Current Challenges and Future Perspectives of CAR-T Cell Therapy in Solid Tumors

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Abstract. Chimeric antigen receptor T-cell (CAR-T) therapy has shown some good results. Clinical Success of Hematological Malignancies and its Efficacy in Solid Tumors. remains constrained. The various biological features of solid tumors are as follows: Heterogeneous antigen expression, poor tumour trafficking and invasion, etc. Immunosuppressive Tumor Microenvironment (TME) and T-cell Exhaustion. It will have little therapeutic effect for CAR-T cells. Although recent studies have shown some good results, these problems are still not fully resolved.This review summarizes the major barriers affecting CAR-T cell therapy in solid tumors and discusses recent advances aimed at overcoming these limitations. First of all, there is tumour antigen heterogeneity and antigen escape; Tumour homing is restricted, intratumoral penetration is poor, there is a suppressive tumour microenvironment, and T-cell function and dysfunction are gradually lost. Recently, some new treatment ideas have been put forward, such as multi-target CAR-T cells, modifications of the TME (tumor microenvironment), and gene-editing technology to construct next-generation CAR-T cells. In short, the above results show that in order to improve the efficacy of CAR-T therapy for solid tumors, many engineering strategies must be integrated to enhance tumour recognition, persistence and antitumor activity. Further progress will be made in cell engineering, gene editing and tumour immunology to advance the development of safer and more effective CAR-T therapy, providing new hope for the treatment of solid tumours.

Keywords: CAR-T therapy; Solid tumors; Tumor microenvironment (TME); Antigen heterogeneity.

1. Introduction

Chimeric antigen receptor T cell (CAR-T) therapy is now one of the top results in cancer immunotherapy. Genetic modification of T cells from malignant tumour patients can be used to recognise tumour-associated antigens, and then these cells are employed in CAR-T therapy to produce a targeted immune response against the malignant cells. Following the approval of the first CD19-targeted CAR-T therapy in 2017, CAR-T treatment has become an effective treatment for several types of blood cancers, such as B-cell acute lymphoblastic leukaemia (B-ALL), diffuse large B-cell lymphoma (DLBCL) and multiple myeloma. Since then, the United States has approved some CAR-T products. Kymriah is approved for the treatment of B-cell acute lymphoblastic leukemia (B-ALL) and diffuse large B-cell lymphoma (DLBCL); Yescarta, Breyanzi, and Tecartus are used for various types of B-cell lymphoma; Abecma and Carvykti are for multiple myeloma.

Although there have been some achievements, the results in haematological malignancies have not been generalised as solid tumours. Therefore, the FDA has not yet approved the use of CAR-T cell therapy for malignant solid tumours. Many current clinical trials have shown little response of CAR-T to many types of solid tumors, and the results are inconsistent. It can be seen that CAR-T technology has not yet solved the problems of solid tumours' disease. Solid tumours are not as simple as blood cancers, and there are many problems in selecting which T cells to use and how to modify them.

Currently, one of the main problems is the diversity of tumour antigens. Many solid tumours do not express the same target antigen uniformly, so antigen-negative tumour cells can survive and eventually cause recurrence of the disease. Due to an abnormal tumour blood vessel system, there is a lack of chemokine signalling and a dense extracellular matrix barrier; thus, CAR-T cells can only be transported to and enter the tumour tissue to some extent. Although CAR-T cells can enter the tumour, they will be faced with an immunosuppressive TME; that is to say, immune cells are

regulated, cytokines are suppressed, there is hypoxia, and nutrients are scarce, and all of these factors can lead to T cell dysfunction and depletion.

Recently, there have been new solutions for the above problems in synthetic biology, gene editing and cell engineering. Dual-targeted CAR-T, armoured CAR-T cells, cytokine-secreting CAR-T, checkpoint anti-CAR-T cells and combination therapy are all in the process of being developed and have shown some positive results in preclinical studies and early clinical trials. In recent years, some clinical studies have shown that CAR-T cell therapy has had some results for glioblastoma, neuroblastoma, gastrointestinal cancer and other solid tumors. Based on the above studies, further improvement and adjustment of the treatment method for these diseases may be carried out in the clinic.

