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Progress and optimization of CRISPR-Cas9/12 application in CAR-T therapy

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Abstract. CAR-T therapy and CRISPR/Cas9 gene editing technology have shown great potential in cancer treatment and gene editing, respectively. Combining these two technologies can significantly improve the effect of cancer treatment. However, CAR-T therapy has problems such as complex production, toxicity and functional failure, while CRISPR/Cas9 technology faces challenges such as off-target effects. This study first reviewed the basic principles of CAR-T therapy and CRISPR/Cas9 technology and their current application status, analyzed the feasibility of applying CRISPR/Cas9 technology to CAR-T therapy, including optimization strategies and examples, and focused on how to reduce off-target effects and enhance T cell function and persistence. The results showed that the application of CRISPR-Cas9/12 technology in CAR-T therapy significantly improved the accuracy of gene editing, therapeutic effects and the realization of personalized medicine. Nevertheless, these advances are also accompanied by ethical issues, especially in areas related to human genes. Therefore, future research needs to pay close attention to and address ethical issues while improving therapeutic effects.

Keywords: CAR-T therapy, CRISPR/Cas9, Cancer treatment.

1. Introduction

CAR-T therapy is currently one of the most popular cancer treatment methods, and its high recognition and therapeutic effect, as well as the characteristic of not requiring MHC recognition of tumor cells, make it highly competitive in cancer treatment. However, simply using Chimeric antigen receptors (CARs) expressed on the surface to assemble with engineered T cells, the resulting CAR-T cells do not possess additional characteristics, making them vulnerable to T cell exhaustion and difficulty in culturing during the tumor process. There have been some preliminary studies and attempts to combine the two, but there are few CRISPR/Cas processes specifically designed for CAR-T.

CRISPR/Cas9 gene editing technology is a widely used technique for specific DNA modification of targeted genes. Under the guidance of sgRNA, Cas9 protein can achieve targeted cleavage of target genes, causing double strand breaks (DSBs) in DNA, which can be repaired through cell autonomous non homologous end joining (NHEJ) or homologous recombination (HR), and target genes can be knocked out, inserted, and mutated. This technology is very suitable for assisting in the construction of CAR-T therapy systems [1].

The understanding of the basic principles of CAR-T and CRISPR/cas has been completed, and the clinical treatment and application based on these two technologies have also gone through many stages. Three main improvement points have been proposed in the application defects of CAR-T itself: the

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toxicity of CAR-T itself brings safety hazards and ethical issues, long-term exposure to the tumor environment will affect the function of CAR-T, and the production complexity of CAR-T cells is relatively high. CRISPR itself already has some issues, such as how to address off target effects in the process of combining the two.

At present, CAR-T therapy has not yet matured, and the CRISPR/Cas system is also constantly being updated. As a commonly used and precise means of cellular gene editing, combining the CRISPR/Cas system for editing and improving CAR-T cells not only facilitates the acquisition of a large number of finished cells, but also enhances the tolerance of CAR-T cells in the tumor environment, and even eliminates the need for repeated assembly of surface chimeric antigen receptors to some extent [2]. This greatly improves the applicability and treatment success rate of CAR-T therapy, and can also explore new applications of the CRISPR/Cas system in the field of cell therapy.

The study will first introduce the basic principles and application status of two biological methods, as well as the problems of their application in treatment. It will also briefly discuss the existing combinations and examples of the two methods. Analyze feasible optimization strategies for CAR-T using the CRISPR/CAS system, and attempt to address some existing issues, with a particular focus on addressing off target effects, increasing T cell production, and enhancing T cell function and persistence. Finally, the future prospects of the CAR-T therapy improvement system based on CRISPR/Cas are discussed, and some feasible therapies under development are discussed.

2. Current application status of CRISPR-Cas9/12 Technology in CAR-T Therapy

2.1. The role of gene editing in CAR-T therapy

There are several crucial steps in the construction of CAR-T systems. Firstly, it is the modification of T cells. As an important component of the immune system, T cells usually need to recognize MHC on the surface of tumors to confirm tumor cells. This process is not very efficient and lacks specificity. Researchers need to equip T cells with chimeric antigen receptors, which are navigation molecules designed based on the target tumor to enable T cells to recognize specific tumor antigens [3]. This process requires the modification of T cells through genetic engineering methods. During the stage where T cells are extensively cultured in vitro, specific tumor antigen receptor genes are embedded on the T cell membrane using genetic engineering methods, enabling them to specifically recognize antigens on the surface of tumor cells. This step can be considered as the most critical link in the entire CAR-T therapy [4]. Only T cells that undergo gene editing and possess additional chimeric antigen receptors can be used for subsequent therapeutic system design.

