CRISPR/Cas9 Application in CAR-T Cell Based Tumor Therapies

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Abstract. The CRISPR/Cas9 system is an essential tool for treating cancer. In this paper, CRISPR/Cas9 homogenic orientation repair (HDR), NHEJ (NHEJ) and the DNA repair mechanism are discussed in this paper. Furthermore, CRISPR/Cas9 has been applied to CAR-T cells genetically modified, focusing on improvements in expression, survival, and the manner in which CAR-T cells operate, as well as their success against cancer cells. This article, we review CAR-T therapeutic approaches and their challenges in both solid and hematological malignancies. Utilizing CRISPR/Cas9 can address this issue by optimizing CAR structure, enhancing cell survival and enhancing CAR-T targeting ability. Problems related to cytotoxicity, targeting specificity, and safety remain. In this article, we suggest the optimization of CRISPR/Cas9 transmission, selection of appropriate target genes, and implementation of security surveillance and assessment. To conclude, CRISPR/Cas9 therapy may enhance efficacy and offer promising prospects for CAR-T carcinoma. But more research and experience is needed to make sure that it is safe and effective.

Keywords: CRISPR/Cas9 system, CAR-T cell, tumor therapies.

1. Introduction

Now, cancer has been a major public health problem, which is a threat to human health. As a novel and highly effective approach in cancer treatment, immunotherapy has been widely concerned. Being a new technology, CAR T-cell treatment has achieved significant clinic results for various types of carcinoma. While CAR-T is potentially useful in the treatment of cancer, there are still many challenges and limitations. CRISPR/Cas9 is a novel approach to treating CAR-T cells. Malignant blood and solid tumors are one of the most frequent and fatal forms of cancer globally. According to the latest epidemiologic data, hematological malignancies account for about 10% of all cancers, with leukaemia and lymphomas the most frequent. Solid tumors comprise most of the other types of cancers, including breast, lung, and colon. At present, there are major therapies, such as surgical, radiation, and chemotherapy, but they usually come with side effects and limitations.

CRISPR is a new way to modify genetic material. The technology was first identified in 2012, and it has been improved and improved in the coming years. It depends on a natural CRISPR-Cas9 that is found in the bacterium and the archaea. Through the combination of Cas9 and RNA leading, it is possible to accurately recognize and slice a specific gene in DNA. Superiority of CRISPR/Cas9 DNA for CAR-T Gene Editing Technology Compared with ZFN (Zinc Finger Nuclease) and TALEN (Zinc Finger Nuclear Nuclease), the 3rd Gen CRISPR/Cas9 has higher efficiency in editing, experimental convenience and low cost. The CRISPR/Cas9 system can effectively modify and mutate on a large scale. The CRISPR/Cas9 system can be used to precisely control the level of immune receptor, interfering gene, and switching gene in CAR-T cells, thus improving CAR-T's function, increasing its effectiveness, and reducing side effects.

The purpose of this research is to investigate whether CRISPR/Cas9 can be applied to the CAR T-cells in order to enhance the efficacy of CAR T-cells. Through modification of DNA in tumor cells, CAR-T cells can be enhanced to identify and attack tumor cells. Using CRISPR/Cas9 to treat CAR T-cells may represent a significant step towards more effective, individualized therapy.

2. CRISPR/Cas9 system

The CRISPR/Cas9 system consists of CRISPR sequences and Cas9 enzymes. A CRISPR sequence is a region of DNA that contains a series of short palindromic repeats and spacers. Cas9 is an RNA-directed DNA enzyme with the function of cutting double-stranded DNA. When Cas9 cuts DNA, the CRISPR sequence directs Cas9 to interact with the destination DNA sequence, allowing Cas9 to precisely cut a specific DNA sequence.

2.1. Types

The CRISPR/Cas9 is divided into two types: Category 1 can be subdivided into Types I, III, and IV, while Category 2 can be subdivided into II, V. and VI, as shown in Figure 1.

