

Advances in CRISPR-Cas Gene Editing Systems and Novel Explorations in Disease Treatment Applications

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ABSTRACT

Developed from the bacteria's immune system, CRISPR-Cas systems have become a critical basis for genome editing with high precision. In this review, we outline in detail the general architecture and mechanism underlying CRISPR-Cas, discuss the variety and evolutionary functions of Cas proteins, and highlight advancements made in improving editing accuracy via the creation of high-fidelity systems. More importantly, this review focuses on the latest clinical achievements made using this gene-editing tool to treat genetic and rare disorders, study multiplex editing and immune reprogramming in the realm of precision oncology, and conduct novel applications of stem cells and tissue repair therapy in treating complex diseases and regeneration. Current literature evidence demonstrates that technological refinement has promoted a number of clinical trials of genome editing in patients with hematologic genetic diseases, not only moving forward with in vitro editing therapy but also extending into in vivo intervention and personalized treatment in accordance with the principles of precision medicine. Future research should pay attention to the remaining technical barriers related to effective delivery, safe monitoring, and ethical regulation.

Keywords: CRISPR-Cas System, Gene Editing, Genetic Disease Treatment, Precision Cancer Treatment, Regenerative Medicine

1. INTRODUCTION

1.1 Research Background and Significance

The CRISPR-Cas system is the result of the evolution of bacterial and archaeal adaptive immunity and functions by guiding the Cas proteins to detect and cut foreign DNA sequences with the assistance of crRNA and tracrRNA. In 2012, the scientists led by Jinek et al., proved in their paper in Science magazine that the Cas9 protein can target particular DNA sequences for double-stranded cutting under guidance of artificially designed guide RNA. Such an innovation redefined the bacterial immune system into a programmable genome editing tool. Later on, the technique was rapidly extended to eukaryotic cells, considerably reducing the difficulty of gene editing. With the development of new generation methods like base editing and prime editing, which are capable of accurate editing of bases and DNA fragments respectively without creating double-stranded breaks, more accurate gene editing was achieved with enhanced security. In 2021, the authors of another study headed by Frangoul showed preliminary clinical outcomes of CRISPR-Cas9 edited autologous hematopoietic stem cells therapy for transfusion-dependent β -thalassemia and sickle cell disease in terms of significant elevation of fetal hemoglobin expression and improvement of the condition of patients^[1]. All these achievements make way to potential cures of monogenic genetic disorders. They have a lot of scientific and medical significance and also contribute greatly to the progress of precision medicine, regenerative medicine, and synthetic biology fields. However, there are still issues, such as off-targeting, delivery efficiency in vivo, safety, and ethics that need to be addressed systematically.

1.2 Current Status of Research at Home and Abroad

Internationally, CRISPR-Cas gene editing research has rapidly evolved from the initial analysis of the Cas9 nuclease mechanism to the construction and clinical translation of diverse precision editing platforms. Base editors achieve precise conversion of C·G-to-T·A or A·T-to-G·C by fusing cytosine or adenine deaminase, while prime editing achieves precise editing of any type of small fragment through the synergistic effect of reverse transcriptase and guide RNA, significantly expanding the spectrum of treatable mutations^[2,3]. In the clinical field, the first CRISPR-Cas9 cell therapy has been approved by regulatory authorities and approved in many countries for the treatment of hematologic monogenic diseases^[4]. Multicenter clinical trials are ongoing, covering areas such as hereditary diseases, tumor immunotherapy, and infectious

diseases. In China, Chinese research institutions and universities actively participate in basic research and tool optimization related to CRISPR, and have carried out a great deal of work in improving gene editing efficiency, developing delivery vectors, and constructing disease models. They have formed a complementary and cooperative relationship with international teams, promoting the rapid follow-up and localization of this technology in Asia. Overall, global research is showing a trend of deepening understanding of basic mechanisms, diversification of tools, and parallel clinical translation, with a focus on editing fidelity, tissue-specific delivery, and improvement of regulatory frameworks, in order to accelerate the expansion into more complex disease treatment scenarios.

2. TECHNOLOGICAL ADVANCEMENTS IN CRISPR-CAS GENE EDITING SYSTEMS

2.1 Basic Structure and Mechanism of Operation of the System

consists of three core parts: Cas9 nuclease, single-stranded guide RNA (sgRNA), and target DNA. Its structure presents a bilobal architecture. Nishimasu ^[5] found that Cas9 has recognition (REC) leaves and nuclease (NUC) leaves in high-resolution crystal structure analysis. REC leaves are mainly responsible for combining with sgRNA and finding the target DNA. NUC leaves have HNH and RuvC domains, which can cut off complementary chains and non-complementary chains respectively. The PAM interaction domain at the C-side plays a role in correctly distinguishing protospacer adjacent motif (PAM).

This mechanism begins with sgRNA guiding to find the target sequence. Next, after PAM is found, part of the DNA will be untied to form an R-loop structure. Finally, HNH and RuvC domain work together to cut off the double strand of DNA. Anders et al. ^[6] also clarified the structural reasons for relying on PAM to distinguish the target DNA, and clarified the mechanism of interaction between important amino acid residues and PAM to maintain the correctness of the target.

This mechanism is the molecular basis of correct genome editing. Especially in the treatment of hereditary diseases, it is very likely to be used in clinic because it can correctly aim at the places that cause disease variation, correct genes and restore functions. However, it is said that this mechanism also has the risk of combining with different targets, so it needs to be further improved in order to improve the safety of treatment.

