Application of CRISPR in Anti-Tumor Immunotherapy

Shuaichen Lin [1], Yibei Wang [2,*]

[1] SHANGHAI CHINON ORIENTAL PRAVITE FOREIGN LANGUAGE SCHOOL, Shanghai, China

[2] School of Life Sciences and Engineering, Southwest Jiaotong University, Chengdu, China

* Corresponding Author Email: Wyb-Besty@my.swjtu.edu.cn

Abstract. The requirements for medicine are getting higher and higher, and the cure rate of cancer is increasingly attracting attention. CRISPR/cas9 genome editing technology has gradually attracted more and more attention, and has gradually become popular in research. At present, CRISPR system has been applied in many tumor immunotherapy methods and cancer research. CRISPR system is one of the key issues today, but there is still a lack of unified cognition about applying CRISPR in immunological therapy about cancer. Therefore, the theme of this study is about CRISPR in anti-tumor immunotherapy. More and more researchers are using CRISPR/cas system to improve and innovate the immunotherapy of various types of tumors, which improves the treatment efficiency and effect. At the same time, they provide innovative ideas and feasible ways for more treatment methods. CRISPR system also has some disadvantages, such as off-target effect. With the development of science and technology and more and more innovation and practice, crispr has been applied to cancer treatment to provide a good experience for patients.

Keywords: CRISPR, gene editing, tumor, therapy.

1. Introduction

A tumor is a newly created organism that results from the body's different carcinogenic agents causing local tissues and cells to proliferate. They usually show local abnormal swelling and the growth of tumor cells is very vigorous, easy to lose control, with relative autonomy. It can still proliferate stably after the initial factors causing tumor proliferation disappear. Malignant tumors and benign tumors are the two categories into which tumors can be classified. A benign tumor is an abnormal proliferation of certain bodily tissues and cells that grows slowly, does not spread to neighboring normal tissues, and causes minimal damage. Malignant tumor is caused by excessive proliferation of cells without normal regulation. Over proliferating cells, also known as cancer cells, usually invade surrounding tissues and even travel through the circulatory system to various organs in the body. Benign tumors have a relatively small impact on the body. They mainly show local compression and obstruction. Their impact is mainly related to the location and secondary changes, and will not directly affect the body. Malignant tumors have a very serious impact on the body because of immature differentiation and rapid growth, invasion and destruction of organ structure and function, and metastasis. It will indirectly lead to malnutrition and fixed pain.

At present, there are five methods to treat tumors, namely, surgery, radiotherapy, chemotherapy, targeted therapy, and immunotherapy. Surgical treatment, in addition to the well-known surgical anesthesia, there are also cryosurgery, laser therapy, hyperthermia, photodynamic therapy and other common treatment methods that do not involve scalpel cutting. Radiotherapy uses radiation to induce changes in DNA in tissues and cells, chromosome aberrations or breaks, and liquid ionization to produce chemical free radicals, which will eventually cause cells or their offspring to lose vitality and rupture or inhibit tumor growth. Low differentiated cells and cells in division stage are sensitive to ionizing radiation and easy to inactivate, so malignant tumors can be treated with radiation therapy. The general radiation therapy will not kill cancer cells immediately, but through several days or weeks of treatment, the DNA of cancer cells will be damaged and die one after another. There are two main types of radiotherapy: external irradiation and internal irradiation. Radiation can damage normal tissues and cells, especially when the amount of light radiation increases, it is easy to damage hematopoietic organs and vascular tissues, and there will be a variety of adverse reactions. The role

of chemotherapy is to prevent or retard the growth of cancer cells, which has been invested in the treatment of cancer. When chemotherapy is combined with other therapies, it can play the following functions: reduce the tumor before surgery or radiotherapy. Carefully remove missing cancer cells, which are usually present in patients after surgery or radiation therapy. However, chemotherapy also has many side effects. It will eliminate fast-growing cancer cells and restrain the growth and division of healthy ones. For example, it will damage the oral cavity, intestines and hair related cells, leading to oral ulcer, nausea and hair loss, and will also cause excessive fatigue. At anatomical and molecular scales, targeted therapy is a means of treating a specific carcinogenic site, which may involve a gene fragment or a protein molecule found in tumor cells. It is possible to design the corresponding therapeutic medications. From the moment as the pharmaceuticals penetrate the circulatory system, they will precisely target the carcinogenic spots and work together to kill the tumor cells while sparing the surrounding normal tissue cells without destruction. Immunotherapy can pass through the internal defense system of the body and achieve the purpose of curbing tumor growth through regulatory function. There are many methods of tumor immunotherapy, which can be divided into active, passive and adoptive immunity, and further divided into specific and non-specific.