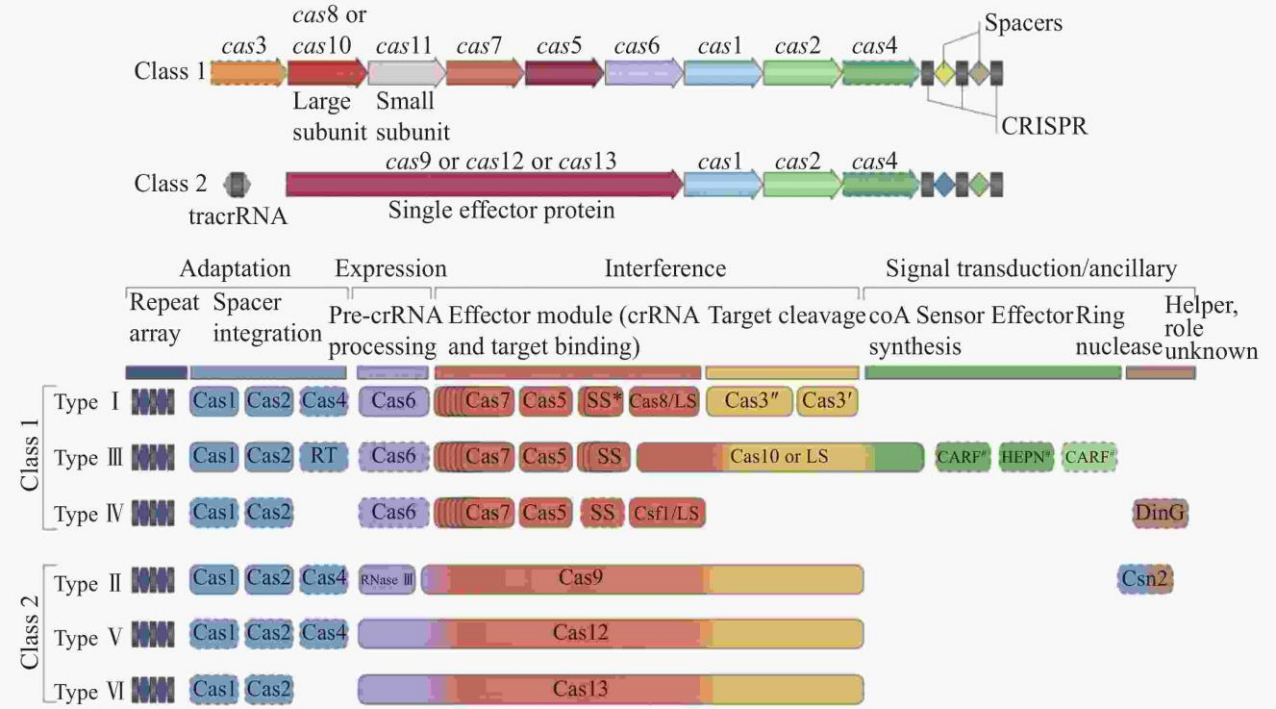


Fig 1. Classification of CRISPR/Cas9 systems [1].

Type I includes types I-A to I-F and I-U. The common feature of these subclasses is that crRNA production is catalyzed by enzymes encoded by Cas6. In type I systems, the interference mechanism involves the involvement of multiple proteins, such as Cas7, Cas5, Cas8, and Cas3 [2]. The adaptation mechanism involves proteins such as Cas1, Cas2 and Cas4 [2]. Types III include types III-A-D. CrRNA production in Type III systems is also done by enzymes encoded by Cas6. The interference mechanism involves the participation of proteins such as Cas7, Cas5 and Cas10 [3]. The adaptation mechanism involves proteins such as Cas1 and Cas2 [2]. Interference mechanisms in type IV systems involve proteins such as Cas7, Cas5, and Csf1 [3].

