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How to enhance the killing ability of CAR-T cells to solid tumors?

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Abstract. Chimeric Antigen Receptor T-cell (CAR-T) therapy is an innovative method of tumor immunotherapy. It has achieved excellent results in treating hematological malignancies and has been applied in a number of clinical situations. However, its therapeutic effect in solid tumors is still limited by multiple factors, including immunosuppression of the neoplastic microenvironment, target heterogeneity, insufficient penetration ability of CAR-T cells and poor persistence, etc. This study aims to explore effective approaches for enhancing the cytotoxicity of CAR-T cells against strong tumors. Through literature review and comprehensive analysis, this paper focuses on evaluating various enhancement methods, including optimizing the design of CAR structures (such as using costimulatory domain improvement, dual/multi-target CAR construction), synergistic therapy with combined immunomodulatory factors (such as IL-12, IL-18, IL-15) or antibody molecules, and targeting the tumor microenvironment, etc. Studies have pointed out that constructing CAR-T cells with stronger penetration, persistent activation ability and anti-exhaustion characteristics is a key direction to break through the bottleneck for treating strong tumors. This project provides theoretical assistance and strategic guidance. It helps us understand more deeply about the potential and problems of CAR-T cell therapy for solid neoplasms.

Keywords: CAR-T, solid tumors, neoplastic microenvironment

1. Introduction

In recent years, regarding tumor cure, particularly in the study of CAR-T cell therapy, certain step-by-step advancements have been accomplished. Car-T therapy has shown remarkable results, revolutionizing cancer treatment in the medical field. Its therapeutic strategies mainly include isolating Peripheral Blood Mononuclear Cells (PBMC) which is from the peripheral blood of patients, extracting T cells and conducting genetic engineering modifications in vitro to make them express specific Chimeric Antigen Receptors (CARs), and then reinfusing them into the patients to achieve precise recognition and efficient clearance of tumor cells [1].

Compared with traditional chemotherapy and radiotherapy, CAR-T cell therapy, as an immunotherapy method, has relatively fewer side effects. Immunotherapy recognizes and eliminates cancer cells by activating and regulating the body's own immune system. In recent years, it has gradually developed into an important direction in cancer treatment and has shown broad application prospects in clinical practice.

Current cancer immunotherapy includes antibodies, vaccines, molecules, and cell therapy. And it can successfully cure a lot of blood tumors, for instance leukemia, lymphoma and many other of various blood cancers. However, there are many limitations. T-cell malignancies differ from B-cell malignancies in that B-cell malignancies have multiple lineage-specific epitopes, and the cells in most solid tumors are heterogeneous [2]. Currently, there are a large number of patients with solid neoplasms. High-incidence solid neoplasms like liver cancer and lung cancer usually have high heterogeneity and the potential to metastasize. Even if CAR-T cells can effectively clear some cancer cells in the early stage, it is still difficult to prevent their recurrence and metastasis in other parts of the body. Therefore, an important research direction in neoplastic immunotherapy is how to effectively improve the penetration and continuous killing ability of CAR-T cells in strong tumors. This article will conduct a systematic discussion around this theme and evaluate the potential and limitations of different strategies in enhancing the killing efficacy of CAR-T cells against solid tumors.

1.1. Advantages of CAR-T cells in hematologic tumors

1.1.1. The target is specific

Hematological malignancies usually have highly specific and stably expressed targeted antigens, including CD19, CD20 and B-Cell Maturation Antigen (BCMA), etc., providing a optimal molecular basis for the precise recognition and targeted clearance of CAR-T cells. These antigens stably exist on tumor cells, but are expressed very little or even completely absent in normal tissues,allowing CAR-T cells to recognize and wipe out tumor cells precisely, with minimal damage to healthy tissues. In contrast, the targets of solid tumors often have expression heterogeneity or overlap with healthy tissues, substantially boosting the likelihood of off-target toxicity. Therefore, the clarity and stability of the target are the key basis for the significant therapeutic effect of CAR-T in the hematological system.As shown in Table 1, CAR-T cell technology is used a lot in different kinds of blood cancers.

Table 1. The current application of CAR-T therapy in the clinical market

CAR-T cell	Brand name	Company	Approval date	Target	Antigen recognition domain	Intracellular signaling	Indication (Targeted disease/Line of Therapy)
tisagenlecleucel	Kymriah	Novartis	08/30/2017	CD19	scFV	41BB-CD3ζ	B-cell precursor ALL (Third Line) Diffuse large B-cell lymphoma (Third Line) Follicular Lymphoma (Third Line)
Axicabtagene ciloleucel	Yescarta	Kite Pharma /Gilead	10/18/2017	CD19	scFV	CD28-CD3ζ	Diffuse large B-cell lymphoma (Second Line) Follicular lymphoma (Third Line)
Brexucabtagene autoleucel	Tecartus	Kite Pharma /Gilead	07/24/2020	CD19	scFV	CD28-CD3ζ	Mantle cell lymphoma (Third Line) B-cell precursor ALL (Third Line)
Lisocabtagene maraleucel	Breyanzi	Juno Therapeutics /BMS	02/05/2021	CD19	scFV	41BB-CD3ζ	Diffuse large B-cell lymphoma (Third Line)
Idecabtagene vicleucel	Abecma	Bluebird Bio/ BMS	03/26/2021	BCMA	scFV	41BB-CD3ζ	Multiple myeloma (Fifth Line)
Ciltacabtagene autoleucel	Carvykti	Janssen /J&J	02/28/2022	BCMA	VHH	41BB-CD3ζ	Multiple myeloma (Fifth Line)

1.1.2. Easily accessible distribution

Hematological malignancies are mainly distributed in the bloodstream, bone marrow, and lymphatic systems. These tissues are part of the human circulatory system, which helps CAR-- to be rapidly distributed through the blood flow and reach the lesion area, thereby achieving efficient targeted killing. In contrast, solid tumors usually have a dense extracellular matrix structure and abnormal angiogenesis, which significantly limit the effective penetration of CAR-T cells and their uniform distribution within the tumor, becoming one of the key obstacles affecting their therapeutic effect. Furthermore, the distribution of hematological tumor cells is relatively uniform, avoiding the limitation problem that CAR-T "cannot kill" in the central area of solid tumors, which is con-ducive to its comprehensive clearance of tumor cells.

