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Principle and Application of CAR-T Cell Therapy for Cancer

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Abstract. CAR-T therapy is a High-profile technology in cancer immunotherapy, which has developed rapidly and has made great achievements in the treatment of hematologic tumors. Its therapeutic principle makes CAR-T cell therapy a great advantage in the treatment of relapsed refractory chronic lymphocytic leukemia. However, CAR-T cell therapy still faces many problems, such as side effects, off-target effects, and the lack of specific antigen deficiency on the surface of solid tumors and so on. At present, it is an important research direction to reduce the impact of side effects on patients and its application in solid tumors. This paper focused on the principles and application of involved in CAR-T cell therapy, and the side effects brought by CAR-T therapy were sorted out. Finally, the further development of CAR-T therapy has prospected.

Keywords: the CAR-T therapy, cancer, chimeric antigen receptor, immunotherapy

1. Introduction

Cancer has long been considered a more serious disease. According to the statistics of the IARC [1]. Cancer is one of the most important reasons of human death. Therefore, the research on cancer treatment is important to mankind. At present, the treatment of cancer mainly includes surgery, chemotherapy, radiotherapy and immunotherapy.

CAR T cell therapy is a relatively advanced cancer treatment in recent years. Current studies have shown that CAR T cell therapy has a good effect on the treatment of lymphatic cancer, leukemia and other hematological malignancies [2,3]. CAR-T cell therapy is the use of genetic engineering technology to modify T lymphocytes, enable T lymphocytes to recognize cancer cells and eliminate the recognized cancer cells to achieve the effect of cancer treatment [4].

Although CAR-T cell therapy has achieved great results in hematological tumors so far, however, there are also many problems, such as it is difficult to use in solid tumors, side effects and expensive treatment.

This review focused on the principle and application of CAR-T cell therapy for cancer. In this paper, the principles, basic technologies and applications of CAR-T cell therapy in cancer treatment are reviewed by analyzing the research and achievements of CAR-T cell therapy in recent years. In addition, this paper analyze the application of CAR-T cell therapy in the treatment of tumors in combination with

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recent studies on solid tumors. The side effects brought by CAR-T therapy were sorted out, and the further development of CAR-T therapy was prospected at last.

2. Principles of CAR T cell therapy

In general, people fight disease through both nonspecific immunity and specific immunity. But faced with cancer, even specific immunity has had little effect. Through gene rearrangement and mutation during T-cell formation, T cells collectively produce thousands of unique receptors that recognize antigens. Subsequently, T cells undergo several screening mechanisms as they develop in the thymus. Among them, the mechanism of negative selection removes T cells that can react with their own auto-antigen proteins, resulting in mature T cells that do not recognize polypeptides derived from their own proteins. Therefore, in the immune response against infection, T cells quickly identify pathogens in vitro, while identifying cancer cells that likes healthy cells is more difficult. In addition, cancer cells will also reduce costimulatory molecules for the purpose of preventing T cell activation and even leading to T cell apoptosis [5].

Traditional antibody therapies to target targeted elimination of intact proteins expressed on cancer cells will also have an effect on the cancer cells. But this method does not kill cancer cells, and is very harmful to healthy cells and is less lethal than normal T cells. It will cause different degrees of allergic reaction, which requires a repeated course of treatment.

CAR-T cell therapy solved these problems. Ordinary T cells are not easy to identify cancer cells, so T cells can be exported, genetically engineered to transform them to specifically identify cancer cells, and provide a second signal to promote the activation of T cells, and then transmitted back to the human body. So far, studies have found at least three effector cell populations participating in this therapy, with specifically activated T lymphocytes, nonspecific responses to macrophages, NK cells. Based on the expression of the CD4 and CD8 genes, T lymphocytes can be divided into two distinct populations. Research suggests both can be induced in response to cancer cell-specific antigens. The main principle of CAR-T therapy is to activate T cells through genetic engineering technology, and identify the specific protein receptor on the surface of tumor cells, make T cells obtain specific anti-tumor ability, specifically identify tumor cells, At the same time, the immune function releases a large number of effectors, which can kill tumor cells and treat malignant tumors [6].