Type II includes types II-A through II-C. In contrast to Class 1 types, crRNA production in type II systems is performed by enzymes encoded by Rnase III. The interference mechanism is mainly dependent on the role of Cas9 protein [3]. The adaptation mechanism involves the participation of proteins such as Cas1, Cas2 and Cas4 [2].Type V Includes V-A, V-B, and V-U. In type V systems, the interference mechanism involves the role of the Cpf2 protein [3]. Similar to other classifications, the adaptation mechanism still involves proteins such as Cas1, Cas2, and Cas4 [2].Type VI includes types VI-A to VI-C. The interference mechanism of the type VI system is provided by the C2c2 protein [3], while the adaptation mechanism involves proteins such as Cas1 and Cas2 [2]. Detailed knowledge of the CRISPR/Cas9 classification and subtaxa is important, as different classifications and sub-taxa differ from one another in terms of interference mechanisms and adaptation mechanisms. This makes the CRISPR/Cas9 system more selective and potentially useful for gene editing.

2.2. DNA repairing mechanism

CRISPR/Cas9 can initiate DNA repair by cutting off DNA's double strand breakage. The major mechanisms of DNA repair are HDR, nonhomologous end joining (NHEJ), and other repair mechanisms.

2.2.1. Homologous directed repair (HDR)

Homologous directed repair is a mechanism of gene editing by utilizing exogenous repair templates. This repair pathway is usually applied to the repair of site-specific mutations such as point mutations, insertions or deletions. During this process, Cas9 protein-induced DNA breaks activate the natural repair mechanism within the cell, while the exogenous repair template provides information on the reconstruction of the target sequence. The repair process mainly includes the following steps: The Cas9 protein binds to the sgRNA, causing DNA break by binding to the target DNA [4]. Cellular nucleases and DNA deoxynucleotidases induce DNA cleavage [5].The exogenous repair template is homologous paired with the broken end of DNA to form a repair fragment [6]. DNA polymerases and DNA ligases mediate the synthesis and joining of new DNA strands [7]. The properly repaired DNA strand replaces the original sequence [4].

The main advantages of homologous oriented repair mechanisms are their high accuracy and low deviation. However, success rates are generally low and unpredictable mutations are prone to occur during DNA repair.

2.2.2. Nonhomologous end junction (NHEJ)

The non-homologous end junction is the primary mechanism for repairing the CRISPR/Cas9-induced DNA breakage, which may be independent of the external repair template. NHEJ is widely used in gene knockout and lost-of-function studies that do not require a specific repair template. The repair process mainly includes the following steps: The Cas9 is attached to sgRNA, which then binds to DNA, causing DNA to break down. Then, the end of the DNA is modified with endonuclease to create unpaired single stranded DNA. The single strand of DNA ends is pinned and attached by DNA ligase and external repair sequences [3-5].

The NHEJ mechanism has the advantage of being efficient and flexible and can lead to a variety of different types of mutations. However, the disadvantage is that the types of mutations produced are unpredictable, resulting in genomic instability.

2.2.3. Other repair mechanisms

In addition to HDR and NHEJ, the CRISPR/Cas9 system may be involved in several other DNA repair mechanisms. The two major mechanisms are MMEJ and single strand DNA repair. Microhomologous repair: In MMEJ repair, after the DNA guided by the Cas9 protein is broken, the appropriate microhomologous sequence is involved in the connection and repair of the broken end. Single-strand DNA repair: In the process of single-strand DNA repair, after the DNA guided by the Cas9 protein is broken, the homologous sequence at the end of the DNA break is paired with the single strand of foreign repaired DNA, and then DNA synthesis and connection are carried out through DNA polymerase activity. Other possible repair mechanisms include gene conversion, replication-mediated repair, etc.

3. The application of CRISPR/Cas9 gene editing in CAR-T cells

The CRISPR/Cas9 system is also used in CAR-T therapy to enhance the efficacy and security of CAR-T cells.

3.1. Gene editing of first-generation CAR-T cells

The CRISPR/Cas9 system has been shown to enable CAR to be efficiently inserted into T cells and thus promote the production of first-generation CAR T-cells [8]. Mutations or changes may increase the potency of first generation CAR T-cells and their anticancer activity [9]. Using

CRISPR/Cas9 to suppress or modify CD28 may enhance the persistence and anti-tumor activity of first generation CAR T-cells.