The reasons for the limitations of CAR-T cell therapy in solid tumours that have been studied are antigenic heterogeneity, tumour transport and poor tumour cell infiltration, an immunosuppressive tumour microenvironment, etc. It also introduces new ways of dealing with these problems and mentions recent changes that may help to promote the development of CAR-T cells in the treatment of solid malignant tumours in the future.

2. Challenges of CAR-T Cell Therapy in Solid Tumors

2.1. Tumor Antigen Heterogeneity and Antigen Escape

Heterogeneity of tumour antigens is frequent in solid tumours and is a problem that hampers CAR-T cell therapy. Most malignant cells of B-cell acute lymphoblastic leukaemia have expressed the target antigen CD19. Solid tumours generally have a number of tumour cell populations, and they also have different antigen expression patterns. Some cells have high expression of the target antigen, and others have low or no expression of it [1,2].

Therefore, it is more difficult to choose the target antigens for solid tumours than for blood cancers. At present, many tumour-associated antigens have been researched, such as HER2, EGFR, mesothelin and claudin18.2. However, the expression of these antigens is varied, and there are different expressions among different patients and even among different tumour cells in the same tumour. Heterogeneity in antigen expression has been reported in breast cancer, glioblastoma, pancreatic cancer and gastrointestinal tumours [3]. Since CAR-T cells need to be exposed to antigens for cytotoxic activity, different numbers of these antigens may result in a different number of target cells that can be attacked by the cells.

Heterogeneity in antigen expression is associated with antigen escape. After treatment, CAR-T cells will destroy the antigen-positive tumour cells; otherwise, the antigen-negative cells in the tumour will remain. The remaining cells can continue to divide and spread disease. After CAR-T cell therapy, there has been a loss of antigen and down-regulation of antigen; this is associated with treatment failure and disease recurrence [4].

Antigen escape has also been observed in clinical trials. Brown and others have been using CAR-T cells directed against IL13Rα2 for the treatment of recurrent glioblastoma in patients. According to imaging, the tumours in the brain and spine have shrunk after therapy. However, in the examination of recurrent tumor tissues, it was found that the expression of IL13Rα2 was significantly reduced compared with that in the pre-treatment samples. Therefore, the change in antigen expression may be caused by treatment and could lead to tumour relapses [5].

The same is also found in some solid tumours. Clinical studies have found that not all tumor cells express the selected antigen at the same level in HER2, EGFR and Mesothelin. In the first phase of clinical trials, researchers have studied the effect of HER2-targeted CAR-T cells on advanced sarcoma in patients and found that the disease was stable for some. However, there is still heterogeneity in HER2 expression, and it has not been completely targeted [6]. There have been fluctuations in antigen expression of gastrointestinal tumours after treatment with Claudin18.2-targeted CAR-T cells [7].

The study of heterogeneous antigen expression has been carried out, and therefore researchers have developed double-target and tandem CAR-T cells. Recently, CAR-T constructs that can recognise various tumour-associated antigens have been introduced in recent years, such as the combined antigen of EGFR and IL13Rα2 for glioblastoma models [2, 3].

2.2. Limited Trafficking and Tumor Infiltration

After CAR-T cells are administered intravenously, they need to travel to the tumour and engage with cancer cells. Many reasons are responsible for the above, such as chemokine signal transduction, blood vessel transport and the physical characteristics of tumour tissue. Research on solid tumors has found that the migration efficiency of CAR-T cells in these tumors is generally lower than that in blood cancers [2].

One problem of CAR-T cell trafficking is that the chemokines released by tumour cells are often not recognized by chemokine receptors on the transferred T cells. T cells should be able to sense chemokine gradients and then migrate to the site of the tumour. However, the chemokine signal produced by many solid tumours is too weak to attract the transferred T cells effectively. A weak chemokine signal is associated with a smaller number of CAR-T cells in the tumour site [1].

