Chimeric-antigen receptor T (CAR-T) cell therapy for solid tumor

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Abstract. Chimeric-antigen receptor T (CAR-T) cell therapy has been researched in the clinical. However, CAR-T cell therapy for solid tumor is faced with lots of challenges such as cytokine storm, how to find the specific antigen and limitation of microenvironment of tumor. In this review, we will discuss the research of CAR-T cell therapy for solid tumors of digestive system, and possible solutions and future direction.

1. Introduction

Cancer is a major killer of human health. It has the characteristics of low early detection rate, high recurrence and metastasis rate and poor prognosis. The latest research has found that 37% people will have cancer in their lifetime and 23% people will die because of it. Currently, surgical resection of cancer tissue, chemotherapy, radiotherapy, targeted anti-cancer drugs are used to cancer treatment. Immunotherapy is a new and rapidly developed cell therapy technology in recent years. Immunotherapy can be divided into three parts: cytokine therapy, checkpoint blockade and adoptive immunotherapy[1-3]. Chimeric antigen receptor (CAR-T) cell therapy is a specific cell immunotherapy and combines the high affinity of antigen and antibody with the killing effect of T lymphocyte[4]. T lymphocyte expresses this specific chimeric antigen receptor through gene transduction and kills target cells[5].

Tumors are usually found in the middle or late stages. At this time, the tumour cells in the patient's body are dominant, which seriously damages the immune function of the body[6]. Under the immune system microenvironment, the efficiency of activating T cells is low, the ability of attacking cancer cells is insufficient and inaccurate; in addition, the tumour cells are inadequate and inaccurate. So, we require the manufacture of precision-guided, precision-hit immune cell weapons to overcome these problems. Compared with the traditional T cell recognition antigen, the recognition of tumor antigen by CAR does not need be restricted by major histocompatibility complex. At the same time, CAR can enhance the anti-tumor killing ability of T cells by increasing costimulatory molecular
signals. Therefore, CAR-T cells can overcome the downregulation of major histocompatibility complex molecules by cancer cells and reduce co-stimulation\cite{7, 8}. Immune escape mechanisms such as excitatory molecule expression. CAR-T cell technology has developed four generations, the first generation of CAR has only one signal region of CD3 receptor in T cells; on this basis, the second generation adds one costimulatory molecular signal; the third generation adds two costimulatory molecular signals (Figure 1)\cite{9}.

![Figure 1. Generation of CAR-T[10]](image)

At present, CAR technology has made remarkable achievements in the treatment of hematological and solid tumors. It has the advantages of reducing the risk of rejection reaction, more precise treatment, and wider range of killing tumors, more precise multi-target, more lasting killing effect and low-dose application\cite{11}. These advantages demonstrated by CAR-T cells provide new solutions for adoptive cellular immunotherapy. This review mainly introduces the CAR-T technology in the treatment of solid tumors of digestive system.

2. Gastric cancer

Mucosal epithelial cells in the inner and outer layer of the gastric wall can form Gastric cancer (gastric cancer or cancer of stomach)\cite{12, 13}. Different parts of the stomach may become cancer and the cancer cells can invade the different depth and breadth of the gastric wall.

According to the different stages of gastric cancer, surgery-based comprehensive treatment was selected. However, the limitation of surgical treatment is that gastric cancer is often found late and these complications will greatly reduce the quality of life of patients. Chemotherapy which has many other side-effects such as bone marrow suppression, heart function damage and kidney. Targeted drugs and interventional therapy all have the problems of drug resistance and recurrence. Therefore, it is particularly important to explore new immunotherapy with fewer complications and lasting killing effects on tumors\cite{14}.