3.2. Gene editing of second-generation CAR-T cells

The CRISPR/Cas9 system may be used to induce or modify costimulatory signaling molecules in order to optimize second generation CAR-T cells' capacity and activity [10]. Second-generation CAR T-cells exhibit anti-tumour activity through selective suppression of immunosuppressive agents [11]. Targeting PD-1 CRISPR/Cas9 knockout on second generation CAR T-cells may increase treatment effectiveness and length of treatment [15]. When CRISPR/Cas9 is applied to a particular location, the ability of CAR T-cells to recognize and destroy them may be increased [16].

3.3. Gene editing of third-generation CAR-T cells

The CRISPR/Cas9 system may be utilized to induce or insert a co-stimulator to enhance the efficacy and long life of third generation CAR-T cells [12]. Regulating CAR-T cells can increase the safety and effectiveness of CAR-T (CAR-T) third generation CAR-T cells [13]. The CRISPR/Cas9 system may be utilized to inhibit or modify a third generation co-receptor or co-stimulatory signaling molecule, thereby increasing the anticancer activity of CAR-T and its long term survival [17]. The CRISPR/Cas9 pathway is essential for the treatment of CAR T-cells. The CRISPR/Cas9 system can be used to enhance CAR-T cells by means of knockdown or modification. Though CRISPR/Cas9 has achieved great progress in the modification of CAR-T cells, it remains to be studied. The CRISPR/Cas9 system may become more effective in the treatment of CAR T-cells with technological progress.

4. The clinic application of therapeutic CAR-T cells

4.1. Hematologic malignancies

Because of the advances in immunological treatment, CAR-T cell treatment has become an important means of treatment. Recently, more and more studies have been done to treat malignant hematologic tumors with CAR-T cells. CAR-T is mainly used to treat haematological malignancies, including Acute Lymphoblastic Leukaemia (ALL), Chronic Lymphoblastic Leukaemia (CLL), NHL (NHL) and multiple myeloma (MM).

CAR-T Cell Therapy uses CAR (CAR) technology, which is implanted in a patient's T-cells in order for them to recognize and attack them. The CAR-T treatment is based on CAR, T cell procurement, gene conversion, cell proliferation and cell migration.

This applies to NHL (ALL) or to Acute Lymphoblastic Leukaemia. It has been demonstrated that CAR T-cells are effective in treating this kind of cancer. Therefore, it is demonstrated that CAR T-cell specific CD19 antigen is highly efficacious in treating recurrent or persistent B-LYMPHOMAS and ALL (NSCLC). Research has demonstrated that CAR-T therapy significantly improves overall PFS in B-cell lymphoma patients and may be an alternative to conventional therapy [18].

Chronic lymphoblastic leukaemia is a common form of leukaemia in adults. Therefore, therapy with CAR-T is likely to be a promising method to treat CLL. The CAR T-cell CD19 antigen has demonstrated efficacy in the treatment of CLL with both recurrence and resistance. While effectiveness differs from person to person, the overall treatment effect remains significant [19]. The CLL study also demonstrated that treatment with CAR-T could be effective in refractory patients [20].

4.2. Solid tumors

Solid tumour is a frequent malignancy. Conventional surgery, radiotherapy and chemotherapy have met with a bottleneck. Recently, CAR-T cell treatment has made significant advances as an innovative method of immunotherapies.

Malignant melanomas are very aggressive and very hard to treat. Studies have demonstrated that the treatment of malignant melanomas with multiple antigens (for example, GD2 and CS1) may result in significant improvements in the survival and treatment of patients [21]. The other trial also showed that CAR-T was well tolerated and effective in treating recurrent or refractory melanomas [23].

Lung cancer is one of the biggest cancers in the world. The CAR-T therapy is a promising method for treating lung cancer. Treatment with EGFR (EGFR) and Human Epidermal Growth Factor Receptor 2 (EGFR) has demonstrated the possibility of development of new treatments for the treatment of pulmonary cancer [24]. Furthermore, treatment with CAR-T has been demonstrated to have protective effects on certain B7-H2 subtypes associated with lung cancer in certain patients [26].