Abnormal tumor blood vessels can also affect the migration of CAR-T cells. The Blood Vessels in Tumors are generally irregular and have poor blood supply. Vascular abnormality reduces the entry of immune cells into the tumour from the blood circulation. Vascular abnormalities have been considered one of the reasons for poor distribution of CAR-T cells in solid tumours [2].

CAR-T cells have reached the tumour, but there are still other problems. Most solid tumours are densely organised, and the structure of this matrix is not easily crossed by immune cells. Proteins of fibroblasts in the tumour microenvironment may restrict the growth of the tumour. Therefore, CAR-T cells may accumulate at the edge of the tumour, and only a few cells are able to enter the deeper part of the tumour [1,2].

Experiments have been carried out to improve the migration of CAR-T cells. Mesothelin-specific CAR-T cells have been modified in the lab to express CCR2b and can bind to the chemokine CCL2. In vitro experiments have shown that the migratory ability of these cells in the presence of CCL2 produced by tumor cells is increased. Based on the mouse model, it was found that CAR-T cells with the expression of CCR2b accumulated more in the tumour than unmodified CAR-T cells and had enhanced anti-tumour effects [8].

Clinical trials have also been carried out for the other ways of delivery. A patient with recurrent glioblastoma received direct delivery of CAR-T cells against IL13Rα2 in the brain. CAR-T cells appeared in the TME later, and some lesions decreased after treatment [5]. Strategies that have been employed by the local government to address other solid tumours have also been studied in order to increase the local accumulation of transferred CAR-T cells [2].

Clinical studies of mesothelin have provided some data on the migration and retention of CAR-T cells. Mesothelin-specific CAR-T cells were found in the peripheral blood of patients with metastatic pancreatic cancer in the first stage of clinical trials, so it was concluded that the infused CAR-T cells had grown successfully after infusion. However, the continuous tumour infiltration is still relatively small, and thus it is not clear how long the CAR-T cells can remain at the tumour site in solid tumours [9]. Another study of Claudin18.2-targeted CAR-T cells for the treatment of gastrointestinal tumours has found CAR-T cells in the peripheral blood of patients after treatment, and some objective responses have been observed in these patients. However, there are different responses among the patients to the treatment, and the effective migration, infiltration and long-term survival of CAR-T cells in solid tumours have not yet been achieved [7].

2.3. Immunosuppressive TME

CAR-T cells should be able to work in the TME (Tumor Microenvironment) after reaching the solid tumour. TME has many immunosuppressive factors that can inhibit the function, growth and spread of metastatic T cells. Compared with hematological malignancies, solid tumors usually have

a more complicated and inhibitory microenvironment; this is one of the main reasons why CAR-T cell therapy is not highly effective in these cases [4,10].

Cellular and Non-cellular Components of the Immunosuppressive TME. Regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs) are all types of immune cells that can suppress the immune system. The cells inhibit the activation of T cells and promote tumour immune evasion in all sorts of ways. Tregs reduce the number and cytotoxicity of effector T cells; MDSCs can suppress the response of T cells through other immunosuppressive factors and metabolic dysregulation of T cells. Some of the TAMs that are M2-like cells release pro-tumour inflammatory factors and growth factors to promote the growth of tumours and suppress immune surveillance [1, 10].

In addition to inhibitory immune cells, there are also many inhibitory cytokines in the TME. Among them, the two main inhibitory factors are transformed growth factor-beta (TGF-β) and interleukin-10 (IL-10). TGF-β can suppress the proliferation, cytokine secretion and cytotoxicity of CAR-T cells, and IL-10 is associated with the suppression of immune activation and the formation of an immunosuppressive microenvironment. Prolonged exposure to the above cytokines will gradually reduce the anti-tumour effect of metastatic T cells and thus cannot treat the disease [2, 4, 10].

The metabolic environment of solid tumours will also harm the function of CAR-T cells. Fast-growing tumour cells have consumed a large amount of glucose and amino acids and reduced the supply of nutrients for invading immune cells. At the same time, there will be an environment of hypoxia in the tumour due to abnormal tumour blood vessels and uncontrolled cell proliferation. Nutritional deficiencies and hypoxia can reduce the proliferation of T cells, inhibit the production of cytokines, and therefore weaken the anti-tumor effect of CAR-T cells. A bad metabolic environment can also result in a decline of T-cell function with the passage of time in anti-tumor therapy [1,3].

