yescarta for the treatment of relapsed or refractory large B-cell lymphoma and relapsed or refractory childhood acute lymphoblastic leukemia, respectively, with CD19 as the treatment target. Solid tumors, such as melanoma, prostate tumors, colon cancer, etc. Solid tumor treatment, among other things, still faces a lot of difficulties. Additionally, similar CAR-T cell therapies have a significant therapeutic impact on a variety of diseases, including autoimmune disorders, cardiometabolic disorders, fibrosis, aging, and others [1]. In addition to outlining the limitations of CAR-T cell therapy in terms of antigen escape, toxicity, CAR-T cell depletion, and drug-resistant relapse, this paper introduces the fundamental structural composition and function of CAR-T cells, compares the benefits and drawbacks of 4 generations of CAR-T cells, and lists the improvement strategies for each limitation separately. By providing a more thorough summary, offering theoretical advice for learning to comprehend the limitations of CAR-T cell therapy, and making it easier for the reader to grasp the most recent CAR-T cell therapy tactics, this paper helps advance the field of CAR-T cell therapy.

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2. Structure, expression , and signalling pathways

CAR-T is composed of three distinct regions: the extracellular area, the transmembrane region, and the intracellular activation region (Figure 1).

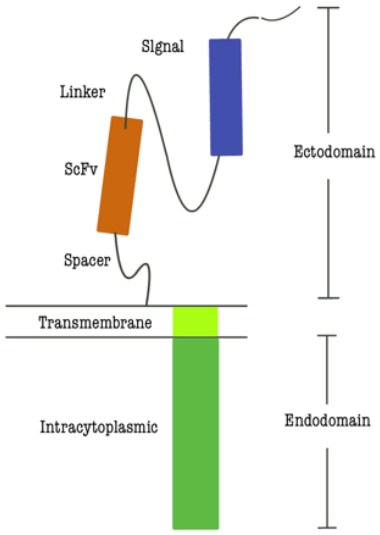


Figure 1. Structure of CAR-T cells.

The scFv region, which is often found outside of cells, is what helps monoclonal antibodies recognize and bind their target antigen. Transmembrane region: the region that acts as a hinge or spacer to join the scFv to the cell membrane. When an antigen is recognized and attached, a stimulatory signal is conveyed to the intracellular signaling domain, activating T lymphocytes to carry out effector functions. The intracellular area is made up of co-stimulatory factors and the CD3 signaling domain. Four generations of CAR structures with various features have been created since the CAR structure was first proposed (Figure 2)

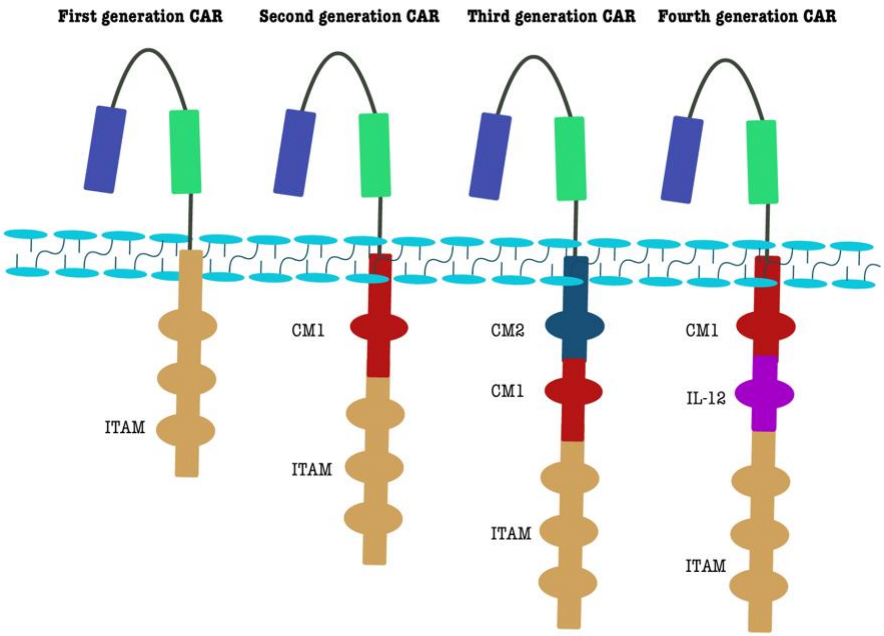


Figure 2. Evolution of the structure of 4 generations of CAT-T cells.