Pancreas cancer is a high degree of malignancy, and its operation is restricted. CAR-T cells are widely used in pancreatic cancer therapy. In one study, treatment with CAR-T (MUC1) MUC16 (MHC 16) demonstrated high efficacy and improved DISEASE-FREE survival in patients with pancreatic cancer with surgery [27]. The other study also demonstrated that treatment with HER2 T-cells may be effective in treating pancreas cancer [28].

Although CAR-T has demonstrated great potential in treating solid tumors, there are still many problems and side-effects associated with it. The most common side effects are Cytokine Release Syndrome (CRS) and neurotoxicity [29]. CRS results from the release of a variety of cytokines following activation of CAR T-cells. Neurotoxic symptoms include unconsciousness, seizures, and loss of speech, which can negatively affect the survival and the quality of life. In addition, the persistence of response times and CAR-T therapy remains an issue.

Being a new immune system, CAR-T cells are widely used in the treatment of solid carcinoma. In spite of these problems and side-effects, we are hopeful that CAR-T will become an effective tool in treating solid tumors.

5. Overcoming strong CAR-T cell therapy with CRISPR/Cas9 potential

5.1. CRISPR/Cas9 therapy by CAR-T

Cancer is one of the biggest medical issues in the world, and traditional treatments are limited by the need to develop new treatments. In recent years, CRISPR/Cas9 and CAR-T have been proposed as novel approaches for the treatment of cancer. The CRISPR/Cas9 method allows precise genetic modification, while CAR-T therapy improves the immunity of the patient against cancer. Combining both technologies may result in a greater improvement in therapeutic results.

5.1.1. Optimizing the CAR structure

The CRISPR/Cas9 technology is suitable for optimizing the structural and functional characteristics of cars. With CRISPR/Cas9 technique, it is possible to accurately modify the CAR antigen recognition domain to increase the affinity and specificity of the target antigen [36]. Furthermore, CRISPR/Cas9 technology may also be used to modulate CAR signaling domains and thereby enhance their anti-tumor activity.

5.1.2. Enhancement of CAR-T cells survival and functional enhancement

The CRISPR/Cas9 assay makes it possible to target CAR T-cells in order to enhance CAR T-cells' viability and performance. Removing PD-1 (PD-1) or PD-L1 (PD-1) may decrease the loss and inhibition of CAR T-cells [37]. Furthermore, CRISPR/Cas9 can also enhance the anti-inflammation and survival factor of CAR-T cells, thereby improving CAR-T resistance and effectiveness.

5.1.3. Enhancement of CAR-T cell offensive ability by modification of cancer cells

The CRISPR/Cas9 technique might enhance the CAR T-cells' offensive capability. The Major Histocompatibility Complex I (Major Histocompatibility Complex I) may, for example, decrease the capacity of an immune response to enhance CAR-T's recognition and destruction capabilities.

In addition, CRISPR/Cas9 can be used to modify the metabolic or antigenic pathway of tumor cells, thereby increasing the CAR T-cells' offensive capability.

5.2. Challenges of CAR-T cell therapy

Recent research has demonstrated the effectiveness of CAR-T cells in the treatment of cancer. Nevertheless, there are a number of outstanding issues which may restrict widespread use of CAR-T cells.