1.1.3. Microenvironment-friendly

The Tumor Microenvironment (TME) of solid tumors is often rich in various immunosuppressive factors, including Transforming Growth Factor -β (TGF-β), Interleukin-10 (IL-10), regulatory T cells (Tregs), and Myeloid-Derived Suppressor Cells (MDSCs), etc. These immunosuppressive components inhibit the activation, proliferation and cytotoxic functions of CAR-T cells through multiple mechanisms, significantly weakening their anti-tumor effects and becoming another important obstacle restricting their application in solid tumors. However, hematological tumors are distributed in the circulatory system and are not prone to form a structured immunosuppressive environment. Therefore, CAR-T cells are more likely to maintain their functional state and continue to proliferate. This is also why CAR-T usually can produce a stronger and more lasting anti-tumor effect in the blood system.

1.2. Challenges and limitations of CAR-T cells therapy

Even though CAR-T cell cure had shown great potential in hematologic tumors in the past few years, there are still some difficulties and challenges. First, serious side effects like cytokine storms and neurotoxicity which can occur during treatment, which can cause patients to need to be closely monitored. Secondly, some patients could develop resistance to drugs following CAR-T cell treatment. These reasons might be that the expression of target antigens on the surface of tumor cells goes down or disappears. Also, tumor cells use many immune escape ways to avoid being recognized and attacked by T cells. These factors significantly affect the sustained efficacy and clinical response percentage of CAR-T management, becoming one of the urgent problems to be overcome in current treatment. In addition, the high cost of treatment and the complexity of cell preparation are also obstacles to the popularity and large-scale application of CAR-T therapy.

1.3. Future prospects and ideas

To overcome these difficulties and challenges, researchers are developing a variety of strategies. For example, the development of new CAR structural designs (dual-target CAR-T) that boost the lasting power and tumor-fighting ability of CAR-T cells and lower side effects.Furthermore, combined therapy with immune checkpoint blockers, traditional chemotherapy or any other immunomodulatory strategies has been proven to improve the anti-tumor activity of CAR-T cells and improve the safety to a certain extent. With the continuous optimization of CAR-T technology and the constant innovation of treatment concepts, the application of this therapy in hematological tumors is becoming increasingly mature and shows broad development prospects. Therefore, this article will focus on the core issue of "How to enhance the killing ability of CAR-T cells against solid tumors", and explore the potential strategies and research progress to overcome the obstacles related to solid tumors.

2. Literature review

2.1. The structure of CAR

The structural design of CAR allows T cells to identify and destroy tumor cells. This receptor is mainly composed of three major functional modules: First, single-chain antibody fragments (scFvs) that can identify tumor-associated antigens specifically usually form the extracellular domain. This domain is in charge of targeted recognition;Second, the transmembrane domain is accountable for anchoring the CAR to the T cell membrane and maintaining structural stability. Thirdly, the intracellular signaling domain, including primary activation signals (such as CD3ζ) and one or more costimulatory signaling elements, is to convey activation signals, leading to the growth of T cells, their survival, and the occurrence of cytotoxic reactions. Once the outer part locks onto a tumor cell, the inner signaling domains—like CD3ζ and costimulatory elements—trigger the T cell to respond. This setup lets the modified T cell react to cancer cells in a precise and powerful way, without relying on the usual pathways the immune system uses.

2.1.1. Ectodomain

The outer domain of the CAR structure is positioned on the outside of the cell membrane. It is one of the key components which determines the specificity of the CAR. This domain generally comprises three components: the signal peptide, the antigen-binding region and spacer region. Signal peptides direct the initial phase of CAR protein production and possess the capability to carry the newly produced polypeptide chain correctly to the endoplasmic reticulum so that further processing and expression could be ensured. The antigen-binding site usually exists in the form of a scFv,which is created as variable regions of the heavy chain and light chain of the same antibody are joined together with an unstructured linking peptide.The scFv is constructed in such a way that it is still able to bind strongly to the target antigen. Also, it causes CAR-T cells to specifically target specific cells. Between the transmembrane domain and the scFv there is a spacer, which provides structural flexibility and proper positioning. Often, a hinge region derived from IgG1 is sufficient to serve as this spacer, ensuring optimal spatial orientation and signal transmission.

2.1.2. Transmembrane

The transmembrane domain is generally made up of a hydrophobic α - helix. Its main job is to firmly attach the CAR to the T cell membrane, ensuring the spatial conformation and functional integrity of the receptor. This domain is located in the transmembrane region of the cell membrane and serves as a bridge connecting the extracellular antigen - binding structure and the intracellular signal-transduction apparatus. It is crucial for the effective conduction and structural stability of CAR signals [3, 4]. The stability of the CAR structure depends a great deal on its transmembrane domain.In some designs, using the

transmembrane region from the native CD3ζ chain can promote interactions between the engineered receptor and the cell's own TCR complex, potentially affecting signaling and overall receptor function. Currently, the most stable and suitable receptor is the transmembrane domain of CD28 [5]. Further studies showed that ICOS TM had a better regulatory effect in terms of antitumor activity and persistence than CD8 TM. In the study of the third-generation CAR-T cells, CAR-T cells with CD8 or 4-1BB as the Transmembrane Domain (TM) exhibited a moderate degree of cancer-fighting ability; in contrast, CAR-T cells using the transmembrane domain of ICOS can achieve 100% tumor clearance in NSG mice within 35 days. More crucially, compared with CD8 TM or 4-1BB TM, CAR-T cells constructed by ICOS TM show better expansion ability and persistence in vivo, and have stronger tumor-killing efficacy at the same time, suggesting that it has potential advantages in CAR structure optimization [6].