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3. The pitfalls of car-t cell therapy and its solutions

3.1. Complex production process of CAR-T cells in vitro and in vivo

The steps involved in creating CAR-T cells are: collecting peripheral blood; isolating single nucleated cells; activating T cells; transferring the CAR gene using viruses or non-viruses; expanding and evaluating the quality of CAR-T cells; and finally, cryopreservation. Cytokines must be used to boost the CAR-T cell expansion process, serum or other nutrients must be added, and CAR-T cell manufacturing needs all of these. For the manufacture of CAR-T cells, there are stringent requirements for raw materials. Second, a number of danger factors such endotoxin, mycoplasma, and chemical reagent residues need to be examined for quality before CAR-T cells are injected into human bodies. The entire preparation procedure takes between two and four weeks, and the entire production process needs to be aseptically controlled. It costs money, takes a lot of time, and delays the best time for patients to receive treatment because of the preparation process.

3.2. Antigenic escape

Many patients who select CAR-T cell therapy for their cancer express part or all of the target antigens in their malignant cells, which is a process known as "antigen escape." Data from surveys show that 30-70% of patients who relapsed after therapy had common disease resistance mechanisms, such as downregulation or deletion of CD19 antigen, and that patients with multiple myeloma who received BCM tarotype CAR-T cells also experienced downregulation of BCMA expression [3].

In an effort to reduce the likelihood of CAR-T treatment relapse, numerous strategies are now being used. For instance, expressing two intact CAR constructs on a single T cell with dual targeting of the antigens BCMA and GPRC5D can prevent BCMA escape and lower the risk of multiple myeloma relapse [4]. An alternative strategy is to alter CAR T cells so that they secrete bispecific T cell attractants (BiTEs), which can actually connect T cells to cancer cells. Research has shown that CAR- T cells that secrete BiTEs can successfully prevent antigen escape, and the FDA has currently approved the use of BiTE blinatumomab targeting CD19 [5].

3.3. Toxicit

At least one in four patients receiving CAR-T therapy need urgent care due to severe cytokine release syndrome (CRS), deadly neurotoxicity, and post-remission recurrence issues. Within the first week of receiving CAR-T cell therapy, CRS typically presents with fever, hypotension, and respiratory insufficiency along with high serum cytokine levels; neurotoxicity presents with transient memory loss, seizures, and infrequently severe cerebral edema. Rapid activation and proliferation of cytokine-secreting T cells are the root causes of both CRS and neurotoxicity[6]. In one examination of the clinical presentation of 25 adult patients at Massachusetts General Hospital, it was discovered that 24 individuals (96%) had CRS, and that 12 of the 25 patients (48%) had neurotoxicity of grades 1-2, 3-4, and/or 3–4 [7].

Decreased toxicity is therefore necessary for effective treatment. The first strategy is creating CAR-T cells, altering the structure of the CAR to lessen toxicity, reducing toxicity by altering the strength of the antigen-binding domain of CAR-T cells, and altering the hinge area and transmembrane region that can control cytokine release. The performance of CAR to elicit an immune response is diminished when using antibody fragments encoded by human or human antibody genes rather than CAR derived from rats, while the co-stimulatory structural domains are altered depending on the tumor type, the extent of tumor damage to the organism, and the antigen density. The second strategy involves altering CAR-transduced T cells and neurotoxicity. For instance, lenzilumab inhibits macrophage activation and monocyte-activated cytokine GM- CSF, which reduces neurotoxicity and CRS and boosts CAR-T cell activity. Implementing a "off-switch" or suicide gene technique is a third method, which aids in reducing cells' ability to be treated with secondary inducers in the event of a negative outcome [7].

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3.4. CAR-T cell exhaustion

When T cells are functionally exhausted from repeated exposure to inflammatory or antigenic signals, they express a variety of inhibitory receptors and have gradually decreased value-added and memory capacities. The anti-tumor actions of CAR- T cells are greatly hampered by Tex development in CAR-T cells, particularly CD8+ T cell depletion. The following strategies are used to control CAR- T cell depletion because it affects the effectiveness of tumor treatment: first, gene editing techniques to over-express or knock down depletion-related genes, second, optimizing and improving CAR- T cell culture media, including both cytokine optimization and inhibitors of T cell differentiation, and third, combining new technologies to screen genes associated with driving T cell depletion. For instance, while treating malignant melanoma, the combination of inhibitors, which is frequently paired with CTLA-4 and PD-1, has been used therapeutically to obtain a 5-year survival rate of 52% [8].