In the first step, select patients with relapsed or refractory DLBCL or ALL. The second step is a referral to the CAR-T treatment center. One week after observation, the third step, is the patient assessment review. After 2 weeks of observation, the cart therapy was evaluated and approved. In the fourth step, patient plasma separation and replacement were performed, mononuclear cells were isolated from the patient, and T cells were further purified by magnetic beads. The CART cells were modified while performing combination therapy with a local oncologist. The process lasts for 3-5 weeks. Step 5, lymphodigestive chemotherapy. In the sixth step, CAR T cells were amplified and CAR T cells were injected into humans via intravenous backtransfusion. The seventh step is to monitor the hospital and make emergency preparation for adverse reactions, about 1-2 months. Finally, the treatment effect was evaluated.

2.1. CAR (Chimeric antigen receptor)

The basic structure of the CAR includes a transmembrane region, a signaling region, and an antigen-binding region. The antigen-binding region is responsible for the specific recognition of the target protein, performing binding, often derived from the scFV segment of the antigen-binding region of the monoclonal antibodies. The signaling region is the intracellular immune receptor tyrosine activation motif.

The antibody are separately encoded by different genes. The antibody recognizes the antigen in two parts, a variable domain (Fv) on the light and heavy chains. These two construct a specific 3D shape, which can bind to antigens selectively. After binding to the target, parts of the underlying constant domain are subsequently cleared by innate immune cells.

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T cell receptors are very similar to antibodies and share the same genetic mechanisms, bringing about the same degree of diversity. Corresponding to the alpha-heavy chain of the antibody, the T cell receptors have alpha and beta chains and also contain constant (C) and variable (F) domains. Both antigen-binding sites are encoded by exons in region V, and by region V, they undergo many gene rearrangements to create fully functional cells with different specialties. The difference is that T cell receptors pass through a transmembrane region, immobilized on the T cell surface. TCR binding to antigen results in tyrosine phosphorylation. Cd3ζ contains tyrosine, an important protein in signal transduction. Phosphorylation triggers a cascade of interactions between the CD3ζ protein and other molecules, transmitting information to other parts. Under the right circumstances, it promotes T-cell activation and produces T-cell effects [7].

Therefore, from a structural point of view, replacing the Fv domain of the target protein antibody with the V region of the TcR can give this protein antibody specificity to T cells, while retaining the extracellular C region, transmembrane segment and cytoplasmic structural segment of the normal TcR, which can normally induce T cell proliferation, the production of effectors and target cell lysis. If this target protein is required for the cancer cells, then the modified CAR-T cells are able to specifically kill the cancer cells without the influence of the autoimmunity.

2.2. The currently selected target protein, CD19

The CD19 protein contains 556 amino acids, with a relative molecular mass of 61,088.36, and an isoelectric point of 4.87. The main amino acid types are leucine (11.3%), glycine (10.6%), and proline (10.3%). The total number of negatively charged residues was 73, and 49 were positively charged, and it was a basic protein with an extinction coefficient of 1.880 at 280nm. The N terminus of CD19 is methionine, with a half-life of 30h in mammalian reticulocytes, a half-life greater than 20h in yeast, and a half-life greater than 10h in E. coli, with a destabilization coefficient of 58.61, and is a labilizing protein in vitro. The total mean hydrophilic level was-0.585. CD19 is a type I transmembrane protein. Amino acids 1 – 19 of CD19 are the signal peptide, amino acids 20 – 291 are the extracellular domain, amino acids 292 – 313 are the transmembrane domain, and amino acids 314 – 556 are the intracellular domain. Among these, the extracellular segment contains two Ig-like domains of C2-type, located at positions 20 – 99 and 177-262, respectively, and is able to participate in the binding function. The intracellular domain is composed of 243 amino acids containing nine conserved tyrosine residues (Y) [8]. The tyrosine residue can be phosphorylated by the Src protein kinase, contributing to the coupling of CD19 proteins to other effector molecules in the signaling cascade, acting as signaling. This is why CD19 was selected as a target protein.