According to clinical trials, at present, an immunosuppressive microenvironment (TME) in the tumour is still one of the reasons for poor performance of CAR-T cells after reaching the tumour. In the first stage of clinical trials for mesothelin-specific CAR-T cells in metastatic pancreatic cancer, after infusion, CAR-T cells were expanded and then found in the peripheral blood. However, only weak and temporary anti-tumor effects were shown; that is to say, although the cells could be grown successfully, the inhibitory microenvironment still restricted the function of CAR-T cells [9]. Similarly, among the patients with gastrointestinal cancer who have received CAR-T cells targeting claudin18.2, some have achieved objective remission, but the duration and scope of this remission vary; thus, it is believed that various factors in the TME still affect the therapeutic effect [7].

To solve the problems above, many modification strategies for CAR-T cells and reshaping approaches to the TME have been developed. For example, CAR-T cells can be modified to produce pro-inflammatory cytokines such as IL-12 and IL-18 in the local environment to enhance the activation of immune cells and extend the lifespan of T cells. Other ways are to alter CAR-T cells so that they are no longer inhibited by TGF-β, or to combine CAR-T therapy with immune checkpoint inhibitors to restore the function of T cells. Research is also being carried out to reduce the number of suppressor cells and change the microenvironment of CAR-T cells to improve anti-tumor effects in solid tumours [1, 2, 4].

In general, the immunosuppressive effect of TME is the main reason why CAR-T cell therapy cannot be used effectively after tumour spread. For the successful treatment of solid tumours, not only is there an effective way to transport CAR-T cells, but also an approach to overcome immunosuppression and sustain prolonged anti-tumour effects.

2.4. T-cell Exhaustion and Dysfunction

Prolonged exposure of antigens to solid tumors will reduce the activity of CAR-T cells over time and lead to T-cell exhaustion and loss of function. Hematological malignancies usually have a small number of CAR-T cells, and most solid tumours are continuously expressed of antigens in the presence of an immunosuppressive microenvironment. Therefore, CAR-T cells will gradually lose

the ability to divide, secrete cytokines and kill cancer cells, and their effect will be relatively short-lived [2,6].

There are many kinds of changes in T-cell exhaustion. Exhausted CAR-T cells have reduced production of cytokines such as interferon-gamma (IFN-γ) and interleukin-2 (IL-2), cannot proliferate, and have lost their cytotoxic function. At the same time, the damaged cells have increased the expression of all kinds of inhibitory receptors, such as programme death protein 1 (PD-1), lymphocyte activation gene 3 (LAG-3) and T cell immunoglobulin and mucin domain protein 3 (Tim-3). An increase in the quantity of the above-mentioned inhibitors will inhibit T-cell activation to a certain extent and thus reduce the anti-tumor effect [2,4,6].

The TME is immunosuppressive and can cause T-cell exhaustion. Inhibitory cytokines, such as TGF-β and IL-10, along with hypoxia and lack of nutrients, can also injure the metabolism and life of CAR-T cells. As shown in the previous analysis, suppressive immune cells, such as regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages, can also constantly emit inhibitory signals and are thus one of the reasons for this phenomenon. Therefore, T-cell exhaustion is not due to a single cause but rather the result of prolonged antigen stimulation and inhibitory signals in the TME [2,3].

Recently, new research has provided some ideas on the molecular reasons for CAR-T cell failure. Single-cell transcriptomics show that after infusion, CAR-T cells gradually change and are eventually dysfunctional. After CD19-targeted CAR-T therapy, both CAR-positive and CAR-negative T cells in the patient showed the same differentiation path upon antigen stimulation and eventually became NK-like cells [7]. Another study has found that this NK-like transition is a particular kind of dysfunctional state; that is to say, it has a reduced proliferation rate and continuous damage, indicating that CAR-T cell exhaustion is caused by dynamic changes in cell differentiation and is not only about the loss of cytotoxic function [8]. Based on the above results, a preliminary understanding of the reason why CAR-T cells gradually lose their therapeutic effect over time can be concluded.