2.1.3. Endodomain

The intracellular part of the CAR receptor, which is important for sending signals, usually includes three Immunoreceptor Tyrosine-based Activation Motifs (ITAMs). After antigen recognition, it can effectively activate the downstream signaling pathways of T cells, initiating immune responses such as cell proliferation, cytotoxic release, and cytokine secretion. The structure of the first-generation CAR just has the ITAM signaling area of the CD3ζ chain. Due to the lack of costimulatory signals, it shows poor in vivo amplification ability and persistence, with limited therapeutic effect, and has gradually been phased out. The second-generation CAR introduces a costimulatory signaling domain, which significantly enhances the function [7]. In recent years, novel costimulatory domains have also demonstrated the potential for tumor clearance in clinical studies. Most of the currently approved CAR-T therapies use CD28 or 4-1BB as costimulatory elements. Therefore, it is given top importance when designing many treatment plans.Although new stimulatory domains have been discovered, the problem of complementary signaling remains unresolved. Putting multiple co-stimulatory domains into CAR-T cells could improve their power to destroy tumors and extend how long they remain active in the body. However, if the selected domains are not well matched, it may result in excessive tonic signaling, which might increase the probability of serious side effects and result in the premature exhaustion of T cells [8].

2.2. The evolution of CAR

The evolution of CAR-T cell technology from the initial generation to the third generation mainly focuses on improving the proliferation ability, persistence, cytotoxicity and specific recognition ability of CAR-T cells (Figure 1). The primary generation is a single CD3ζ chain which function is recognizing and activating T cells to attack tumor cells [9]. But the problem is they have inability to produce enough Interleukin-2 (IL-2), resulting in insufficient cell proliferation and lifespan in vivo, need to supplement exogenous IL-22. Second-generation CARs incorporate additional intracellular co-stimulatory domains which have been proven to greatly improve T cell growth, cytotoxic activity, and the ability to maintain long-term anti-tumor responses [10]. The advantage is that the basic structure of CAR was determined, and the two characteristics of highly specific tumor recognition and highly activated t-cell. The third-generation CAR-T cells are further optimized products taking the second-generation ones as the basis, integrating multiple costimulatory signaling domains—such as the combined use of CD28 and 4-1BB (i.e., CD3ζ-CD28-4-1BB)—to synergistic enhance the activation.

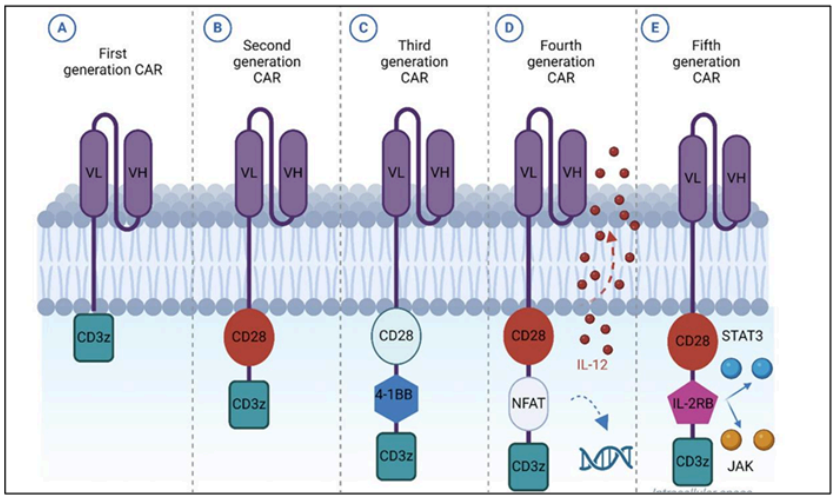

Figure 1. The evolution of CAR

The next-generation CAR-T cells further introduce Cytokine expression elements. Through engineering modification, these T cells can locally secrete immune activators after recognizing tumor antigens. They can kill tumor cells directly and also make the tumor microenvironment less likely to support tumor growth, recruit and activate other immune cells to fight together, thereby further enhancing the overall anti-tumor immune response [11]. Enhanced T cell activation not only significantly improves their power to directly destroy tumor cells and also remodels the immune environment of tumors through secreting specific cytokines, effectively recruiting, thereby mediating the indirect clearance escape tumor cells. Furthermore, the fourth - generation "TRUCK" CAR-T cells have been investigated as a way to treat viral infections, metabolic diseases, and autoimmune diseases. They don't just help to directly destroy tumor cells more effectively, but they also rebuild the immune microenvironment of tumors by secreting specific cytokines due to their modulatable immunomodulatory functions, demonstrating broad therapeutic Potential.

2.3. Limitation of CAR-T cells therapy for solid tumors

The main obstacles include: Solid tumors lack highly specific ideal target antigens, making it difficult to balance therapeutic targeting and safety; the dense tumor stromal structure and abnormal vascular system limit the effective penetration of CAR-T cells into the core area of the tumor. In addition, immunosuppressive factors and inhibitory immune cells (such as Tregs and MDSCs) are widespread which significantly weaken the activation. These factors have severely weakened the killing efficacy and persistent activity of CAR-T cells, becoming an important bottleneck for the expansion of their clinical application (Figure 2).