CD19 is usually found on the surface of immature B cells, and most cancerous B cells express CD19 protein, as this may be essential for their survival. In healthy individuals, CD19 transmits B cell receptor information to B cell cells to recognize an antigen; in cancer individuals, this process is dysregulated, and b cells deliver false information without recognizing the antigen, leading to inappropriate cell activation, survival, and growth. Without CD19, cancer cells are very difficult to survive. Since CD19 is not a protein unique to cancer cells, healthy b-cells are killed together while CAR-T works. But the lack of B cells is slightly less affected health relative to the lack of T cells, especially plasma cells. So CD19 was selected as a good target protein.

2.3. Cooperative activation mechanism of T cells

The mechanism of T cell activation is cooperative stimulation, referring to the need for two different sources for activation. The signal comes from the interaction of the MHC complex with the TCR complex. Another costimulatory signal is non-antigen specific costimulatory molecules expressed by antigen presenting cells that interact with T cells. Only the first signal does not induce an immune response in T cells, and lacking a second signal, T cells will enter an unreactive state, or undergo apoptosis [9].

The human T cell antigen CD28 provides a second signal. CD28 provides signals that produce interleukin 2 and other cytokines and chemokines during T cell activation and can act synergistically

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with T cell antigen receptor stimulation to activate T cell proliferation and secrete lymphokines. Moreover, it is involved in the regulation of T cell nonreactive state and T cell apoptosis. Multiple signaling cascades are independent of or dependent on activation of protein tyrosine kinases. In CAR-T cells, a costimulatory signaling receptor was incorporated into the CAR construct, addressing the need for cancer cells to emit a second signaling stimulus. Car-t cell modifications help provide a second signal that significantly improves efficacy and persistence [10]. Furthermore, the current study demonstrated that this costimulatory signal can block the induction of energy deficit in t-cell clones. There are currently many co-stimulatory molecules, except for CD28, 4-1BB (also called CD137) related to the current CAR-T cell technology.

2.4. Tumor escape from the immune response

Tumor cells become cancerous from their own normal cells and express proteins that are recognized by the immune system and induce an immune response. Yet tumor cells can still evade detection by the immune system. In animal experiments, it can be observed that the number of T cells increases significantly during tumor growth, but the tumor growth is not inhibited. The tumor cyst fluid creates an environment suitable for cancer cell proliferation that is not suitable for T and B cells, reducing the strength of the immune response. In addition, the cyst fluid contains immunosuppressive factors, growth factors, and so on. All this evidence suggests that tumors can evade immune responses by inhibiting lymphocyte factors while promoting their own growth.

3. Gene transduction techniques

There are many technologies used for CART gene transduction, including three more common viral vector gene transduction technologies (alpha retrovirus, gamma retrovirus, and lentivirus), transposon, and mRNA electroporation.

Retroviruses are ideal for gene transduction vectors for two reasons. First, most of the genome of the virus can be replaced by the desired transgene, and second, after successful transduction, the genome carried by the virus remain stable in the host cell genome for a long time. Because of these prominent advantages, one of the first retroviruses to be programmed as gene transduction vectors were gamma retroviruses.

3.1. Gamma-based retroviral vectors

In the process of gene transduction in CART cells, the Y-retroviral coding sequence was replaced with the CAR gene to generate the vector carrying the target gene. The recombinant genome still requires parts of the virus to infect host cells, so the Gag, pol and env genes, which encode capsid and envelope proteins, need to be left and expressed. The coding and regulatory sequences will be separated into different nucleic acid molecules, making their transfer more difficult and increasing safety. 5'LTR and 3'LTR are long terminal repeats of retroviruses, in which CD3 encodes intracellular information transduction structures, CD28 encodes transmembrane domains, 41BB encodes coactivation signaling molecules, and ScFv is the specific recognition part.