CRISPR is mainly used for the treatment of tumors, including chimeric antigen receptor T cell (CAR-T) immunotherapy, PD-1 immunotherapy, adoptive T cell therapy, gene editing therapy, and combining with chemotherapy to reduce the chemotherapy resistance of tumor cells [1]. The basic principle of CAR-T immunotherapy is to extract the patient's own immune cells (T lymphocytes) through genetic modification, and then through modification, processing, and cultivation, obtain the necessary T cells, which are then reintroduced into the patient's body to eliminate cancer cells. Therefore, in general, modified T lymphocytes can make tumor cells accurately locked in normal cell groups and promote the expression of antigen targets, thus making T lymphocytes in an effective excited state and enhancing their lethality to leukemia and tumor cells. PD-1 immunotherapy utilizes the specific binding of PD-1 and programmed death receptor 1 (PD-L1) to affect the nature of tumor immune escape. PD-1 generally refers to programmed cell death molecules, which are predetermined and strictly controlled normal cell death that occurs in multicellular organisms. During this process, cells die and disappear from normal tissues in a dispersed and gradual manner, with no inflammatory response in the body. PD-1 is a checkpoint that can limit T cell-mediated immune responses. The combination of PD-1 ligand on cell surface and PD-1 receptor on immune effector cells promotes tumor cells to escape immune response. Therefore, the target population for PD-1 immunotherapy mainly includes patients with autoimmune diseases, transplant rejection, and tumors. The principle of adoptive immune cell therapy is to transfuse or reinfuse activated cytotoxic immune cells to tumor patients, allowing them to inherit specific immunity and improve the body's anti-tumor ability. The methods of adoptive immune cell therapy include CIK cells, DC cells, DC-CIK cells, NK cells, etc. In addition, there are lymphokine activated killer cells (LAK), tumor infiltrating lymphocytes (TIL), and cytotoxic T cells (CTL). In gene editing therapy, classification can also continue, with the main methods including constructing tumor models and identifying tumor suppressor genes.

Compared to the previous methods of constructing tumor models, CRISPR-Cas9 gene editing technology can not only construct tumor models by inoculating tumor cells edited in vitro into animal bodies, but also directly introduce constructed plasmids into animal bodies in situ, inducing the production of in situ tumor models [2]. In addition, this technology can also introduce multiple genetic mutations simultaneously in the same individual, allowing scientists to quickly study the mechanisms by which different genetic mutations interact to produce cancer, as well as some potential drugs that may affect tumors with a genetic profile cannot be ignored. There are other methods for identifying or screening tumor suppressor genes, such as chromosome fragment separation hybridization, cell culture transformation, and so on. Compared with these operations, CRISPR fundamentally changes the cell genome sequence by directly editing genes. When cultured, the results can be seen more intuitively and quickly, thereby judging the association between knocked out genes and tumor proliferation, which is more efficient. From this, it can be seen that there are already many methods

that use CRISPR for tumor treatment, each with certain characteristics and advantages. Therefore, this research will analyze the application of CRISPR for anti-tumor immunotherapy.