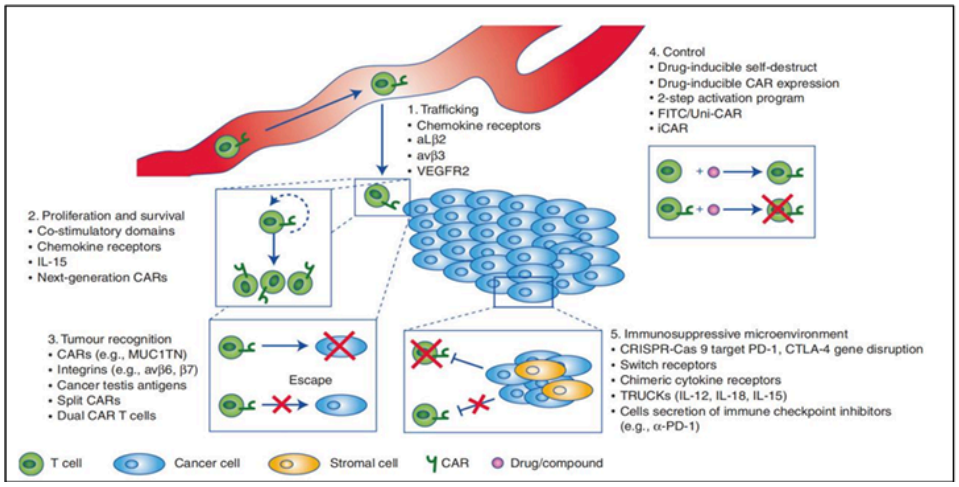

Figure 2. Limitation of CAR-T cells therapy for solid tumors

2.3.1. Treatment status

Solid tumors possess a unique microenvironment and employ various immune evasion strategies, making CAR-T cell cure more difficult to apply effectively in these settings. There are significant antigen heterogeneity, immunosuppressive TME, and limited penetration ability of CAR-T cells in strong tumors. These reasons together cause the effect of CAR-T therapy in treating solid tumors to be much worse than that in treating blood cancers. Nevertheless, current research is still trying to enhance the effectiveness of CAR-T in solid neoplastic treatment by optimizing targets, enhancing cytokines, and improving cell permeability.

2.3.2. Limitation

2.3.2.1. Tumor antigen heterogeneity

The cell population of solid tumors is usually highly heterogeneous, and different tumor cells may express different antigens [12]. As a result, CAR-T cell therapies aim at only one antigen often face difficulties such as drug resistance and variable efficacy. For example, some cells in a tumor may express a target antigen, while others may lack that antigen, leading to medical incompleteness or drug resistance This policy has been clinically proved to be safe and feasible in different large B cell lymphoid tumors.

2.3.2.2. Immunosuppressive effect of tumor microenvironment

Solid tumors are usually accompanied by a strong immunosuppressive microenvironment, which includes tumor-associated phagocytes, T cell suppressors (such as PD-L1, CTLA-4), and connective tissue and proteins in the tumor matrix. These factors inhibit the activity of CAR-T cells, make it difficult for them to play an effective role to against the tumor. Activation of immune checkpoints often leads to immune escape of CAR-T cells, weakening their ability to fight strong tumors. Methods that aim at a single antigen tend to encounter challenges.

2.3.2.3. Permeability and viability of CAR-T cells

The vascular network of solid tumors is usually structurally abnormal, with poor vascular permeability, and the surrounding tumor matrix is dense, which severely limits the penetration and directional migration of CAR-T cells. These physical barriers prevent CAR-T cells from effectively reaching the core area of the tumor. Furthermore, adverse conditions such as hypoxia, acidic pH value and nutritional deficiency in the tumor microenvironment can weaken the activity, reproductive capacity and persistence of CAR-T cells, further limiting their anti-tumor effects.

2.3.2.4. Adverse reaction

Though CAR-T cell therapy has shown excellent effects in treating certain blood cancers, its potential side effects, still exist and may be exacerbated when treating solid tumors, becoming an important challenge in clinical applications [13]. Cytokine release syndrome can induce a strong inflammatory response, which may result in serious health complications for patients undergoing treatment.

2.4. Strategies to enhance CAR-T efficacy: target optimization and tumor microenvironment modulation

2.4.1. Target optimization

A. Target selection

Antigen expression in solid tumors is heterogeneous, so selecting an appropriate target is critical. Conventional CAR-T cells typically target B-cell-associated antigens (e.g., CD19), while solid tumors require more refined selection. For example, targeting antigens such as Human Epidermal Growth Factor Receptor 2 (HER2), Melanoma-associated Antigen A3 (MAGE-A3), CD2 and other antigens has shown potential in many clinical therapy plans for kinds of solid tumors.

B. Multi-targeted CAR-T

To solve the problem of different tumor antigens, scientists are developing CAR-T cells that can aim at several tumor antigens simultaneously. This helps to improve the treatment outcome and reduce the possibility of the cancer developing resistance to the drugs.

2.4.2. Permeability modification of tumor microenvironment

A. Tumor matrix degradation

The stroma of solid tumors often inhibits the penetration and performance of immune cells. By degrading tumor matrix (such as using hyaluronidase, matrix metalloproteinases) the permeability of CAR-T cells can be improved. Research has found that using hyaluronidase can break down hyaluronic acid in the tumor tissue. This makes it easier for CAR-T cells to pass through and helps them get deeper into the tumors.

B. Improved vascular permeability

The structural and functional abnormalities of tumor blood vessels significantly influence the delivery and distribution of CAR-T cells. By improving vascular permeability, such as using anti-VEGF antibodies, the capability of CAR-T cells to enter neoplastic tissues can be effectively enhanced, thereby improving their therapeutic effect.