There were many researches on the treatment of gastric cancer by CAR-T cells. People used Cha21 as a gastric cancer cell specific antigen and evaluated the ability of anti-tumor of CART-HER2 cells in vivo. The researchers treated the mice with CART-HER2 cells 12 days after inoculating the tumor cells under the axilla, and measured the growth of the tumors twice a week until the maximum diameter of the tumors was greater than 2 cm. In this research, the tumors can be inhibited by CART-HEAR2 cells. The survival rate also increased. These results indicate that CART-HER2 cells
have specific anti-tumor activity[15].

In addition, cancer stem cells (CSC) are the initiating cells of tumors, responsible for drug resistance, recurrence, progression and the eradication of CSC is a promising method for the treatment of cancer[16]. GCSC (gastric cancer stem cells) has also been identified using specific markers, such as CD44; some studies have found that HER2 plays an important role in maintaining CSC subsets of GC cells. Therefore, HER2 targeted therapy can kill CSCs and improve the therapeutic effect of HER2 positive GC patients. Researchers have successfully verified the self-renewal and tumor initiation ability of CD44+ HER2+ GCSC in primary gastric tumors. It was also found that CART-HER2 cells could effectively degrade tumor spheres and inhibit the growth of transplanted tumors in nude mice. These results suggest that CART-HER2 cells have a strong cytotoxic effect on GCSCs, suggesting that CART-T cells have potential efficacy in preventing recurrence and metastasis of GC. However, because of the functional and genetic heterogeneity of CSCs and the complexity of tumor microenvironment, the actual impact of this treatment needs to be further validated in future clinical trials.

3. Esophageal cancer

Esophageal cancer is a malignant tumor occurring in the epithelial tissue of the esophagus. Every year, about 220,000 people die because of its worldwide[17]. The number of people who die of esophageal cancer is in the second class and only less than gastric cancer. The occurrence of esophageal cancer is related to chronic nitrosamine stimulation, inflammation and trauma, genetic factors and trace elements in drinking water, food and vegetables.

The treatment of early stage esophageal cancer contains surgery, radiotherapy and chemotherapy and so on[18]. In recent years, CAR-T cell therapy has gradually become the main research on the treatment of esophageal cancer. People used EPHA2. CAR - T cells and studied its killing ability to esophageal squamous cell carcinoma (ESCC) cells in vitro. EPHA2 was up-regulated in ESCC tissues and cells and expressed on ESCC cell membrane and can be used as tumor-associated surface antigen (TAA) of CAR for the treatment of ESCC. After the successful construction of EPHA. CAR, researchers first considered the killing effect of co-culture of EPHA. CAR-T cells with target cancer cells ECA109 and TE-1[19]. In this research, the killing effect of EPHA2.CAR-T cells on ECA109 and TE-1 cells was better than that of T cells. Crystal violet staining and IDH release test further confirmed the results. These results indicated that EPHA 2.CAR-T cells also have a dose-dependent and specific killing effect on ESCC in vitro. Next, TNF-alpha and IFN-gamma which are produced by EPHA2.CAR-T cells was detected. The results showed that TNF-alpha and IFN-gamma increased significantly, which proved that CAR-T cells could play an important role in cell killing by increasing the release of cytokines[20]. However, the experiment is only supported by cell experiment, and needs further clinical experiments.

4. Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors which is higher in middle-aged males[21, 22]. Progressive enlargement of the liver, hard texture, irregular margins and nodular sensation on the surface are the main signs of hepatocellular carcinoma, often accompanied by pain in the liver area and digestive tract symptoms. Comprehensive treatment should be carried out according to different conditions, such as chemical drugs, radiation, immunity, freezing, perfusion and
so on. However, because of the early metastasis of hepatocellular carcinoma cells, the radical cure rate is not ideal[22].

High expression of alpha-fetoprotein (AFP) is a secretory glycoprotein that is usually overexpressed in endodermal tumors. AFP is an attractive candidate for T cell immunotherapy[23, 24]. The expression of AFP in tumors and serum was 60%-80% in HCC patients, which was associated with poor prognosis. Although the function of AFP is still unclear, it is reported that AFP can increase the proliferative capacity, inhibit cell apoptosis, and so on, suggesting that AFP may be important in disease progression.