2. CRISPR-based anti-tumor immunotherapy methods

2.1. CAR-T cell therapy

This therapy precisely targets tumors, transforming T cells into CAR-T ones carrying chimeric antigen receptors through genetic engineering technology. By utilizing the immune effect activated by T cells, it specifically kills tumor cells [1]. It is widely used in hematological malignancies [1], and gene modified T cells expressing chimeric antigen receptors have shown unprecedented therapeutic effects in hematological malignancies [2]. However, the preparation technology of CAR-T cells is complex, production costs are expensive, and cytokine release syndrome may often occur during the treatment process, with some even exhibiting embedded carcinogenic effects. The majority of T cells employed in clinical studies are taken from and altered from tumor patients' peripheral blood. This approach is labor- and time-intensive, and many patients are unable to obtain sufficient numbers of high-quality T cells for collection, which significantly restricts the use of CAR-T treatment. Although allogeneic T cells can compensate for the insufficient quantity and quality of autologous T cells, due to differences in the main histocompatibility complex (MHC) and T cell receptor (TCR) between donors and recipients, donor derived T cells may lead to severe allogeneic reactions and graft-versus-host disease (GVHD) in the recipient's body. The corresponding solution is to utilize the characteristics of CRISPR/Cas9 technology for multi genome editing, which can simultaneously interfere with multiple loci, producing universal CAR-T cells with both endogenous MHC and TCR expression defects, thereby preventing GVHD and solving the problem of insufficient T cell sources. This technology can accurately insert the CAR gene into the TARC site, efficiently achieving target gene introduction while knocking out the TCR gene. In addition to directly knocking out genes that inhibit T cell activity, CRISPR/Cas9 technology can also be used to reduce adverse reactions during CAR-T treatment [1]. CAR-T therapy can also make killer T cells specifically attack tumor cells by attaching human leukocyte antigen (HLA) to the surface of T cells. However, HLA varies greatly among individuals, so this therapy is only targeted at a few types of cancer such as leukemia and lymphoma [3]. Widely using universal CAR-T cells in clinical practice is made feasible by the ability of CRISPR gene-edited human primary T cells to make allogeneic T cells with increased anti-tumor activity and fewer side effects, in addition to certain advances in immunooncology [4].

2.2. PD-L1 immunotherapy

PD-L1, binding to its ligand, PD-1 immunotherapy, is a critical immune checkpoint in the body that adversely controls T cell activation and is essential for both tumor immune evasion and normal immunological regulation. Despite the approval of multiple targeted PD-1/PD-L1 inhibitors for clinical usage, immune-related adverse reactions remain a possibility, particularly given the increased frequency of adverse reaction events primarily treated with PD-1 inhibitors. The researchers' suggested remedy is to create PD-1 defective CAR-T cells using CRISPR/Cas9, which not only increases cell survival and boosts anti-tumor activity but also lessens the toxicity of systemic immune checkpoint inhibition. The method that also can make the therapeutic effect equally remarkable is to use CRISPR/Cas9 technology to block the expression of related genes in CAR-T cells. However, it is worth noting that in the tumor microenvironment, immunosuppressive factors such as adenosine can promote tumor cell immune escape under hypoxic conditions. For other inhibitory factors, CRISPR/Cas9 can release its inhibitory effect on CAR-T cells through receptor gene knockout [1].

2.3. Construction of tumor animal models

Using CRISPR/Cas9 technology to establish personalized cell and animal experimental systems, comprehensive analysis of individual patient cancers is conducted, and genotype specific defects are quickly identified on this platform. This method can also simulate the treatment of patients to quickly

reveal the resistance mechanism of specific anti-cancer drugs and help establish effective treatment methods for permanently correcting cancer-related gene mutations [5]. Researchers processed a non metastatic mouse non-small cell lung cancer cell line using the mGeCKOa library, and then transplanted virus infected cells to immunodeficient mice to prepare a subcutaneous transplant tumor model. After tumor formation, the primary and metastatic tumors are separated and sequenced for comparison and analysis of the degree of sgRNA enrichment. The sequencing results indicate that the deletion of genes such as Nf2, PTEN, CDKN2a, trim72, and FGA is closely related to tumor metastasis. By knocking out multiple different genes, it is possible to determine whether they are closely related to tumor metastasis or proliferation, and thus infer possible immunotherapies. Constructing corresponding diseased animal models using CRISPR/Cas9 gene editing technology to analyze the inhibitory/promoting effects of relevant genes is helpful for the study of the interaction between different molecules during the occurrence of diseases and laying a theoretical foundation for developing precision medical solutions for diseases [3]. A mouse model was established by disrupting tumor suppressor genes Pten and P53 in the liver of mice using CRISPR/Cas9. The liver tumors produced by this model exhibited a similar cancer phenotype to those produced by traditional cre loxp technology. Several researchers used CRISPR technology to establish a zebrafish model of hereditary SPRED1 deficient mucosal melanoma in their study, and found that SPRED1 has an anti-tumor effect [6]. CRISPR/Cas9 has been widely used to establish cancer models, identify indispensable genes related to drug targets, study drug resistance mechanisms, and further discover the functions of non-coding regions of genes [2], as shown in Fig. 1.