2.4.3. Enhance persistence and function of CAR-T cells

A. Enhanced persistence

CAR-T cells may be interfered with by immunosuppressive factors in the neoplastic microenvironment, thereby causing weakened functions. Through genetic modification, such as introducing co-stimulatory signals like IL-15 receptor or 4-1BB, the proliferation, survival ability and perseverance of CAR-T cells can be improved, thereby improving their anti-tumor function. For instance, IL-15 has been proven to effectively accelerate the proliferation of T cells and enhance their persistence, thereby strengthening the therapeutic effect.

B. Immune tolerance

Increased immune tolerance of CAR-T cells can reduce their function failure during long-term therapy. By introducing tolerance genes or improving the metabolic state of T cell, the occurrence of immune escape can be reduced.

2.4.4. Co-stimulatory molecules and cytokines enhance

A. Co-stimulatory signal enhancement

The introduction of costimulatory molecules can improve the activation, multiplication ability and longevity of CAR-T cells. Especially 4-1BB has been proven to promote their long-term survival, thereby enhancing the therapeutic effect.

B. Cytokine support

By introducing cytokines into CAR-T cells, the function of the cells and the anti-tumor effect enhanced. IL-12 has shown its potential to enhance the immune response, especially when treating solid tumors.

2.4.5 Synergistic approaches: CAR-T Cells and immune checkpoint inhibitors

A. Immune checkpoint inhibitor combination

Strong tumors often stop the immune system from attacking them in many different ways. Using CAR-T cells together with immune checkpoint inhibitors (antibodies that target PD - 1/PD - L1 or CTLA - 4) can fight against this immune suppression and make the treatment work better. Clinical studies have shown that the combined application of PD-1/PD-L1 inhibitors and CAR-T cells has achieved encouraging results in certain cancer types, demonstrating promising clinical prospects.

B. Chemotherapy combination

Chemotherapy can improve the tumor microenvironment to a certain extent and provide favorable conditions for the useful infiltration of CAR-T cells. For example, chemotherapy alleviates the immunosuppressive effect, thereby helping CAR-T cells exert their anti-tumor influence better.

3. Discussion

Although CAR-T cells have made breakthroughs and opened up new paths for immunotherapy, their killing efficacy still faces multiple challenges when dealing with more complex solid tumors or refractory tumors. These problems include T cell problems, an environment that stops the immune system from working well, and tumor antigens getting away. Therefore, how to further improve the killing ability of CAR-T cells has become a key research direction at present. In order to overcome these challenges, researchers have conducted in-depth studies from multiple perspectives such as CAR structure optimization, cell metabolic reprogramming, genetic engineering modification, and combination treatment strategies, aiming to improve its ability to cure more tumor types.

3.1. Target optimization

Target optimization of CAR-T therapy is also an important part of cancer cure improvement and can increase efficacy and safety. It involves the selection and identification of specific tumor-associated antigens that can be highly expressed on cancer cells. Researchers can use advanced technologies such as bioinformatics and large-scale screening such as CD19 and BCMA. In addition, enhanced CAR design that also includes co-stimulatory domains and single-stranded variable fragments (scFv) can significantly enhance T cell activation, persistence, and antitumor activity. Continued optimization of targets is needed to enhance and expand the applicability of CAR-T regulation for aggressive tumors and to reduce risks and immune escape.

• Advantage

Safety: Safety of CAR-T cell therapy greatly relies on the accuracy of antigen selection. When the antigen targeted by the treatment is present in normal tissues, even at a low level, it may activate T cells in an unanticipated manner and induce cytotoxicity in non-tumor tissues, thus causing dangerous side effects like organ damage or even lethal immune responses. Therefore, optimizing target selection, focusing on tumor-specific or tumor-associated antigens, and making sure that they are expressed at an extremely low level in normal tissues is the key to making CAR-T therapy safer in clinical use.Furthermore, advanced CAR designs such as logic gate systems (such as AND gate or inhibitory CAR) can hinder the activation conditions of T cells and activate the effector function only when multiple tumor antigens are recognized simultaneously, thereby further reducing the attack on normal cells. For example, designing CAR-T cells that are activated only when multiple tumor antigens are present can effectively avoid causing damage to normal tissues that express only a single antigen.

• Disadvantage

Tumor heterogeneity: Solid tumors often exhibit strong tumor heterogeneity, meaning that different tumors cells may express different antigens. The expression of tumor antigens can show significant differences within the same anatomical region of

patients. The expression level and type of tumor antigens can differ significantly between early and advanced disease stages, as well as between primary and recurrent tumors. Most CAR-T cells are designed to recognize a single surface antigen, which makes them vulnerable to antigen loss or downregulation. This limitation can lead to immune evasion and tumor relapse due to the absence or reduced expression of the targeted antigen.

Expression of normal tissues: Certain tumor antigens may also have low expression in normal cells. (e.g. HER2, EGFR, etc.). Which can lead to de effects that damage normal tissue.

Refute: Some opponents may argue that target optimization cannot completely solve the immune escape problem of solid tumors, because tumor cells can still evade immune surveillance through mechanisms such as antigen downregulation, rapid division and mutation.