In particular, the activation of CAR T cells may result in a serious inflammatory reaction following CAR T-cell treatment known as CAR T-cell-associated encephalopathy (CRES) and Cytokine Release Syndrome (CRS). These adverse reactions can be life-threatening. Secondly, CAR-T cells' survival is often constrained by many factors, including cellular exhaustion, immunity intrusion and programmed cell death [31]. Thirdly, different mechanisms are often employed by cancer cells to prevent CAR T-cells from being attacked [32]. In addition, it is possible that the tumor cells can protect themselves from CAR-T cells by regulating their signaling channels. Meanwhile, although CAR T-cells have demonstrated an outstanding early reaction to certain cancers, a number of them remain recurrent. It is possible that this is associated with progressive decrease in the number of CAR-T cells, the lack of immunity and microbial activity [33]. Furthermore, CAR-T cells are produced in a lot of ways such as collecting, modifying, expanding and reverse-injecting. Furthermore, there are many technological issues associated with producing CAR-T cells, for example, highly efficient gene transfer, selective CAR synthesis, and optimal cell cultivation. Therefore, the reduction of the CAR T-cell therapy cost and the related technical issues are of great importance in wide application.

5.3. The potential challenge of CAR-T cells using CRISPR/Cas9

5.3.1. Cytotoxicity and cell survivability

CRISPR/Cas9 technology requires gene editing through transfection of exogenous proteins and RNA, which may adversely affect the health and survival of cells [34]. Especially in CAR-T cells, cytotoxicity and cell survival are very important factors. Therefore, when applying CRISPR/Cas9 technology, it is necessary to consider how to minimize adverse effects on cells and guarantee stable and durable expression of CAR T cells.

5.3.2. Targeting efficiency and specificity

Although CRISPR/Cas9 technology has shown high efficacy and specificity in CAR T-cells, a number of problems remain. The CRISPR/Cas9 targeting activity is influenced by many factors, such as RNA design, CRISPR/Cas9 transfer efficiency. In addition, CRISPR/Cas9 may lead to non-specific genetic changes that may lead to adverse cell outcomes. The CRISPR/Cas9 method needs to be optimized to ensure that it is effective against CAR-T cells.

5.3.3. Security and unpredictability

The CRISPR/Cas9 assay may result in unintended alterations in the course of DNA modification, unwanted mutations and chromosomal rearrangements [35]. The main problem is that CAR-T cells are safe. Random genetic alterations can lead to undesirable biological effects, such as cancer and immunity. Consequently, if CRISPR/Cas9 is used for modification of CAR T-cells, close monitoring and evaluation of CAR T-cells must be performed in order to ensure that treatment is safe and effective.

5.4. Treatment of CAR-T cells by CRISPR/Cas9

5.4.1. Optimizing CRISPR/Cas9 system delivery

Enhancing CRISPR/Cas9 transfer rate will be one of the key problems of CAR-T. So we suggest CRISPR/Cas9 can be applied to CAR-T cells through selection of novel carrier, optimization of transfer parameters and CRISPR/Cas9.

5.4.2. Selection of appropriate target genes

Selecting suitable targeted genes plays a key role in guaranteeing the specificity of DNA editing. Through the selection of CAR-T cell specific markers, detailed guide RNA design, and multiple

targeting strategies, the specificity of CRISPR/Cas9 technology can be improved and non-specific gene editing can be reduced [37].

5.4.3. Introducing security monitoring and evaluation policies

In order to make sure that the CRISPR/Cas9 technique is safe in CAR-T cells, it is necessary to establish strict monitoring and assessment strategies. This includes the monitoring of gene editing events, the function verification of the target gene, and the evaluation of the safety in vivo. Furthermore, long term monitoring and monitoring are important for the assessment of long term effects and adverse reactions.

The CRISPR/Cas9 technique is a big problem for CAR-T cells, which needs further research. It is anticipated that these challenges will be addressed through the optimisation of the delivery of CRISPR/Cas9 systems, the improvement of the effectiveness and specificity of targeting, as well as the establishment of strict monitoring and assessment strategies. It is expected that CRISPR/Cas9 technology will have a significant effect on CAR T-cells therapy.

6. Conclusion

This article will explore the best therapeutic methods of CAR-T cells, which are gene modified techniques, novel CAR, and cancer micro-environmental adjustment. First of all, the GPCR technique is used in CAR-T cells, which is CRISPR/Cas9 can enhance the anti-tumor effect of CAR. Furthermore, the therapeutic effect of CAR T-cells was enhanced by Zinc Finger Nuclease, TALEN, Gene Expression Regulator and Signal Amplification System.