The specific CAR-T gene transduction process is that, when transfected into packaging cell lines, the vector plasmid allows the synthesis of many viral genome copies, and it is subsequently packaged into viral particles by structural proteins. The post-transduction events are very similar to those of true infection, and, after γ -retrovirus and activated T cell membrane fusion, the virion core is released into the cytosols and transported along the microtubules to the nucleus. Subsequently, the carrier integrates the carried information into the genetic information of the target T cells, and the T cells undergo the normal replication, transcription, and translation processes to produce the modified T cells with the specific recognition ability of the tumor antigen. But gamma retroviruses do not have an NLS (nuclear localization sequence), so they can only infect cells in a dividing state.

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3.2. Lentiviral vectors

The gene transduction process of lentivirus and retrovirus is similar, and also requires the transfer of REV gene, except Gag, PPL and env. The Rev protein binds to REE, a Rev response element, to amplify the expression and nuclear export of GAG-pol transcripts. A cis-acting element unique to lentiviral vectors is the central polyuria tract (cPPT)/central termination signal (CTS), which functions to facilitate nuclear import of the preintegrated complex upon infection. Thus, compared with gamma retroviruses, lentiviral vectors can infect dividing cells, expanding the target cell population.

The REV and Env packaging structures and vector plasmids carrying CAR structural genes were used for transfection of the packaging structures expressing GAG-pol. The lentivirus supernatant was purified by gel filtration, anion exchange chromatography and ultra-centrifugation. Lentiviruses are unable to transduce unactivated T cells and require stimulation to enter a phase of the Glb cell cycle. In addition, anti-CD28 and anti-CD3 antibodies were used to induce T cells out of the Go state and sensitize them to lentiviral transduction. The integration of transduction and genome results in a stable functioning CAR structure. Car-t cells, a lentiviral vector targeting CD19, have achieved remarkable results in clinical trials.

3.3. DNA transposon technology

DNA transposons are another form of stable gene transfer and exist independently of viral vectors. In nature, transposons are flanked by terminal inverted repeats (TIRs), which contain transposase binding sites. Transposonase binds to TIRs, cleaves transposons, and transfers them to a transposon-based vector system such as the SB system, which then uses the NE transposon as a way to introduce the target gene into the host genome. SB transposons are integrated completely into DNA, and they carry no risk of random mutations or gene rearrangements compared with retroviruses. Similar to the previous transduction modalities, stabilizing the genome implies efficient and stable expression of target genes.

Modified T cells successfully expressed anti-CD19-specific CAR. Transposon and transposase plasmid DNA will be electroporated into peripheral blood mononuclear cells for proliferation. Artificial antigen presenting cells (aAPCs) can produce sufficient CAR T cells for clinical use within one month after electroporation. The efficacy and safety of T cells edited by DNA transposon technology are still being tested in clinical trials.

3.4. mRNA electroporation

Different from the viral vectors mentioned above, gene expression mediated by mRNA transfer has gradually attracted the attention of researchers due to its higher safety characteristics. MRNA transfer is only based on the cytoplasmic level and does not need to enter the nucleus to play a role. Therefore, the system can transfect quiescent or proliferating cells without genome integration, and the mutation probability is much lower than that of viral vectors. Not only that, but the mRNA transposon technology is relatively simple and easy to manipulate.

The stability of mRNA itself is low, and the existence time is relatively short, which is easy to be degraded. This is the limitation of mRNA transfer strategy, because the expression of the encoded protein will be relatively short. In some cases, however, this disadvantage can favor CAR-T strategies. Some Cars cause serious side effects due to cross reaction with normal human tissues, resulting in safety threats. Transient CAR expression may reduce this risk, especially when tissue-specific OSS response CAR is unknown. Such a strategy could provide a faster regulatory pathway for newly designed mRNA electroporation, using transient CAR expression to evaluate the potential extra-temporal reactivity of novel CAR therapeutic products. The lack of neurotoxicity in these trials could be used to treat more persistent antigenic targets with CAR.