Respond: While this rejoinder makes sense, attempts can be made to increase the comprehensiveness of therapy and overcome tumor heterogeneity by developing multi-target CAR-T cell (That it, targeting multiple antigens at the same time). CAR designs that incorporate natural ligands or domains in vitro, which bind to a variety of markers on malignant cells, offer a potential solution for antigen escape. Members of the TNF ligand superfamily: proliferation-inducing ligands and B-cell Activating Factor of the TNF Family (BAFF). To counter antigen escape, they have been integrated into multi-target CAR constructs aimed at simultaneously recognizing receptors like B-cell Activation Factor Receptor (BAFFR), B-cell Maturation Antigen (BCMA), and TAC19, thereby enhancing therapeutic resilience [14]. Clinical studies have shown that CAR-T cell strategies based on A Proliferation-Inducing Ligand (APRIL) or BAFF can effectively eliminate various B-cell malignancies and retain antitumor activity even in the absence of traditional targets like BCMA or CD19 [15]. After recognizing the basic trimer structure of these two ligands, their binding and potency to target cells was enhanced while maintaining their natural conformation anchored to cells [16]. Studies have reported that incorporating the 4-1BB transmembrane domain can promote trimerization of the APRIL ligand on the cell surface, resulting in improved therapeutic efficacy compared to its monomeric form. Some of these methods help prevent the tumor from escaping through a single antigen [17].

3.2. Tumor microenvironment permeability modification

The modification of the permeability of the tumor microenvironment is a very good idea for the treatment of solid tumors, aiming to improve the delivery and effectiveness of therapeutic drugs. People enhance the permeability of TME to achieve the best effect of the therapeutic agent. Strategies for altering permeability may include the use of enzymes to degrade the extracellular matrix, drugs that normalize vascular structure and function, or the design of nanocarrier systems to enhance drug delivery. By enhancing the permeability of the TME, this approach may overcome a major obstacle in tumor treatment and ultimately improve the therapeutic effect and survival rate.

• Advantage

Enhance immune cell infiltration: Tumor-Associated Macrophages (TAMs) are the main components of tumor-infiltrating immune cells and are usually classified into classically activated M1-type macrophages and alternately activated M2-type macrophages. M1 macrophages are generally considered to kill oncologic macrophages, primarily enhancing immunity and participating in anti-tumor activity, while M2 macrophages can suppress tumor immune responses by promoting IL-8 secretion from tregs, resulting in the production of transforming growth factor β (TGF-β). Therefore, developing methods to consume and reprogram TAM could enhance the invasiveness of immune cells [18]. MDSCs can also reshape the Extracellular Matrix (ECM) by producing Matrix Metalloproteinase-9 (MMP-9), which can promote angiogenesis, tumor invasion, and tumor spread [19].

• Disadvantage

Risk of side affects:Excessive destruction of tumor matrix may lead to damage of normal tissue. It also may become a dissolution of the tumor and release a large number of inflammatory factors, causing systemic inflammatory response. Many retrospective clinical trials of breast cancer use peripheral blood inflammatory markers to measure TpCR in NAT. However, the prediction potential of peripheral blood inflammation index for DpCR is lacking.

Still difficult: Although osmotic modification can improve the infiltration efficiency, some solid tumors such as pancreatic cancer, liver cancer and other tumor matrices are extremely dense, and improving permeability is still a great challenge.

Refute: Some may argue that strategies to modify the tumor microenvironment have high side effects and may not see significant results in the short term.

Respond: Although there are certain risks in the short term, with advances in precision therapy technology, osmotic modification strategies can reduce side effects under precise control. By combining the application of small molecule drugs (such as the use of hyaluronidase, metalloproteinases, etc.), a balance can be found between improving CAR-T cell permeability and reducing side effects. The TMCC platform is an advanced technology platform that simulates the complex microenvironment around tumors through microfluidic technology and cell culture technology [20]. By integrating extracellular matrix structures such as collagen, it offers a physiologically relevant environment for cell growth. Combined with advanced imaging technology for real-time dynamic monitoring, it provides rich data for experimental therapy.

3.3. Persistence and functional enhancement of CAR-T cells

The persistence and functional enhancement are the key factors determining the therapeutic effect. Persistence refers to the survival and long-term activity, which can continuously fight against cancer cells. The methods to improve persistence include the use of cytokines, the optimization of CAR design, and the combination of costimulatory domains that enhance T cell survival. Functional enhancement refers to improving the activation, proliferation and cytotoxicity capabilities of CAR-T cells. Techniques such as gene modification to express memory or anti-exhaustion phenotypes, as well as the combined use of immune checkpoint inhibitors, all contribute to enhancing the efficacy of CAR-T cells. By constantly improving these strategies, researchers are developing more durable and effective CAR-T therapies.

• Advantage

Increased tolerance: Enhancing the anti-exhaustion ability of CAR-T cells through genetic engineering means (such as increasing IL-15 receptors, enhancing co-stimulatory signals, etc.) can promote the long-term survival of CAR-T cells in the body and their continuous attack on tumors. Common γ-chain cytokine families, play a crucial role in cytokine differentiation, proliferation and in vivo immune balance. The IL-2/15 receptor binds to CD25 or IL-15 to form a receptor with high affinity, activating downstream signal molecules, especially the STAT protein family. In the case of IL-2 receptors, DTAT5A can induce homologous or heterologous dimerization and promote nuclear translocation, thereby activating the expression of multiple cell cycles and anti-apoptotic genes [21].

