Car-t cells in the treatment of chronic lymphocytic leukemia

Abstract

Cancer has always been a difficult problem to be solved by humans, of which leukemia is one of them. With the development of gene recombination technology and our in-depth understanding of cancer, chimeric antigen receptor T cells (CAR-T) can be carried out in clinical trials. Recently, CAR-T has made new progress in the treatment of acute and chronic lymphocytic leukemia. CAR-T cells are T-cell receptor gene and anti-CD19 antibody gene binding, transfection to T cells, in vitro amplification after transfer to patients for the treatment of leukemia new immunotherapy. The surface of the modified CAR-T cells has a specific binding site, which can recognize the CD19 antigen on the surface of B cells in lymphoblastic leukemia. CD19 antigen can stimulate the continuous activation and proliferation of CAR-T, CAR-T in the patient's body can be multiplied hundreds of times, effectively killing acute and chronic lymphocytic leukemia cells.

Keywords: car-t cell therapy, chronic lymphocytic leukemia

1. CAR-T principle

With the development of gene recombination technology, the specific antibody can be stably expressed on the surface of T cells, so that it has a specific antigen epitope. Chimeric antigen receptor T cell immunotherapy is carried out on the basis of a cellular immunotherapy. The chimeric anti-gen receptor (CARs) is an antigen-recognition domain composed of a specific antibody in the extracellular domain and an antigen-chimeric protein composed of the intracellular CDC3-ζ chain or FcγI protein linked to the transmembrane domain [1] . After the CARs are recognized and stimulated by

specific antigens, they can provide activation signals for T cells and conduct the signals through the intracellular domain, which results in the activation of cells, which are CARs dependent cell activation and cytotoxicity, and cytokines Release [2] . In order to increase the cytotoxicity of CARs, the proliferation of signal transduction was achieved by constructing co-stimulatory molecules connected to the extracellular CD3Cζ in the intracellular domain, resulting in a multiplication of the cell killing effect, which greatly enhanced the CARs Cell killing effect [3] . Generation of CARs intracellular contains only one activation domain, so its specificity in the identification of tumor cell-associated antigens after killing effect is very limited [4] . Second-generation CARs contain an activation domain and a co-stimulatory domain, such as CD28 or 4-1BB [5-6] . The three-generation CARs are composed of the activation domain and multiple co-stimulatory domains, such as CD27, CD28, 4-1BB and OX40. The increase of these domains not only increases the ability of CAR-T cells to specifically recognize TAA and binding , More able to significantly extend the extracellular area of the cell signal transmission, causing lower levels of cell killing cascade [7] . With the improvement of structural design of CARs and the improvement of tumor targeting and killing, the researchers have constructed many other intracellular co-stimulatory molecular structures, including CD134, Lck, ICOS and DAP10 [8] . In addition, CD19-derived CAR-T cells were further engineered by researchers at the Duke University Center for Immunology to autocrine IL-12, which may or may not be required in specific syngeneic tumor models Pretreatment chemotherapy, if further extended to clinical patients can be in the lower side effects to obtain better efficacy [9] .

Therefore, the continuous innovation of CARs related technologies is not only the structural optimization, but also the construction of more costimulatory molecules in function. The efficiency and function of CAR-T cells will be further improved.

2. Obtain specific CAR-T cells

CD19 is a potential target for B-cell neoplasms and can be expressed in normal B cells, follicular dendritic cells, malignant B cells, and precursor B cells in addition to hematopoietic stem cells [10] . Acute and chronic lymphocytic leukemia is usually accompanied by CD19 expression, but in other lymphoid system tumor expression is not the same. Jena and others through genetic modification technology for the first time applied to chronic viral vector, the CD19 $^{+}$ specific chimeric antigen receptorand expression of B cells, transfected into the patient's T cells, the transformation of T cells called CD19-specific CAR -T cells [11] . These T cells in vitro after a large number of amplification, re-enter the acute and chronic lymphocytic leukemia patients play a role in the body. CD19-specific CAR-T cells are able to recognize leukemia specific CD19 targets and release B-cells from CD19 cells by releasing a variety of cytokines, thereby promoting the clearance of malignant tumor cells. The results show that, after retroviral gene transfection of T cells, in clinical applications is safe and effective [12] . CD19-specific CAR-T cells have been shown to be effective in attacking CD19 + tumor cell lines and in vivo B-cell tumors in animals [13] . In immunodeficient mice, CD19 + T cells can be effectively removed by the addition of CD19 + T cells [14] .