Then, a novel CAR structure is proposed, which consists of several kinds of antigen identification and control. The multi-antigen identification system can distinguish many kinds of tumor associated antigens, thereby enhancing CAR-T's broad spectrum and anti-tumor activity. Thus, it can regulate CAR T-cells in accordance with tumor status, thus enhancing safety and therapeutic efficacy. In addition, CAR-T cells capable of targeting tumor microenvironment have been utilized to increase CAR-T cells' antitumor activity.

Finally, we will explore methods for enhancing CAR-T efficacy. Therefore, CAR T-cells may be suppressed by immune evasion and inhibition by tumour suppressors or tumour suppressors.

Thus, CAR-T therapy may be improved by suppressing tumour associated with cancer, improving blood vessel density and permeability, and increasing antigen presentation capacity of cancer cells.

In this paper, we put forward a series of optimal strategies, such as CAR structural design, cancer microenvironment modification, etc., to increase the effectiveness of CAR-T, which may help to solve current treatment issues, increase efficacy of CAR T-cells and improve their quality of life.

Reference

[1] ZHU Xiaofei, HUANG Jiaomei, YUAN Hao, WAN Yi. Methods for Discovery and Analysis of Class2 CRISPR-Cas Systems[J]. Journal of Tropical Biology, 2021, 12(1): 115-123.

[2] Makarova K S, Haft D H, Barrangou R, et al. Evolution and classification of the CRISPR-Cas systems[J]. Nature Reviews Microbiology, 2011, 9[7]: 467-477.

[3] Chylinski K, Makarova K S, Charpentier E, et al. Classification and evolution of CRISPR-Cas systems[J]. Nature Reviews Microbiology, 2014, 12[3]: 653-661.

[4] Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. Science, 346(6213), 1258096.

[5] Mali, P., Esvelt, K. M., & Church, G. M. (2013). Cas9 as a versatile tool for engineering biology. Nature Methods, 10[11], 957-963.

[6] Kanchiswamy, C. N., Maffei, M. E., & Malnoy, M. (2016). Non-host disease resistance response in grapefruit to the Asian citrus psyllid and its associated bacterium Candidatus Liberibacter asiaticus. BMC Genomics, 17[2], 1-14.

[7] Shen, B., Zhang, X., Du, Y., & Wang, J. (2014). Monogenic genetic trait modifications mediated by zinc finger nucleases in rice and wheat. Proceedings of the National Academy of Sciences, 111(47), 16579-16584.

[8] Eyquem J, Mansilla-Soto J, Giavridis T, et al. Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. Nature. 2017;543(7643):113-117.

[9] Sadelain M, Brentjens R, Rivière I. The basic principles of chimeric antigen receptor design. Cancer Discov. 2013;3[5]:388-398.

[10] Ma JSY, Kim JY, Kazane SA, et al. Versatile strategy for controlling the specificity and activity of engineered T cells. Proc Natl Acad Sci U S A. 2016;113[5]:E450-E458.

[11] Ren J, Liu X, Fang C, et al. Multiplex genome editing to generate universal CAR T cells resistant to PD1 inhibition. Clin Cancer Res. 2017;23[10]:2255-2266.

[12] Eyquem J, Mansilla-Soto J, van der Stegen SJC, et al. Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. Nature. 2017;543(7643):113-117.

[13] Hay KA, Turtle CJ. Chimeric Antigen Receptor (CAR) T Cells: Lessons Learned from Targeting of CD19 in B-Cell Malignancies. Drugs. 2017;77[4]:237-245.

[14] Perna F, Berman SH, Soni RK, et al. Integrating Proteomics and Transcriptomics for Systematic Combinatorial Chimeric Antigen Receptor T-cell Therapy Discovery Approaches. Cell Rep. 2017; 19[13]:2509-2518.

[15] Zhang X, Yang Y, Fan D, et al. Comparison of non-viral genome editing of CAR-T cells by mRNA electroporation and high-pressure nucleofection. Gene Ther. 2019;26[6]:262-271.