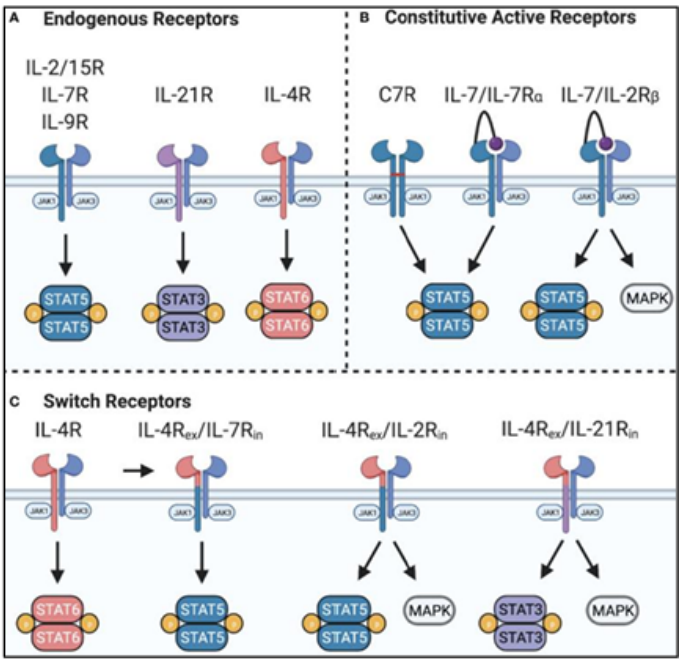

Figure 3. Common gamm chain cytokine signaling and synthetic receptors

In clinical studies, high doses of IL-2 have been used to extend BCMA-CAR products [22], although high doses of IL-2 are a strong inducer of T cell reproduction, where it can also promote terminating differentiation and T cell failure. Therefore, the study found that IL-15 is an effective substitution for high doses of IL-2, because they have the same mitotic characteristics of T cells, but IL-15 does not cause T cell failure. In clinical experiments, the replacement of IL-2 with IL-15 or IL-17 can effectively improve its survival activity and persistence [23]. In addition, IL-15-driven ARI2h has less phenotypic differentiation, less functional obstruction, and is more like memory stem cells.

Lasting efficacy: Enhancing the persistence and function is not only for improving the initial tumor clearance effect, but more importantly, it endows them with the ability to continuously monitor and clear residual tumor cells in the body, thereby achieving more durable clinical remission. Although many patients with hematological malignancies achieve rapid remission after the initial treatment with CAR-T, there is still a risk of recurrence due to the functional failure or decreased number of CAR-T cells in the body. Therefore, by regulating the differentiation state of T cells, delaying the process of effector cells differentiating into terminal cells, and maintaining their "stemness" or "memory-like" phenotypes, their long-term survival and functional retention can be significantly improved. For example, inducing the generation of central memory or stem cell-like memory T cells helps to build a stable and continuous immune defense line. In addition, the continuously expressed CAR structure, the persistent release of supportive cytokines and the optimization of the costimulatory structure can all enhance the sustained activity. The

"persistent" CAR-T cells constructed through these strategies can patrol in the body, detect and eliminate latent tumor cells, thereby minimizing the risk of recurrence and enhancing the depth and sustainability of treatment.

• Disadvantage

Cytokine storm: Overactivation of CAR-T cells can trigger cytokine storm, which is a serious side effect that can lead to a strong inflammatory response in patients. This toxic side effect is commonly associated with CAR-T cell therapy in patients with chemotherapy-resistant hematologic cancers and solid tumors. IL-6 plays a central role in driving CAR-T-related cytokine storms. Beyond this, IL-6 also contributes to tumor development and resistance to apoptosis, and serves as a critical biomarker for cancer risk assessment and diagnosis.

Refute: Some believe that enhancing the persistence may trigger more side effects, especially when the immune system is out of balance, and the cytokine storm is a risk that cannot be ignored.

Respond: This concern is valid, but by precisely controlling the activity (for example, by adding negative regulatory molecules to the surface or using smart CAR design), overactivation can be avoided. In addition, the use of immunomodulatory drugs (such as tocilizumab) can also be effective in alleviating cytokine storms. CAR-T cells engineered to express CD40L and CD28-ζ signaling domains have demonstrated enhanced activation and improved stimulation of antigen-presenting capabilities in CLL cells. Efforts have also been made to leverage CD40L-mediated activation of CLL cells to promote differentiation toward an inflammatory phenotype. In addition, the direct impact of augmented co-stimulatory signals on the function of 4-1BB-based CAR-T cells has been actively investigated. This design provides a good idea for future target innovation [24].

3.4. Costimulatory molecule or cytokine enhancement

Costimulatory molecules and cytokines play a key role in enhancing the efficacy and durability of CAR-T cell therapy. Costimulatory molecules, such as CD28 and 4-1BB, are core components for T cell activation and survival. By adding additional costimulatory domains to engineered CAR-T cells, their proliferation and function can be significantly enhanced, thereby eliminating tumors more effectively. Furthermore, cytokines are equally crucial in the functions of T cells. Enhancing the ability to produce specific cytokines helps promote their activation and survival. Through the combination of these two strategies, not only the immediate anti-tumor response is enhanced, but also the long-term therapeutic effect is supported, ultimately improving the clinical efficacy of patients.

• Advantage

Improve function: By adding co-stimulatory molecules like 4-1BB, CD28, etc., or cytokines, the proliferation and inhibition can be enhanced, and their anti-tumor effects can be improved. One study found that ICOS are mainly expressed on ILC3s in human tissue and can be upregulated in response to IL-7/2/23. ICOSL stimulation of ICOS can improve the survival time, proliferation and activation of ILC3s, and also enhance the expression of CD69. Thus improving the anti-tumor efficiency [25]. The CD40 Ligand (CD40L) is a membrane-bound protein critical for orchestrating both humoral and cellular immune responses. Its interaction with CD40 on B cells promotes their activation, supports antigen presentation, and is indispensable for immunoglobulin class switching. Furthermore, CD40L signaling is vital for the formation and maintenance of germinal centers, thereby contributing to effective adaptive immunity.Moreover, t cell proliferation and cytokine secretion are also via CD40L [26]. These is a good idea to improve function.