At present, many methods have been developed to delay or reverse CAR-T cell exhaustion. The other is that immune checkpoint inhibitors can be used to activate T cells after CAR-T therapy. Gene editing technology can be employed to disrupt the inhibitory signal pathway of PD-1 knockout and extend the life and anti-tumor effect of CAR-T cells. Recently, scholars have been studying how to optimise the structure of CARs, costimulatory domains and cytokine signalling for the purpose of reducing chronic activation and preserving the functional phenotype of T cells after prolonged use [2, 4].

T-cell exhaustion and dysfunction are still the main reasons why CAR-T cells have failed in the long run for solid tumours. Continuous exposure to tumour antigens and the inhibitory microenvironment of the TME will gradually reduce the activity of CAR-T cells, and they will fail to respond to therapy. To help the development of a new generation of CAR-T cells with extended lifespan and prolonged anti-tumor effects, it is needed to understand the molecular reasons for T-cell exhaustion; thus, this paper introduces a multi-target CAR-T cell.

3. Strategies to Overcome the Biological Barriers

3.1. Multi-Target CAR-T Cells

Tumour antigen heterogeneity and antigen escape are still the main reasons why CAR-T cell therapy has not been fully realised in solid tumours. As shown in Section 2.1, CAR-T cells that target a single antigen can destroy antigen-positive tumour cells but will not affect antigen-negative cells; thus, there will eventually be a disease relapse. Therefore, multi-target CAR-T cells have begun to be used to enhance the tumour recognition rate and reduce the problem of antigen escape [3,4].

In order to make CAR-T cells recognise many different tumor-associated antigens, many methods have been developed. Another is to construct T cells that have two separate CARs to recognise different antigens. Tandem CAR (tanCAR) is another type; the two antigen-binding domains are linked together to form one CAR construct that can recognise several antigens at the same time.

Recently, some people have also been studying logic-gated CAR systems to improve tumour specificity and only activate CAR-T cells upon detection of a certain combination of antigens. The purpose of the above engineering plan is to increase the area of the tumour and avoid damage to healthy tissue [3, 9].

Some multi-target CAR-T cells have already shown the ability to kill all types of solid tumors in animals at present. CAR-T cells that target both EGFR and il13rα2 were more effective in treating glioblastoma in mice than those directed only at EGFR or only at il13rα2. Since the expression levels of these antigens are different in different populations of tumour cells, targeting can increase the proportion of tumour cells recognized by CAR-T cells and reduce the occurrence of antigen-negative tumour cells [1, 2]. Based on the above results, it can be concluded that multi-antigen recognition may have more extensive tumour coverage than conventional CAR-T therapy.

Clinical trials have also shown that there needs to be a certain degree of antigen expression. Brown and others. It has been reported that for patients with recurrent glioblastoma who had received il13rα2-targeted CAR-T cells, there was a considerable reduction in the tumours of the intracranial and spinal cord. However, the expression of il13rα2 in recurrent tumour tissue was significantly reduced after treatment; thus, it can be concluded that antigen loss is one of the reasons for disease relapse [5]. Similarly, the phase I studied of HER2 targeted CAR-T cells for advanced sarcomas also showed that the condition of some patients was stable, but the heterogeneity of HER2 expression was still a main problem preventing complete tumour clearance [6]. Some good results have also been obtained in the clinical trials for patients with gastrointestinal tumours treated with claudin18.2-specific CAR-T cells; however, differences in antigen expression among various patients may still be one of the reasons for poor treatment effects [10]. In short, the above research has found that there are problems with single-antigen targeting and supports the development of multi-target CAR-T strategies.