However, AFP is expressed and secreted inside cells, so traditional CAR-T cells cannot find this target. In view of the fact that the peptides derived from intracellular/secretory proteins are treated and presented on the surface of cancer cells by class I MHC, the researchers designed a highly specific antibody against AFP/MHC complex and engineered it into CAR. Researchers have proved that it is feasible to target this peptide-MHC complex by engineering methods. In addition, they designed a specific peptide-MHC complex scFv to enter the second generation of CAR platform and avoided some shortcomings related to CAR. The second generation of CARS can effectively regulate T cell proliferation and persistence through its dual-signal receptor (CD28/CD3Z). Therefore, researchers have been able to selectively target intracellular and secreted antigens, while ensuring the effectiveness of CARS. It is noteworthy that the researchers found that the targeted T cells produced by the second generation of CAR constructed by antibody showed strong anti-tumor activity in the treatment of multiple preclinical models of AFP/HLA-A2:01 expressing human liver cancer. Therefore, by expanding the types of targeting antigens, including cancer-specific intracellular or secreted protein products, they have overcome the major obstacles to successful targeting of solid tumors through T-cell therapy[25].

So far, the clinical trials of CAR-T in the treatment of hepatocellular carcinoma have not been completed[25]. However, effective antineoplastic effects were shown in vitro and in vivo preclinical studies. Targets such as glypican-3, epithelial cell adhesion molecule and BRYONIC antigen are currently being studied. Researchers’ work shows that CAR-T cell therapy can be successfully applied to target secretion/intracellular antigen therapy for hepatocellular carcinoma. Importantly, this approach will be extended to other cancers.

5. Future Directions

CAR-T technology is a new immunotherapy, which can effectively treat leukemia and some solid tumors[26]. It can specifically recognize and bind cancer cells, and release cytokines, so as to achieve the effect of killing cancer cells. However, in the course of clinical trials, researchers have also found some drawbacks of CAR-T treatment.

5.1. Cytokine storm

Cytokine storm is one of the main adverse reactions in the clinical application of CAR-T technology[27]. The release of cytokines caused by the proliferation of T cells causes fever or myalgia, hypotension, respiratory failure and other symptoms. For the cytokine storm caused by CAR-T cell transfusion, the clinical use of IL-6 receptor antagonist tocilizumab can alleviate it. At the same time, some research groups also observed that cytokine storms were correlated with disease progression or tumor load, and cytokine release was higher in patients with high disease load. The clinical
manifestation is that the patient's super high fever does not subside, if not well controlled, there may be life-threatening[28].

5.2. Targeting toxicity

Targeting toxicity is another major problem in CAR-T technology[28]. Because of the strong targeting ability of CAR-T antigen, it is impossible to distinguish the cancer cells and normal cells expressing the corresponding antigen. Therefore, both cancer cells and normal cells expressing the corresponding antigen are aggressive. Therefore, CAR-T synthesized with appropriate tumor-specific antigen can differentiate normal cells from cancer cells is important (Table 1).

Table 1: Different target of different types of cancer

| Target | Type of cancer          |
|--------|-------------------------|
| HER2   | Gastric cancer          |
| EPHA2  | Esophageal cancer       |
| AFP    | Hepatocellular carcinoma|

5.3. obstacles in treatment

CAR-T cell therapy in the treatment of solid tumors has many obstacles include immunosuppressive microenvironment of tumors, permeation of CAR-T cells to tumors, lack of limited transport and specific tumor antigen[29, 30]. In addition, some strategies to control the side effects of CAR-T were developed. In theory, the best way to solve the "miss-target effect" is to select specific tumor targets. However, CART cells can only recognize antigen targets on the surface of cancer cells, so it is not easy to find targets that are specifically expressed on the membrane of cancer cells. Usually, the gene encoding CAR is transfected into T cell DNA through virus, so that it can be permanently expressed. However, considering that most of the available solid tumors are related antigen (TAA), permanent expression of CART cells may cause damage to normal tissues with the same TAA. Because the expression of some specific receptors decreases gradually during the growth of cancer cells, the development of a new kind of CAR-T cells that can recognize a variety of tumor-specific antigens has become a new research direction. This new type of CAR-T can ensure the persistent and effective killing of cancer cells, and can effectively reduce the damage to normal human tissues.