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4. Current problems with CAR T cell therapy

4.1. CAR-T therapy side effects

Although CAR-T cell therapy has many advantages over conventional therapies, there are still many risks involved in the treatment process, with adverse reactions following infusion posing a significant risk. The main side effects are tumour lysis syndrome, target toxicity, cytokine storm and neurotoxic syndrome. Cytokine storm (CAS), for example, is one of the more common and fatal side effects of CAR-T cell therapy for cancer patients [11]. after CAR-T cell infusion, T lymphocytes are activated and proliferate rapidly, from which high circulating levels of a variety of pro-inflammatory cytokines may be detected, including excessive cytokine release fromTNF-α, interferon-g and IL-6 release. These cytokines are also important indicators for detecting CAS. Monitoring and control of CAS is critical because CAS can trigger various immune responses in patients, such as fever, hypotension, dyspnoea and other adverse effects, and excessive immune responses can be life-threatening. However, treatment of CAS can lead to a diminished effect of CAR-T cell therapy, so a clinical analysis of the extent to which patients are affected is required prior to treatment.

4.2. Application of C A R-T therapy in solid tumors and the difficulties encountered

At present, a variety of solid tumor target antigens have been developed for CART cell therapy, and some clinical trials have demonstrated the efficacy and safety of this therapy in a variety of solid tumors. When comparing tumor samples before and after treatment, decreased antigen expression and increased presence of inhibitory immune checkpoint molecules and regulatory t-cell infiltration were found after treatment, indicating an escape immune response in tumors. Of all solid tumors, one studies has occurred in the use of the gd2-targeted cart for neuroblastoma treatment, with three out of the 11 treated patients achieving complete remission. However, in sharp contrast to the efficacy of hematological malignancies, the absence of CAR t-cell therapy has been shown to induce persistent, durable solid tumor regression in human patients [12].

For CART cells to clear solid tumor cells, they must be able to infiltrate, migrate, proliferate, and maintain the ability to consistently eliminate tumor cells. To date, however, cart cells to achieve these goals has been limited by the following properties of solid tumor cells.

Firstly, Selection of the antigenic targets. Solid tumors arise from the accumulation of different mutations, some of which lead to the generation of tumor-exclusive epitopes. However, tumorigenesis occurs through the evolution and adaptation of individual malignant cells. Thus, solid tumors consisted of subsets that were highly molecular heterogeneous, expressing a distinct and overlapping spectrum of unique TAAs. None of the current potential antigen repertoires for CAR development in solid tumors are optimal targets and lack homogeneous expression or tumor exclusivity. CART is designed to target antigens expressed by malignant cells to prevent damage to healthy tissue However, after CART treatment against an overexpressed autoantigen, such as CEA (solid tumor-associated antigen), unexpected destruction was observed.

The expression of tumor epitopes is not consistent between tissues, which makes it more complicated to identify feasible tumor-specific antigenic targets for CART immunotherapy [13]. There may be a subset of malignant cells that lack expression of a given target antigen and escape destruction by the corresponding CAR T cells. This, in addition to reducing the overall therapeutic effect, creates selective pressure for the accumulation of target antigen-expressing cells and leads to tumor recurrence [14].

Second, lymphocyte transport. Unlike cancer cells in the blood, solid tumors have difficulty migrating and invading. CART persistence and intratumoral accumulation following adoptive metastases are poorly characterized in both humans and mice [15]. Some studies have shown that organs such as the lung, spleen and liver do not have any preferential accumulation at tumor sites. CART immunotherapy for solid tumors becomes a time war, with adoptively transferred cells having only a short time to reach and destroy malignant cells.

Thirdly, the tumor-induced immunosuppression. The complex signaling network of malignant and non-malignant cells in a tumor is conducive to tumor persistence. Through the refinement of

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immunosuppressive cytokines, other mediators, recruitment of leukocytes, and activation of immune checkpoints, tumors are resistant to immune-mediated destruction in a microenvironment that inhibits effector cell activity.