3. CAR-T cells in the treatment of chronic lymphocytic leukemia

Chronic lymphocytic leukemia (CLL) is a slow-growing, inert B-cell leukemia, usually occurring in adults, and many patients can have no symptoms for several years, compared with other types of leukemia. Currently CLL has no specific treatment options, no obvious symptoms of CLL advocates observation and wait, mainly symptomatic treatment, drug therapy is difficult to achieve long-term remission and clinical cure. Genetically modified CAR-T cells have a significant therapeutic effect on B-cell malignancies. The New England Journal reported in June that the June Task Force [15] successfully treated 3 patients with CLL with CAR-T cells for the first time, 2 of whom were still in complete remission after 2 years of follow-up. They subsequently found that [16] , the infusion of CAR-T cells in patients with peripheral blood and bone marrow in a large number of survival, proliferation in the body more than 1000 times, effective removal of CLL cells function can be maintained for more than 6 months. Not only that, some CAR-T cells are even in the form of memory cells that produce a rapid response when re-exposed to CLL cells. The mechanisms by which CAR-T cells proliferate and survive in vivo are unclear, probably due to the activation or release of cytokines by normal B cells and CD19-expressing leukemic cells in the internal environment. The cytokines such as IFN-r, CXCL9, IL-6 and soluble IL-2 receptor increased significantly after CAR-T cells entered the body, reaching a peak on the 23rd day after transfusion. Elevated cytokines in bone marrow were consistent with reduced levels of leukemic cells, but TNF levels in peripheral blood and bone marrow did not

vary significantly. The number of CAR-T cells in vivo was detected by RT-PCR, and the ratio of cells increased 1000-fold on the 21st day after transfection, accounting for more than 20% of the peripheral blood lymphocytes. The number of CAR-T cells was consistent with the time of oncolytic syndrome and elevated levels of cytokines. The doubling time of CAR-T cells in peripheral blood was about 1. 2 days and the half-life was 31 days. It is noteworthy that cytotoxic side effects such as cytokine release syndrome and macrophage activation syndrome may occur after treatment with CAR-T cell immunotherapy. These symptoms and children hemophagocytic syndrome, lymphoproliferative disease occurs in similar cytokine storm [17] . Cytokine storm is due to CAR-T cells kill B cells caused by tumor cell lysis, characterized by inflammation, long-term fever, hepatosplenomegaly, cell reduction. At this point the laboratory examination of patients with ferritin, triglyceride, transaminase, bilirubin, soluble IL-2 receptor a chain were increased and fibrinogen reduction [18] . CAR-T cells after the input, the patient’s peripheral blood and bone marrow loss of B cells and hypogammaglobulinaemia up to 6 months or more, but patients do not necessarily have recurrent infection. If the clinical symptoms require symptomatic treatment, the number of CAR-T cells in the patient’s body or the anti-tumor effect will not be significantly affected. In the past, patients treated with rituximab, after a few months of treatment, B cells can gradually pick up. Whether this phenomenon will occur in patients with CAR-T cell immunotherapy is still unclear. Because CAR-T cells can proliferate extensively and produce cytotoxicity in vivo, CAR-T cell-specific detection is

needed in the course of clinical treatment [19] , and timely prevention of adverse reactions occurs.