There are some problems in addition to the above. It is necessary to choose an appropriate combination of antigens; that is to say, the target antigen should be highly expressed in tumour cells but relatively less so in normal tissues, in order to reduce off-target toxicity and non-tumour toxicity. In addition, with the increase in complexity of CAR, production will be more difficult, and it may affect the expression, signal transduction and long-term stability of CAR. Therefore, before the generalisation of multi-target CAR-T cells in clinical trials, optimisation of antigen selection and CAR design still needs to be done [4,9].

In general, multi-target CAR-T cell therapy is a good way to address the problem of antigen heterogeneity and antigen escape in solid tumours. With the continuous progress of CAR engineering technology and deeper research on the expression of tumor antigens, it is expected that CAR-T therapy will be more effective and safer in the future.

3.2. Engineering CAR-T Cells to Overcome the TME

Immunosuppressive TME is one of the major obstacles limiting the efficacy of CAR-T cell therapy in solid tumors. As described in Section 2.3, inhibitory immune cells, inhibitory cytokines and adverse metabolic conditions can impair the proliferation, persistence and cytotoxicity of CAR-T cells. Therefore, people have invested a lot of energy to modify CAR-T cells to make them resistant to these factors.

An important strategy is to develop "armored" CAR-T cells. Different from traditional CAR-T cells, armored CAR-T cells can secrete immune stimulating cytokines or express other functional molecules through genetic engineering, so as to enhance the immune response in tumor. Cytokines such as interleukin-12 (IL-12), IL-15 and IL-18 have been integrated into CAR-T cell design to improve the proliferation, persistence and cytotoxic activity of T cells. In addition to directly enhancing the function of CAR-T cells, these cytokines can also activate the surrounding immune cells and promote the proliferation and clearance of tumor cells.

Another method focuses on reducing the inhibitory effect of immunosuppressive signaling pathway. Transforming growth factor - β (TGF - β) is one of the main inhibitory cytokines in solid

tumors, which can significantly damage the activity of CAR-T cells. To overcome this limitation, researchers constructed CAR-T cells expressing dominant inactivated TGF - β receptors or other modified signaling molecules to reduce TGF - β mediated inhibition. These modifications enable CAR-T cells to maintain higher proliferation and cytotoxic function even in the presence of inhibitory cytokines in the TME [2,3].

In addition to the internal modification of CAR-T cells, combination therapy is also used to improve the therapeutic effect. Immune checkpoint inhibitors targeting PD-1/PD-L1 pathway can restore T cell activation by blocking inhibitory signal transduction, so they have been used in combination with CAR-T therapy in preclinical and early clinical studies. Other combined strategies include chemotherapy and radiotherapy, which may increase tumor antigen release and improve CAR-T cell infiltration; And oncolytic viruses, which can selectively infect tumor cells and promote local immune activation. Although the clinical benefits of these combined therapies are still under study, they may further enhance the function of CAR-T cells when combined with the modified CAR-T therapy [9].

Recent clinical studies also support the feasibility of improving the efficacy of CAR-T cells by changing the TME. In patients with gastrointestinal cancer treated with claudin18.2 specific CAR-T cells, encouraging objective remission shows that improving CAR design and treatment strategies can enhance the therapeutic effect of specific solid tumors [10]. Similarly, studies targeting CAR-T cells with mesothelin have shown that although CAR-T cells can be successfully expanded after infusion, overcoming the inhibitory effect of TME is still the key to obtain lasting clinical efficacy [11,12]. These findings highlight the importance of combining improved CAR engineering with strategies to enhance the activity of CAR-T cells in tumors.

In general, the modification of CAR-T cells to resist immunosuppression and reshape the TME is an important direction for future development. Enhancing the immune tolerance of CAR-T cells, improving their resistance to inhibitory cytokines and reasonable combination therapy are expected to jointly improve the durability and anti-tumor function of CAR-T cells. Continuous optimization of these methods is expected to further improve the clinical efficacy of CAR-T cell therapy in the treatment of solid tumors.

3.3. Gene Editing and Next-Generation CAR-T Cells

The latest progress in gene editing technology has accelerated the development of a new generation of CAR-T cell therapy for the treatment of solid tumors. Traditional CAR-T therapy is often limited by poor persistence of CAR-T cells, depletion of T cells and immunosuppressive signal transduction in TME. Gene editing provides a new opportunity to improve the function of CAR-T cells by modifying specific genes that regulate T cell activation, survival and anti-tumor activity [2,3,4].