T cells usually achieve the effect of killing target cells by guiding apoptosis and secreting perforin, which also means that by modifying some genes of T cells to enhance these two processes, the overall killing efficiency of T cells is improved. In addition to the killing effect of T cells themselves, the general tumor environment can also become an important limiting factor. When CAR-T cells are exposed to the tumor environment to perform cancer cell clearance tasks, their various physiological activities will decrease due to the special nature of the tumor environment. At this time, strengthening the metabolic level and adaptability of T cells through gene editing can ensure their normal functioning [5]. That is to say, gene editing plays a very important role in many aspects of the construction of CAR-T systems. It not only assists in the construction of CAR-T cells, but also ensures the function of T cells in subsequent physiological activities, and can enhance therapeutic efficacy.

2.2. Specific application cases of CRISPR-Cas9/12 in CAR-T therapy

CD19 is an antigen primarily expressed on the surface of B cells and is a key biomarker for B cell development and maturation. Due to the expression of CD19 antigen in B-cell malignancies such as acute lymphoblastic leukemia and non Hodgkin lymphoma, chimeric antigen receptors targeting CD19 are combined with T cells to achieve targeted killing of mutated B cells.

In 2022, a patient with severe anti synthetase syndrome received CD19-CAR-T therapy, which is a complex autoimmune disease with evidence indicating the involvement of B cells and T cells. Rapid

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clinical improvement was observed after CAR T cell infusion targeting CD19. After eight months of treatment, the patient's overall evaluation by the doctor and scores in muscle and lung function tests improved, and there were no detectable signs of myositis on magnetic resonance imaging. At the same time, serum muscle enzymes (alanine aminotransferase, aspartate aminotransferase, creatine kinase, and lactate dehydrogenase), CD8+T cell subsets, and inflammatory cytokine secretion (interferon gamma, interleukin-1 [IL-1], IL-6, and IL-13) in peripheral blood monocytes were normalized. In addition, the levels of anti-Jo-1 antibodies decreased, and IgA (restored to 67% of normal values), IgG (restored to 87%), and IgM (restored to 58%) partially recovered [6]. That is to say, CD19 targeting CAR T cells can destroy pathological B cells. This also confirms the qualified specificity recognition of CD19 antigen as a T-cell guide.

2.3. Problems and challenges encountered in applications

It is not difficult to see that programming and modifying CAR-T therapy using the CRISPR system is a significant advancement in future cancer treatment. However, this treatment system still faces many technical and ethical issues. Firstly, there is a phenomenon of insufficient accuracy or incorrect cutting of the target gene using CRISPR. The CRISPR-Cas9/12 system may cause non-specific DNA cleavage, which can lead to loss of cellular function or other unpredictable side effects. In addition, how to effectively deliver the CRISPR-Cas9/12 system to T cells is also a technical challenge.

Secondly, ethical issues also need to be considered. The use of CRISPR-Cas9/12 technology for gene editing may trigger some moral and ethical controversies. For example, in situations where the functions of many gene fragments are not yet clear. In addition, gene editing may lead to social inequality. As an efficient means of curing cancer, if technological barriers are formed due to high costs, this treatment method that effectively prolongs the lifespan of the human population will only be affordable for a small number of people. As a new technology, ethical issues still cannot be ignored.

3. Optimization strategies for CRISPR-Cas9/12 technology

3.1. Methods to improve editing efficiency

The CRISPR system has not been discovered and applied in the field of genetic engineering for too long, which also means that this technology still has many shortcomings that can be improved. The problem that has been troubling researchers is the insufficient conversion efficiency and poor accuracy of CRISPR.

The first step is to combine the genetic background of the host cell, understand and select the host cell type suitable for CRISPR Cas editing, and select the appropriate Cas protein based on the characteristics of the target gene to improve editing efficiency. And by controlling the expression level of Cas9/12 to conform to the corresponding cellular environment, the editing efficiency can be optimized. Overexpression may lead to cytotoxicity, while moderate expression helps maintain cell health and improve editing efficiency. Using homologs of Cas: Cas9 and Cas12 in the CRISPR system have different characteristics, such as different PAM sequence requirements and cleavage properties. Secondly, there are process improvements in the system, such as post modification strategies: by using small molecule compounds or post translation modifications, the activity of Cas can be regulated, thereby optimizing the efficiency and success rate of editing. The delivery process of the CRISPR system also greatly affects overall efficiency. Improving the methods of delivering CRISPR systems to cells, such as using more efficient viral vectors or non viral delivery methods, can enhance editing efficiency [7].