5. Development direction

At present, cell therapy has become the most promising new hot spot for tumor immunotherapy in recent years, and has achieved better clinical results in the treatment of hematological tumors. Figure 1 shows the time trend of global patent applications for CAR-T therapy between 1998 and 2015, with the number of applications hovering in single digits at the beginning of 1998 until 2013, when the number of applications began to rise significantly, occupying 46% in 2015. proportion [16].

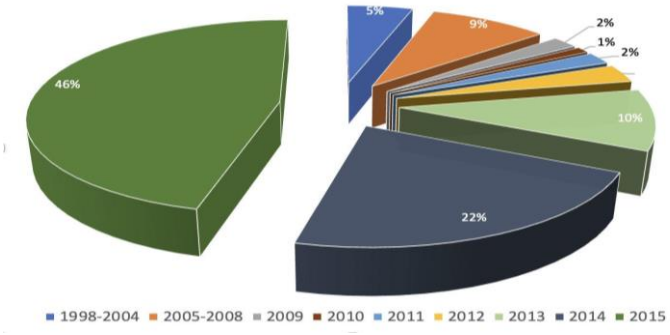

Figure1. Global patent filing time trend for car-t therapy.

In addition, the clinical trial results achieved by CAR-T therapies so far are also very impressive. It is documented that two CAR-T products (Kymriah and Yescarta) received marketing approval from the FDA in 2017, respectively. Table 1 shows the hematologic tumors involved in clinical studies and their major targets. It can be found that most of the current clinical treatments are mainly focused on hematologic tumors, while the most studied one is CD19, which accounts for 40% of the clinical programs. Therefore, based on this general trend environment, we have an outlook on its future development direction.

Table 1. Hematological tumors involved in clinical studies and their main targets.

Hematologic Tumors	Target name
B-cell malignant tumor	CD19、CD20、CD22、CD23、ROR1
Acute Myelogenous Leukemia	CD123、CD33、CD44、CD174
Hodgkin’s Lymphoma	CD30
Myeloma Multiforme	CD38、CD138、BCMA

First, the technical difficulties of CAR-T therapy should be overcome. CAR-T cell therapy targets mostly tumor-associated antigens, therefore, CAR-T identifies and kills tumor cells while damaging normal tissues with low expression of target antigens, producing off-target effects that can seriously endanger patients' lives. Therefore, it is crucial to optimize the off-target effects produced by CAR-T cell therapy [17]. For example, bispecific CAR, i.e., scFv targeting 2 different antigens are connected to activation signal and co-stimulation signal, the scFv recognizing the first antigen is connected to activation signal and the scFv recognizing the second antigen is connected to co-stimulation signal, CAR-T cells can only target tissues or cells expressing these 2 antigens at the same time to enhance their specificity and thus avoid off-target effect. In addition, there are methods such as introduction of

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suicide genes, exo-expression of antigens in tumor cells, and targeting of tumor-specific glycosylation sites [18].

Second, the future pharmaceutical costs of CAR-T therapies should be adjusted. In recent years, CAR-T cell therapy products have been marketed in China, and the price of the first domestic CAR-T product is as high as one million. How such expensive treatment costs can be borne by ordinary families is a hot spot of current concern.

Globally, the market cost of CAR-T products in the United States, Germany, the United Kingdom and other developed countries is as high as RMB 2 million, and several CAR-T products have been included in health insurance payments. Therefore, China can learn from other countries' health insurance payment ideas and explore new payment methods to reduce the price of CAR-T cell therapy products and further control the cost.

6. Conclusion

Currently, CAR-T therapy is an emerging technology for the treatment of haematological malignancies, which is developing rapidly and has achieved good results in clinical trials. However, CAR-T cell therapy still faces many problems, such as, how to reduce its side effects, how to apply it in solid tumours and how to reduce its medical costs. This article summarises the principles and basic techniques involved in CAR-T cell therapy in tumour treatment, collates the side effects brought about by CAR-T cell therapy, and provides an outlook on the further development of CAR-T therapy. It is believed that with the continuous improvement of CAR-T technology, the problems faced by CAR-T cell therapy will be solved and will bring gospel to tumour patients in the near future.

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