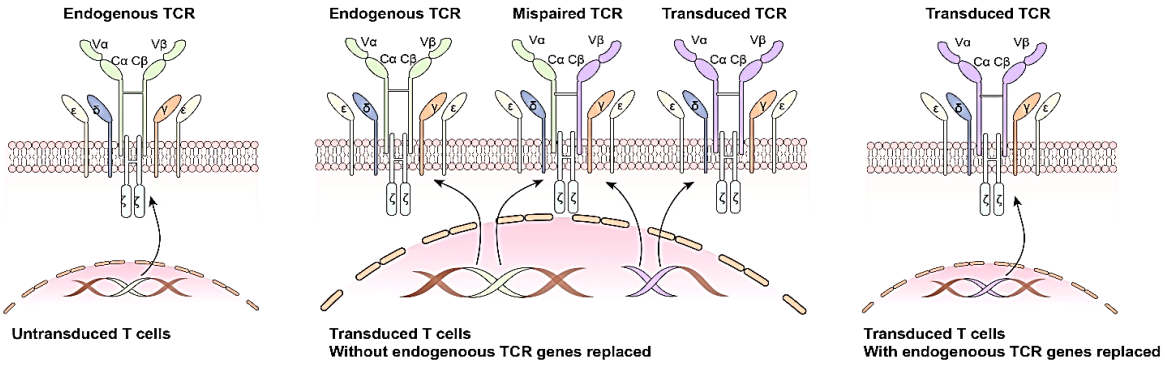

Figure 1. Application of CRISPR/Cas9 technology for cell therapy [2]

2.4. Gene editing therapy

CRISPR/Cas9 technology can generate gene function deletion mutations, precise genomic site knockout proteins, site modifications, transcriptome and epigenomic changes. Gene therapy mainly works through two ways. For directly acting on tumor related genes, CRISPR/Cas9 technology can be used to knock out E6 and E7 protein related genes that is to be pivotal in the development and maintenance of cervical cancer malignant tumors. The results showed that knocking out severely damaged the survival ability of cells, but cut no ice with HPV negative cells, proving the effectiveness and specificity of the CRISPR/Cas9 system. Based on the traditional CRISPR/Cas9 system, design an adapter that can complement the sgRNA guiding region, and in the absence of specific signals, pair the sgRNA with the designed adapter without binding to the target DNA. When specific signals exist, they bind to the aptamer, allowing the guiding sequence of sgRNA to bind to the corresponding DNA sequence and affect the transcription of another signal, achieving interference with specific signaling pathways within tumor cells without affecting normal cells [5]. The experiments of cell proliferation include: by mediating the deletion of CD133 gene, down-regulating the expression of Vimentin in colon cancer cells, and inhibiting the movement and invasion of cancer cells. The invasion and tumorigenicity of pancreatic cancer cells were prevented by knocking down miR-3064 gene. By knocking out YB-1 gene, a transcription factor, in tumor stem cells, the proliferation of breast cancer and melanoma stem cells can be inhibited, and their cycle arrest, apoptosis and irreversible differentiation can be significantly improved [6]. Knocking out endogenous TGF in CAR-

T cells with the use of CRISPR/Cas9 technology-β Receptor II (TGFBR2) can reduce the depletion of CART cells and improve the killing effect of solid tumors in vitro and in vivo [2]. Using a mouse in situ model, whole genome CRISPR/Cas9 gene knockout technology can be used to identify potential genes controlling the peritoneal spread of ovarian cancer (OC) [2]. Through the study of human papillomavirus cells, it is found that HPV negative cells are not affected when the CRISPR/Cas9 system targets HPV18-E6 or HPV16-E6 to promote apoptosis and reduce the proliferation ability of HPV positive cervical cancer cell lines, which provides a new strategy for cancer gene therapy [4].