Enhancing immune effect: The enhancement of cytokines can not only improve the killing ability, but also enhance their survival ability in the tumor microenvironment and prolong the therapeutic effect. Tumor-associated macrophages (Tams) are the most common infiltrating immune cells in the tumor microenvironment, and their accumulation in various tumor types is usually associated with a poor prognosis [27].

• Disadvantage

Risk of immune storm: Increased expression of co-stimulatory signal or cytokines may trigger immune side effects such as cytokine storm. Excess cytokines may lead to local or systemic inflammatory responses. And different patients respnd differently to cytokines, and over-activation of certain cytokines may be less effective in some patients or even exacerbate side effects.

Refute: Some believe that cytokine enhancement strategies may lead to an excessive immune response, which in turn puts patients at greater risk. Although secondary genetic modifications are usually used to enhance the function, these modifications may also aggravate CRS, which is a serious and potentially fatal adverse reaction related to this therapy. Especially related to cytokines, the enhanced proliferation of CAR-T cells may lead to an increase in the toxicity levels of effector molecules, further exacerbating the severity of CRS. Thus, methods to improve CAR-T cell expansion with the risk of dose-limiting toxicity.

Respond: Overactivation can be avoided by finely regulating the expression level of costimulatory molecules. In addition, the use of controlled cytokine release systems, such as nanotechnology or degradable materials, can enable dynamic adjustments during treatment, thereby reducing the risk of side affects.

3.5. Combination treatment strategy

The combined treatment strategy aims to improve the therapeutic effect and overcome the limitations of a single treatment method. By combining CAR-T cells with other therapeutic methods, T cell exhaustion can be alleviated and the anti-tumor ability can be enhanced. Especially immune checkpoint inhibitors can relieve the immunosuppression and enhance the effect. Furthermore, the combined use of CAR-T cell therapy with methods such as radiotherapy can promote the release of tumor antigens, thereby further enhancing the therapeutic effect of CAR-T cells. Researchers are still exploring the combination with new therapies, such as oncolytic viruses or immunomodulators, to further stimulate the immune response. This multi-faceted approach also reflects the complexity of tumors, provides comprehensive efficacy for tumor treatment, ultimately improves the therapeutic effect of patients, and expands the applicability in different tumor types.

• Advantage

Synergies: Combining CAR-T cells with other therapies (chemotherapy, targeted therapy, etc.) can take advantage of the synergies of different mechanisms to enhance the therapeutic effect. To enhance the efficacy of CAR-T cells, multiple strategies have been employed to suppress Programmed Death 1 (PD-1) signaling, including Short Hairpin RNA (shRNA)-mediated downregulation, genetic knockout, the use of endoplasmic reticulum retention peptides to sequester PD-1 intracellularly, and the introduction of Dominant-Negative PD-1 Receptors (DNRs) [28].

Overcoming immune escape: Through combination immunotherapy, CAR-T cells are able to overcome the immune escape mechanism of tumors and produce a stronger anti-tumor response. The combined use of PD-1 inhibitors and TIM-3 inhibitors can overcome the drug resistance problem that occurs when PD-1 inhibitors are used alone. The combined use of PD-1 and LAG-3 inhibitors shows a stronger inhibitory effect on tumors than a single drug.

• Disadvantage

Treatment complexity: Combination therapy requires precise drug combinations and treatment regiments, otherwise it may lead to the addition of side effects, especially in the dose of drugs and treatment timing may be difficult to control. And it can also lead to tumor-related immune storms and organ damage.

Refute: Some believe that the complexity of combination therapy makes it difficult to be widely used in the clinic, especially in the case of the need to carefully adjust the drug does and treatment time point, the risk of side effects cannot be ignored.

Respond: Although the complexly of combination therapy does exist, with the development of precision medicine, we can respond to the needs of different patients through personalized treatment plans, the use of modern drug monitoring technology and precision regulation means, can completely reduce the side effects of treatment.

4. Conclusion

CAR-T cell therapy technology represents a significant advancement. Its clinical success highlights the potential and sustainability of personalized immunotherapy. However, extending CAR-T cell therapy to the treatment of solid tumors still faces huge challenges, which is also one of the main directions of current research. This article reviews the structural composition, the maintenance of progenitor cells and their developmental background, and simultaneously analyzes the challenges in related treatments. Several new ideas have been proposed for CAR-T cell therapy of solid tumors, including target optimization of multi-target CAR-T methods, aiming to improve specificity and reduce the risk of immune escape. By regulating the tumor microenvironment, enhancing tumor permeability and immune cell infiltration, and further improving the function, it is the key to improving the therapeutic effect. In addition, making CAR-T cells more persistent and active through genetic engineering and enhancing the costimulatory domain can address the limitations faced in solid tumors. Finally, the combined use of immune checkpoint inhibitors and chemotherapy provides a promising new strategy. CAR-T cell therapy is undoubtedly a cutting-edge medical technology that has attracted much attention at present, demonstrating great therapeutic potential. With the continuous development and update of CAR-T cells, solving these challenges will lay the foundation for more effective treatment. The therapeutic development and new technology of CAR-T for tumor have great prospects, and continuous innovation in overcoming the limitations indicates a new era of personalized cancer therapy.

In the process of writing a literature review on CAR-T cell therapy, substantial academic knowledge and specialized skills are acquired, with a particular emphasis on developing a comprehensive understanding of this frontier field. Through the discussion of the success in the treatment of blood tumors, the challenges of their application in solid tumors and the coping strategies, the author not only deepened my understanding of the mechanism of cancer immunotherapy, but also mastered the relevant biomedical terminology and research methods. This process enhances the recognition of the transformative potential of personalized immunotherapy, while simultaneously illuminating the existing gaps in current research and future directions.

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