CRISPR/cas9 has become one of the most widely used gene editing tools in CAR-T cell engineering. This technology can precisely modify the target gene, so that researchers can improve the function of CAR-T cells and reduce adverse immune reactions. One of the most commonly used research strategies is to destroy the programmed cell death protein 1 (PD-1) gene. PD-1 deficient CAR-T cells exhibit enhanced proliferation, cytokine production and cytotoxic activity by reducing inhibitory signal transduction in the TME. These findings suggest that gene editing may help maintain the function of CAR-T cells during long-term antigen exposure [3,4,9].

Gene editing technology has also been used to optimize CAR integration and generate universal CAR-T cells. Targeted insertion of CAR constructs into specific genomic sites, such as the T cell receptor α constant region (TRAC) site, can more accurately control CAR expression, and may improve the persistence of T cells, while reducing the risk of unpredictable gene insertion. In addition, the destruction of endogenous T cell receptor genes makes it possible to develop allogeneic or "ready-made" CAR-T cells, which can shorten the production time and improve the treatment accessibility compared with the traditional autologous CAR-T therapy [2,9].

In addition to gene editing, researchers are also constantly developing the next generation CAR-T cells with higher safety and effectiveness. The new CAR design integrates the optimized

costimulatory domain, inducible signal system and safety switch, which can better control the activity of CAR-T cells after infusion. Other methods combine a variety of engineering strategies, including enhancing antigen recognition, improving resistance to immunosuppressive signals and enhancing persistence, to overcome many obstacles in solid tumors at the same time. These advances highlight the increasingly close integration of synthetic biology and genetic engineering in the development of CAR-T cells [2,9].

Although these technologies are still under active research, they represent a promising development direction of CAR-T cell therapy in the future. With the continuous progress of gene editing technology and a deeper understanding of tumor biology and immune regulation mechanism, it is expected to develop CAR-T cell products that are safer, more effective and more accessible. The combination of precise genetic engineering and reasonable CAR design may ultimately overcome many limitations of CAR-T therapy in the treatment of solid tumors.

4. Conclusion

CAR-T cell therapy has achieved remarkable success in the treatment of hematological malignancies, but its application in solid tumors is still limited by a variety of biological barriers. This article reviews the main challenges affecting CAR-T cell therapy in the treatment of solid tumors, including tumor antigen heterogeneity and antigen escape, CAR-T cell migration and tumor invasion limitation, immunosuppressive TME, and T-cell exhaustion and dysfunction. These factors reduce the persistence, cytotoxic activity and overall therapeutic effect of CAR-T cells, resulting in limited and unstable clinical efficacy.

In order to overcome these limitations, many engineering strategies have been developed. The design of multi-target CAR-T cells aims to improve tumor recognition ability and reduce antigen escape. Engineering methods such as enhancing CAR-T cells, immunosuppressive signal resistance and rational combination therapy have shown the potential to enhance the function of CAR-T cells in the TME. In addition, the progress of gene editing technology and new generation CAR design also provides a new opportunity to improve the durability, safety and anti-tumor activity of CAR-T cells. In conclusion, these strategies suggest that overcoming the complex barrier of solid tumors may require the integration of multiple methods rather than relying on a single means of transformation.

Although encouraging progress has been made, there are still some limitations. At present, most strategies are still in the stage of preclinical research or early clinical trials, and their long-term safety and effectiveness still need further study. In addition, the biological heterogeneity of different solid tumors means that any single treatment strategy is unlikely to be effective for all tumors. In the future, the continuous progress of synthetic biology, gene editing technology and a deeper understanding of tumor biology are expected to promote the research and development of safer and more effective CAR-T cell therapy. The further optimization of CAR design and the development of individualized treatment strategies may ultimately expand the clinical application scope of CAR-T therapy and improve the treatment effect of patients with solid tumors.

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