2.5. Engineering T cell therapy

The CRISPR/Cas technology can be also used to treat human malignancies in 2016. Twelve of the 22 patients with advanced non-small cell lung cancer (NSCLC) who were enrolled in this clinical trial were able to receive treatment, and 17 of them had enough modified T cells for infusion. The primary procedure entails isolating T cells from the patient's body, targeting and knocking off the immunosuppressive PD-1 gene in T cells using Cas9 and sgRNA plasmid electroporation co transfection, and then injecting the modified T cells back into the patient's body. Peripheral blood contains the modified T cells that were infused. According to the survey, the median overall survival of 95% patients in this group is about six times that of the median progression-free survival, in which the median progression-free survival is 7.7 weeks and the median overall survival is 42.6 weeks. Combined with second-generation sequencing, the median mutation frequency of off target events at 18 candidate sites was found to be 0.05%. These results indicate that using the CRISPR/Cas system to edit T cell clinical applications is usually safe and feasible [7]. Engineered TCR-T cell therapy has a greater potential to target a wider range of antigens than CAR-T immunotherapy, leading to an expansion of cancer treatments [2]. CRISPR/cas9 mediated knockout of endogenous TCR in T cells-β. Simultaneously conducting cancer responsive receptor transduction significantly increases expressing tgTCR and enhances the killing effect of engineered T cells on target cancer cells [4].

2.6. Adoptive T cell therapy (ACT)

Adoptive T cell therapy (ACT) refers to the expression of a synthetic (transgenic) TCR by the patient's own T cells through genetic engineering, which can specifically detect and kill tumor cells. Using transgenic TCRs with specific immunogenic NY-ESO-1 tumor antigen is a safe and effective method for adoptive T cell metastasis in patients with myeloma, melanoma, and sarcoma [8].

2.7. Identifying tumor suppressor genes

Glioblastoma may appear in the brains of mice with CRISPR/cas9 deficiency because of the existence of many tumor suppressor genes. The non-anchored growth of pancreatic cancer Panc02, prostate cancer MyC Cap, breast cancer 168FARN and 67NR cells, colon cancer CT26, glioma GL261 and bladder cancer MB49 cells was markedly boosted by the loss of LATS1/2 in numerous mice cancer cell lines with CRISPR/Cas9. Apoptosis and cell death can result from using CRISPR/Cas9 to knock down the E6 and E7 genes since doing so restores the levels of the tumor suppressor p53 and the retinoblastoma (Rb) protein [6].

2.8. Reducing chemotherapy resistance of cancer cells

In a mouse model of lung cancer xenotransplantation, knocking down NRF2 by CRISPR/cas9 can enhance sensitivity to cisplatin and carboplatin. It was discovered that SKA3 knockdown via CRISPR/cas9 increased the susceptibility of laryngeal cancer cells to cisplatin in preclinical settings. The CRISPR/Cas9 method of RSF1 knockdown can greatly raise the paclitaxel sensitivity of H460 cells [6].

2.9. Disadvantages of CRISPR/Cas9 system

Although CRISPR/Cas9 has been widely used, the inherent target specificity and recognition process of the CRISPR/Cas9 system have not been thoroughly studied [9]. Meanwhile, the chimeric phenomenon of gene knockout is a potential issue of this technology [9]. Gene knockout causes permanent changes in genetic material and poses a risk of mutation [5]. The method of importing and expressing the CRISPR/Cas system needs improvement when using this system. Some cells or tissues are difficult to transform through ordinary carriers, optimizing the CRISPR/Cas system and its introduction methods can make this technology more effective [9]. The efficiency of delivering CRISPR components determines the efficiency of gene editing. Currently, viral vectors are the most widely used, and there is still a need to develop safer and more effective delivery tools for non viral vectors such as polymers, liposomes, nanomaterials, etc., or to find and develop Cas9 subtypes with wider targeting range, higher specificity, and smaller molecules [5], that is, to find better ways to more efficiently and safely import CRISPR/Cas systems.

In terms of safety, it has been recently discovered that more and more CRISPR/Cas systems have toxic effects on embryonic development and cell growth. The reasons for their occurrence should be explored, and safer Cas tools should be developed to pose significant safety risks to human health and growth. Editing human primary T cells using the CRISPR/Cas system resulted in chromosome loss in 5% to 20% of cells. There is still a lack of clinical data about the long-term safety and effectiveness of gene editing in immune cell therapy-more animal model and clinical trials are needed, while improving the method of introducing the CRISPR/Cas system to enhance safety [7]. Gene editing may be less effective in humans due to pre-existing humoral and cell-mediated adaptive immunological responses to Cas9 [6]. Anti SaCas9 T cells were detected in 78% of donor serum, and 67% of donors produced anti SpCas9 T cells, indicating that humans' potential immunity to Cas9 protein, which is also a major safety hazard for gene editing [6, 9]. In terms of ethics and ethics, CRISPR/Cas9 is a potential pathway for targeted treatment of human genetic diseases, but gene editing of human embryos and germ cells has brought a storm about many scientists' ears [3, 9].