3.2. Strategies for reducing off target effects

In addition, the system has been plagued by a phenomenon called off target effects. Off target effect refers to the phenomenon of Cas9 protein cutting DNA at non target sites. This non-specific cleavage may lead to unwanted gene mutations, resulting in a series of side effects. This not only seriously affects the editing efficiency of the target DNA, but also generates unpredictable mutations, leading to severe

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toxicity. In the face of this problem, improving the specific binding ability of Cas variants or selecting Cas9 variants with lower non-specific binding ability, such as eSpCas9, SpCas9-HF1, etc., can enhance the accuracy of editing. [8]. It is also possible to improve accuracy by selectively editing and evolving existing CAS systems to enhance their specificity for specific targets. In addition, improvements to sgRNA will significantly increase specificity. sgRNA is a key component in this system that can guide Cas9 protein to recognize and cleave specific DNA sequences. SgRNA is a short synthetic RNA, typically only 20 bp in size, but it can bind to the Cas9 protein to form Cas9 sgRNA complexes. This complex binds highly specifically to the target DNA through the spacer sequence of sgRNA, triggering the endonuclease activity of Cas9 and causing DSBs in the target DNA. At present, by integrating large-scale empirical data, researchers can improve the computational design rules and create optimized sgRNA libraries to select different sgRNAs for different design purposes, maximizing targeting activity and minimizing off target effects [9,10].

Currently, there have been several successful cases of CRISPR based modification of CAR-T, such as the work published by Doench et al. in 2016. Researchers used the CRISPR-Cas9 system to create universal CAR T cells resistant to PD-1 inhibition through multiple gene disruption of endogenous T cell receptors (TRAC), beta-2 microglobulin (B2M), and PD-1 (PDCD1). Three gene edited CAR T cells exhibit enhanced activity in preclinical glioma models. Mice carrying intracranial tumors had an extended survival time after intracerebral administration, but not after intravenous injection. CRISPR-Cas9 gene editing not only provides a potential source of allogeneic universal donor cells, but also can simultaneously disrupt checkpoint signals, otherwise hindering maximum anti-tumor function [11].

4. Future prospects of CRISPR-Cas9/12 technology in CAR-T therapy

With the development of medical technology, the independent physiological characteristics exhibited by each patient are becoming increasingly important, making personalized medicine even more crucial. Personalized medicine is rooted in the belief that individuals may need to provide interventions for their illnesses to adapt to these subtle differences and unique characteristics at the molecular, physiological, environmental exposure, and behavioral levels. As a highly programmable and compatible treatment system, CAR-T itself can also be designed to meet the needs of personalized medicine. As discussed earlier, different tumor environments have a significant impact on the killing effect and function of T cells, and everyone has their own unique internal environment system, even the tumor environment system is completely different. And as a treatment method that relies on specific recognition, the off-target tolerance of different individuals varies, and the targeted binding effect of different sgRNAs in vivo is also different. If uniform CAR-T cell design and assembly are still adopted based solely on tumor type in this situation, it may not only make it difficult to achieve the expected therapeutic effect, but also pose additional risks. At this point, CRISPR technology, as a relatively mature targeted programming technique, can effectively select appropriate recognition sites based on the patient's physiological conditions to reduce off target effects and improve the efficiency of specific recognition. It is also possible to design T cells that are more suitable for a specific individual's tumor environment, making CAR-T cell activity more persistent and achieving better therapeutic effects. If a comprehensive database is established based on this regarding different types of physiological or tumor characteristics, recording the reactions of CAR-T cells edited to varying degrees, it will be more conducive to personalized treatment for patients, maximizing treatment effectiveness and safety [12].

As mentioned earlier, the current progress of CRISPR technology in CAR-T therapy mainly focuses on the design of CAR-T and the enhancement of T cells. In addition to these two points, the combination of CAR-T with other drugs or small molecules can also greatly enhance its therapeutic effect, which means that CRISPR can also play an important role in antigen presentation and search stages. In addition, there are currently not many databases and validated specific targets, and the design paths available for CAR-T therapy are also relatively rare, often with poor recognition effects [13]. This means that CRISPR can enhance its binding efficiency by editing and correcting antibodies to existing targets, or by designing relay molecules to link T cells with cancer cell targets, thereby achieving the effect of expanding the number of targets.

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5. Conclusion

The application of CRISPR-Cas9/12 technology in CAR-T therapy has made significant progress, reflected in the precision of gene editing, improved treatment efficacy, reduced side effects, increased personalized treatment, and expanded treatment scope. These advances not only enhance the targeting and effectiveness of treatment, promote the development of personalized medicine, but also increase the safety of treatment. Make CAR-T therapy more flexible and safe. Looking ahead, the research and application of CRISPR-Cas9/12 technology in CAR-T therapy will be dedicated to further optimizing editing efficiency and accuracy, exploring new therapeutic targets, addressing immune escape issues, simplifying production processes and reducing costs, as well as conducting more clinical trials. These efforts indicate that CRISPR-Cas9/12 technology will not only enhance cancer treatment efficacy, but also open up new paths for future medical treatments, bringing revolutionary changes. However, as mentioned at the beginning of the article, the ethical issues brought about by the combination of two technologies have still not been widely recognized by the scientific community. Researchers tend to view this artificial product therapy that has undergone special gene editing with a more optimistic and conservative attitude towards traditional targeted therapies. Anyway, the potential dangers behind it and the ethical implications related to human genetic ethics still deserve the attention and discussion.

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