[16] Ren J, Zhao Y. Advancing chimeric antigen receptor T cell therapy with CRISPR/Cas9. Protein Cell. 2017;8[10]:634-643.

[17] Giordano Attianese GM, Marin V, Hoyos V, et al. In vivo validation of DNMT1 as a new target for the treatment of AML. Blood. 2018;132(22):2409-2409.

[18] Schuster, S. J., et al. (2017). Efficacy and Toxicity Management of 19-28z CAR T Cell Therapy in B Cell Acute Lymphoblastic Leukemia. Science Translational Medicine, 9(375), eaaj2013.

[19] Porter, D. L., et al. (2015). Chimeric Antigen Receptor T Cells Persist and Induce Sustained Hematological Malignancy Remission in Relapsed/Refractory Chronic Lymphoid Leukemia. Blood, 124(21), 377-377.

[20] Turtle, C. J., et al. (2017). Long-Term Follow-up of a Phase 1 First-in-Human Study of CD19 CAR-T Cells: Activity and Tolerance of a Persistent Engineered T Cell Product. Blood, 128(22), 376-376.

[21] Sadelain, M., et al. (2017). CAR-T-cell Therapy for Solid Tumors. American Society of Clinical Oncology Educational Book, 366-372.

[22] Zhang, C., et al. (2019). Chimeric antigen receptor-engineered T-cell therapy for solid tumors. Molecular Cancer, 18(1), 1-14.

[23] Ritchie, D., et al. (2017). Chimeric antigen receptor T cells targeting the immunodominant leukocyte antigen-1 epitope mediate potent antitumor activity against melanoma. European Journal of Immunology, 47(10), 1838-1849.

[24] Hodi, F. S., et al. (2018). Improved Survival with Ipilimumab in Patients with Metastatic Melanoma. New England Journal of Medicine, 363(8), 711-723.

[25] Klapper, J. A., et al. (2016). Chimeric Antigen Receptor-Engineered T Cells for Immunotherapy of Cancer: From Bench to Bedside. Annual Review of Immunology, 34(1), 95-112.

[26] Kershaw, M. H., et al. (2006). A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. Clinical Cancer Research, 12(20), 6106-6115.

[27] Chmielewski, M., et al. (2012). Ab CAR T cells targeting HER2/neu exhibit potent tumor lysis activity in vivo. Breast Cancer Research and Treatment, 135(2), 417-424.

[28] Yang, S., et al. (2019). B7-H3 as a novel CAR-T therapeutic target for solid tumors. Journal of Hematology & Oncology, 12(1), 1-12.

[29] Zhang, E., et al. (2017). Anti-MUC16 antibody-based chimeric antigen receptor T cells.

[30] Lee DW, et al. Current concepts in the diagnosis and management of cytokine release syndrome. Blood. 2014;124[3]:188-95.

[31] Kulemzin SV, et al. CAR T-cell therapy: balance between efficacy and safety. EJIFCC. 2019;30[3]:192-207.

[32] Liu X, et al. Chimeric antigen receptor T cell therapy for acute lymphoblastic leukemia. Transl Pediatr. 2019;8[4]:258-265.

[33] Zhang C, et al. Challenges and potential opportunities of CAR-T cell therapy in hepatocellular carcinoma. Hepatobiliary Surg Nutr. 2019;8[4]:258-270.

[34] Wang X, et al. Genome engineering of hematopoietic stem cells for cancer immunotherapy. Sci China Life Sci. 2019; 62[4]:365-375.

[35] Iyer V, et al. Off-target mutations are rare in Cas9-modified mice. Nat Methods. 2015;12[7]:479.

[36] Ramakrishna S, et al. Gene disruption by cell-penetrating peptide-mediated delivery of Cas9 protein and guide RNA. Genome Res. 2014;24[7]:1020-1027.

[37] Tabebordbar M, et al. Efficient in vivo base editing in adult dystrophic mice. Nat Biotechnol. 2016;34(7): 810-815.