In PD-1 therapy, PD-1 monoclonal antibodies have the ability to mobilize the host immune system to recognize, combat, and ultimately destroy tumor cells. T lymphocytes play an indispensable role in combating the occurrence and development of tumors. The effectiveness of PD-1 monoclonal antibody, as one of the immune checkpoint inhibitors, depends on the infiltration of effector T cells (Teff) in the tumor and the degree of immune suppression of regulatory T cells (Treg). Therefore, it is crucial to improve Teff infiltration and Treg inhibition in tumors and enhance the effectiveness of PD-1 monoclonal antibody therapy [10].

In a certain experiment, plasmids expressing Cas9 and sgRNA were delivered to mouse liver using fluid dynamics injection, directly targeting tumor suppressor genes Pten and p53 to induce liver tumors. The experiment demonstrated the feasibility of using the CRISPR/Cas system to directly mutate tumor suppressor and oncogenes in the liver, providing a new approach for the rapid development of liver cancer models and functional genomics. The fluid dynamic injection is used, which may cause certain damage to other cells during the injection process. Therefore, it is necessary to consider how to reduce the damage in other ways while introducing plasmids expressing Cas9 and sgRNA into the mouse liver [5]. Another method of introducing the CRISPR/Cas9 system is electrical conduction. Studies have shown that knocking out PD-1 from plasmids encoding the CRISPR/Cas9 system through electroporation is technically feasible and does not affect the survival ability of T cells in vitro [2]. Most CRISPR engineered T cells used in clinical trials are transduced through electroporation, which may lead to cell damage and hinder T cell proliferation in vitro [6].

The main drawback of the CRISPR system is the off target effect. The research suggests that the off target probability of the Cas9 system varies and is difficult to predict. Although DNA methylation does not affect genome editing by Cas9, the structure of chromatin may affect off target effects. There are reports that the off target effect is related to the ratio of gRNA and Cas9 protein, and increasing the concentration of gRNA can help reduce the off target rate. High concentration of Cas9 enhances the system's fault tolerance. Reducing the concentration of Cas9 can significantly improve the

positive/off target rate, but some studies have found that the off target rate and positive target rate decrease synchronously after reducing the concentration of Cas9. Therefore, more research is needed to explore how much the concentration of Cas9 is most suitable to reduce [9].

The irreversible consequences caused by off target are a major obstacle that restricts the CRISPR/Cas system from basic research to clinical use. Among them, PAM sequences (identifying incorrect PAM sequences: Cas9 may recognize low-frequency NAGs, leading to off target [3]), the structure, length, and targeting position of sgRNA can all affect targeting efficiency (sgRNA mismatches with target sequences [3]). By optimizing PAM and appropriately designing and modifying sgRNA sequences [3, 5] Changing the Cas9 protein domain (using Cas9 mutants [3, 6]), enhancing gene editing efficiency, improving specificity, and fully tapping into the in vivo therapeutic potential of CRISPR/Cas9 technology [5].

3. Conclusion

More and more researchers are using the CRISPR/Cas system to improve and innovate immune therapies for various types of tumors, such as CAR-T cell therapy, adoptive cell therapy, engineered T cell therapy and gene editing. These therapies have significant advantages such as strong targeting, fast response, and good results. At the same time, they provide innovative ideas and feasible approaches for more treatment methods. However, CRISPR also has many shortcomings, and some methods of importing CRISPR systems can cause damage to cells, and gene editing may lead to the occurrence of other diseases or potential mutations in other genes. The off target effect leads to unpaired and mismatched outcomes, resulting in more serious consequences for patients. For the CRISPR system, although researchers are constantly making new progress, there are still many areas worth exploring and researching. In order to introduce the CRISPR system, efforts should be made to find natural carriers that are harmless to the human body to reduce cell damage. Regarding the off target effect, it is necessary to find the most suitable concentration of Cas9 to ensure a high positive target rate while minimizing off target rates. It is also possible to search for or construct more specific Cas9 mutants. Future research is needed to investigate the effects of mismatches or gRNA length on splicing efficiency and specificity. Of course, CRISPR has a promising future, and as people continue to explore, the application of CRISPR will become increasingly sophisticated, with more and more innovation and practice, ultimately being able to be invested in the treatment of cancer patients

Authors Contribution

All the authors contributed equally and their names were listed in alphabetical order.

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