5.4. Side effect and strategy

CAR-T cell therapy has shown clinical effectiveness, but also some clinical side effects. How to control these adverse reactions is also a challenge for CAR-T cell therapy[31]. 1) Reduce the damage to normal tissues by introducing suicide gene system or improving CAR structure. 2) Optimizing cell culture and transfusion schemes to reduce the number of T cell transfusions or segmental transfusions to reduce the production of inflammatory cytokines. 3) Some immunosuppressive agents, such as IL-1, IL-6 antagonists and corticosteroids, were used to reduce the serious consequences of CARS. Therefore, close monitoring, timely detection and effective and strong medical intervention should be carried out during the treatment. Currently, under the correct and effective treatment conditions, cytokine release syndrome is completely reversible in clinic.

Based on the above considerations, one strategy is to improve the specificity of antigen
recognition of CAR-T cells. For example, the dual-targeting combination antigen method is to introduce two CARs into T cells, one is responsible for transmitting killing signals and the other is responsible for transmitting costimulatory signals. The dual-targeting strategy can not only avoid miss-target effect as much as possible, but also overcome immune escape. Another strategy is to fine-tune the affinity of CAR to reduce the damage to normal tissues. It is found that the maximum activation of T cells by CAR binding antigen does not depend on the affinity of antigen binding. Increasing the affinity of CAR antigen often accompanies the down-regulation of tumor antigen expression while enhancing the targeting of tumors. In addition, once the binding of CAR with antigen is too close, the effect of T is fine. Cells may not be able to bind to more target molecules, which limits their killing efficacy. Low affinity receptors can differentiate normal tissues from tumors by differentiating the high and low expression of target antigens.

There are many molecules that can be used as target antigens of CAR-T cells in theory, but only a few antigen molecules are feasible based on the distribution and function of these molecules in normal tissues. At present, most of the targets targeted by CAR-T cells in clinical application are not specific to cancer cells[32]. Because of the specific killing mechanism of CAR-T cells, it is particularly important to find tumor-specific antigens, which is the key to ensure the effectiveness and safety of CAR-T cells. On the one hand, all tumors have their own unique gene mutations. It is possible to find mutant targets that can be recognized by immune cells by gene sequencing or biological detection. On the other hand, there are few ideal tumor-specific antigens. For cancer-related antigens, we can analyze their different expressions in normal tissues and tumor tissues. To reach the level, evaluate the patient's condition and the related side effects of treatment and select the best target antigen[33].

6. Conclusion

The modern biomedicine develops rapidly, we realize that it is very important to maintain a healthy immune system. Under the control of the immune system, cancer cells of the body can exist harmlessly for a long time in vivo. The use of immune mechanism for cancer treatment can achieve efficient, specific and lasting purposes. Its economic and social benefits have great potential. As a green therapy, CAR-T has been used in medicine. It is generally believed that the main development direction of cancer treatment in the future is broad prospects for treatment. In cancer immunotherapy, CAR-T cells represent the advanced cancer immunotherapy technology, but still faces many challenges. In the future, the hope of cancer treatment must be based on the combination of immunotherapy. How to continuously improve the specific killing ability of CAR-T and ensure the safety of clinical treatment has become a hot research topic in this field. It is believed that CAR-T cells will be further optimized through the continuous efforts of researchers. We firmly believe that it is expected to change the status of clinical treatment of some cancers and benefit more cancer patients.

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