4. Adverse reactions and treatment

Although CAR-T cell therapy has achieved surprising clinical results, but have to admit that there are still many cell treatment process risk, adverse reactions after treatment for the treatment of a great test. Because CAR-T can proliferate in vivo and produce severe cytotoxic effects on target cells, the most common and serious is cytokine release syndrome (CRS) [20] . In the current report of CAR-T cells in the treatment of blood cancer cases, almost all appeared in different severity of the CRS response. CRS is mainly due to the large number of cells after activation, including circulating IL-6, ferritin, INFγ, IL-2, granulocyte colony stimulating factor IL-10, IL-8, IL-5, including rapid increase in cytokines Caused by fever without cause of infection, persistent hypotension, and even reports of neurological toxicity such as seizures [21-22] . CRS was observed in 48 of the 51 patients treated with CD19-modified CAR-T cells (94%) and serum ferritin levels were greater than 1000 mg / dl in all patients with severe CRS 4-5 C The relationship between the increase of CRP and the course of disease was similar to that of ferritin, but serum C – reactive protein and ferritin level did not have significant effect on the prediction of CRS progression. Further, they used 10 healthy volunteers as baseline and found that severe CRS responses within 1 month after CAR-T cell therapy were associated with 24 cytokines including IFNγ, IL-6, SGP-130, and SIL6R Serum levels of the peak correlation [23] . Patients with severe CRS response to the survival of patients posed a huge risk, patients with severe hypotension or shock, respiratory distress

syndrome, neurotoxicity, liver and kidney dysfunction, it must actively deal with the situation. In addition to the use of vasoactive drugs, tracheotomy and enhanced supportive therapy, the application of IL-6 receptor inhibitor tincture of monoclonal antibody is necessary by inhibiting IL-6 binding to cells and soluble IL-6 And block its classic and bypass IL-6 signal pathway, so after receiving the monoclonal antibody treatment, many patients quickly achieved a good clinical response [24] . CRS can be divided into 5 levels, different levels of recommended CRS treatment is different, it is generally recommended priority early adequate use of trastuzumab to prevent severe CRS response, but because IL-6 mAb is not easy through the blood-brain barrier, 3 to 4 neurotoxic patients, may be preferred to use glucocorticoids to prevent severe CRS. Therefore, IL-6 monoclonal antibody can be relatively early application, can be controlled for CRS and does not affect the efficacy of CAR-T

5. Summary and outlook

Nowadays, the clinical application of CAR-T cell therapy, especially the treatment of CD19 CAR-T in hematologic tumors, has made great achievements and the application potential of adoptive immunotherapy as the main representative of CAR-T cell immunotherapy Is very broad. Not only in the blood disease, but also in solid tumors and many benign or chronic diseases, it also has a lot of potential applications. With the development of gene recombination technology and antigen protein construction technology, more CAR-T has been applied to precise targeted therapy. In addition, epitopes expressed on the surface of different blood tumor cells, such as: CD20, CD22, CD34, etc. may also be used as future treatment of other

refractory blood diseases targeted to disease treatment to bring a new direction. One of the patients treated with CD19-modified CAR-T cells for MLL rearrangement of B-cell acute lymphoblastic leukemia showed that two patients developed AML after one month of treatment, which may be related to the CD19-negative cellular immune escape Of a mechanism-related [25] . Therefore, the application of CAR-T in combination with multiple epitopes and the sustained killing effect of CAR-T cells in vivo may bring new hope to these cases. There have also been advances in the use of checkpoint inhibitors in combination with CAR-T in the treatment of animals such as AML [26] . However, CAR-T therapy is still a significant clinical risk of adverse treatment, therefore, CAR-T therapy to avoid the safety and toxicity is also a clinical problem to be solved. At present, CAR-T cell therapy for specific antigenic epitopes is combined with nonspecific traditional therapy for tumor cells. It is also a safe and reasonable regimen for the treatment of these diseases, not only killing the tumor cells completely, reducing immune escape and ineffective CAR-T cell immunotherapy, in turn, reduces the dose of non-targeted therapies such as prior chemotherapy and the consequent toxic reactions. Therefore, in the process of discovering new target antigens and constructing precise immunotherapy, The combination of non-targeted drugs and hematopoietic stem cell transplantation is also the research direction of CAR-T cell therapy for a long time. Thus, CAR-T cell therapy has provided new hope for refractory hematologic malignancies, and although there is a pleasing therapeutic effect, more research and further clinical trials are needed, Multi-angle, combined with previous and recent targeted

therapy experience, will give CAR-T cell therapy to bring a broader application prospect and exciting clinical efficacy.

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