3.5. Relapse of drug resistance in CAR- T cell therapy

The rate of drug resistance recurrence among patients undergoing CD19-targeted CAR-T cell therapy is as high as 50%, and most relapses happen within 12 months of treatment [9], despite significant advancements in the field. Resistance to CAR-T cell therapy is influenced by three factors: the tumor, the tumor microenvironment, and the CAR-T cells themselves.

3.5.1. The CAR-T cell factor. Expansion, retention time, and cytotoxicity are the primary factors that affect the therapeutic efficacy of CAR-T cells. T cells from cancer patients typically have a poor prognosis and a deficiency in their natural cytotoxicity. Age-related changes in T cell structure result in undifferentiated T cells becoming differentiated effector/memory T cells, but differentiated T cells lack CD28 expression and have a lower proliferative potential in response to antigens. The failure of CAR-T cell therapy is also a result of CAR- T cell failure [10]. In order to improve the universality and safety of CAR-T cells, the current coping approach mostly relies on the advantages of gene editing technology or the inherent benefits of umbilical cord blood and hematopoietic stem cells.

3.5.2. Tumor factors. Tumor factors are divided into two categories: targeted antigen-negative relapses and antigen-positive relapses. The main mechanism of antigen-negative relapse is antigen loss, which mainly includes splice mutation, epitope concealment, and cell lineage alteration due to target epitope loss. However, even if antigen is not completely lost, reduced expression or density of antigen through immunomodulation is sufficient to allow tumor cells to escape. The main cause of antigen-positive relapse is the exhaustion of CAR- T cells, which become hypofunctional due to long-term exposure to high levels of antigen. Based on this, we can enhance the level of CAR molecule expression or the affinity of immune synapses between CAR-T cells and tumors by enhancing the sensitivity of CAR-T cells. Some investigators have designed a novel high antigen sensitivity CAR through the TCR working principle, and they found that by embedding CD3ε or growth factor receptor binding protein 2 (GRB2) structural domain in the 4-1BB/CD3ζ sequence can make CAR-T cells activate at lower antigen threshold The novel CAR-T cells have shown advantages over conventional 4-1BB/CD3ζ in constructed mouse tumor models of leukemia, lymphoma and breast cancer [11].

3.5.3. Tumor microenvironment. The primary factor undermining the efficacy of immunotherapy in solid tumors is the tumor microenvironment. The microenvironment can have a significant impact on the immune system's effector cells. For example, hypoxia and glucose deprivation can cause lactate to build up and further reduce the pH of the microenvironment, which can limit the activity of effector T cells. Additionally, tumor cells might conceal themselves in the microenvironment to avoid detection by immune cells [10]. It is possible to genetically modify CAR T cells to release particular cytokines in order to combat the medication resistance brought on by the tumor microenvironment.

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4. Advantages and disadvantages of anti-pd1 and anti-pd-l1 in rcc treatment

In past decades, anti-PD-1 and anti-PD-L1 therapy achieved excellent success. Immunotherapy anti-PD-1 and anti-PD-L1 blocks the immune checkpoint PD-1 and PD-L1, which has pro-tumour activity [15]. In the study of the safety, activity, and immune correlation of anti-PD-1 therapy investigation among patients with RCC, the rates for all doses were 27%. There were 21% of patients died due to disease progression mostly. However, most patients did not show clinical responses, so they cannot benefit from anti-PD-1/PD-L1 therapy. Despite patients responding to anti-PD-1/PD-L1 therapy, acquired resistance developed in a large number of responders after initial responses [15].

5. Conclusion

In conclusion, a cure for refractory and recurrent hematologic malignancies is now conceivable because to CAR-T cell immunotherapy, which has given patients with these illnesses fresh hope. Although immunogenicity, drug resistance, and toxicity are only a few of the current problems with CAR-T cell treatment. Due to the development of gene editing technology, other immune target drugs, ongoing design optimization, and combination with other immunotherapies, CAR-T cells are considered to be one of the most promising cancer immunotherapies with the ability to eventually penetrate solid tumors. In the future, it is hoped that CAR-T cells can overcome the restrictions of solid tumors, broaden the indications, and treat other diseases. However, this research does not summarize the efficacy of CAR-T cell therapy in autoimmune illnesses, cardiometabolic diseases, fibrosis, or aging. Future study should focus on the mechanism and pathophysiology of CAR-T cell treatment for conditions other than malignancies so that it can contribute more to the advancement of humankind.

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