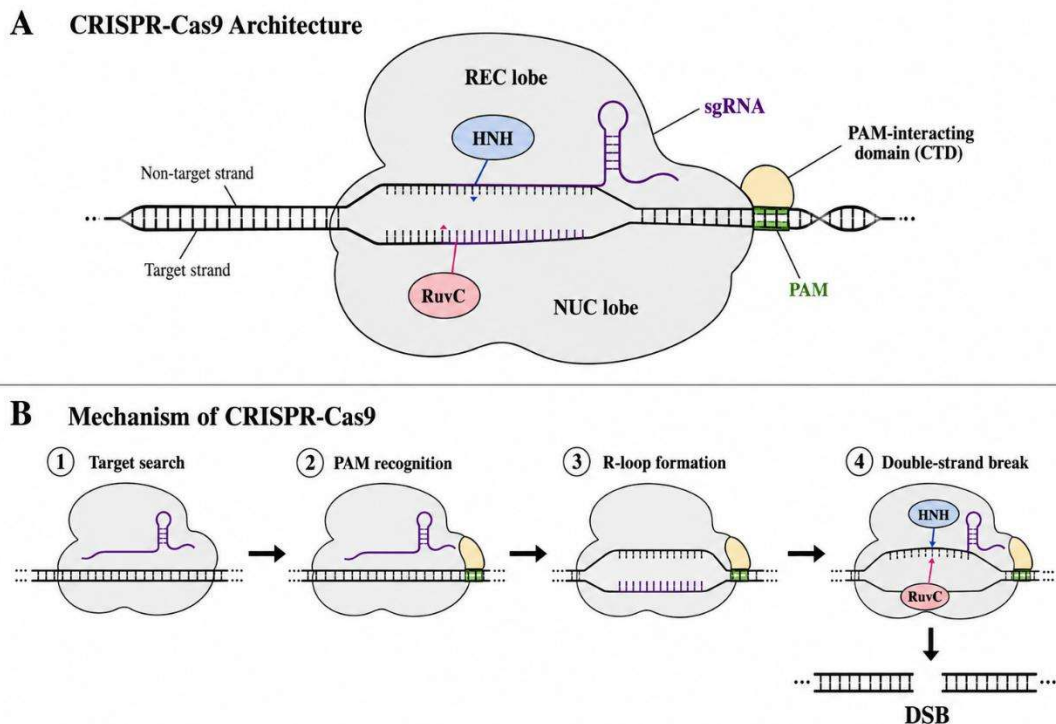


Figure 1. Structure and working principle of CRISPR-CAS9 system.

CRISPR-Cas9 system can be composed of Cas9 protein and single-stranded guide RNA (sgRNA), as shown in Figure 1. The overall shape consists of two parts, including recognition lobe (rec lobe) and nuclease lobe (Nuclease lobe). REC lobe mainly plays the role of bonding sgRNA and target DNA, and NUC lobe has HNH and RuvC domains, which cut complementary strand and non-complementary strand respectively. C-terminal PAM interaction domain can accurately distinguish protospacer adjacent motif (PAM). The working mechanism is as follows: finding the target sequence, distinguishing PAM, making R-loop, and making the slit of double helix. The function of this correct molecule is to improve the efficiency and correctness of gene editing and make the treatment of hereditary diseases and cancer available in clinic.

2.2 Diversity and Functional Evolution of the Cas Protein Family

CRISPR-Cas system has many kinds in prokaryotes. According to the difference of effector proteins and structure, it can be divided into several type. Makarova^[7] carefully investigated the evolutionary classification of CRISPR-Cas. Moreover, this system comes from the adaptive immunity of archaea and bacteria, and has been evolving in horizontal gene transfer and modular recombination, forming two big groups. The first one is Class 1 (effector complex with multiple components). The second is class2 (individual effector proteins, such as ca S9, Cas12 and Cas13).

Cas9 of Type II is mainly used for DNA cutting. Cas12 of Type V has different preferences and cutting methods for PAM. On the other hand, Cas13 of Type VI targets RNA and may be used for post-transcriptional regulation.

The evolution of function is reflected in the diversity of PAM recognition, the characteristics of cut products and the optimization of the size of effector protein. Because of these differences, the system can adapt to various host environments, and the used scenarios have also increased.

In the new measures of disease treatment, the diversity of Cas protein family has become a collection of rich tools for developing reduced delivery methods, RNA-targeted treatment and various editing methods. Then, we are changing from a basic immune mechanism to a tool of precision medicine.

2.3 Progress in Optimizing Gene Editing Efficiency and Specificity

In order to surpass the limit of target risk and editing efficiency of wild-type Cas9, researchers have made great improvements in protein engineering using structure. Kleinstiver^[8] made a high-precision variant named SpCas9-HF1. The redundant contact with DNA was reduced by mutation, and the effect of off-target found in the whole genome was eliminated while maintaining the function of cutting the target position. Through this method, the accuracy of editing is greatly improved and it becomes a safer tool for treatment. In the following research, the powerful promoter, well-designed sgRNA and small molecules were combined to improve the editing efficiency. Especially in cells and animals that are difficult to put genes into, the effect has become better. These improvements directly contribute to the new research of disease treatment. For example, treating genetic diseases and editing tumor immune cells, striking a balance between efficiency and accuracy, can reduce the risk of treatment and speed up treatment. We hope that, with the help of artificial intelligence, combining design with the development of new variant will be able to surmount more obstacles in the trade-off between efficiency and accuracy in the future.

3. NEW EXPLORATIONS IN THE APPLICATION OF CRISPR-CAS IN DISEASE TREATMENT

3.1 Recent Clinical Advances in the Treatment of Genetic and Rare Diseases

CRISPR-Cas gene editing technology in the treatment of genetic diseases and rare disease has entered the stage of practical treatment from the initial “proven” stage. Using peripheral blood stem cells to edit in vitro, patients only need infusion once to achieve the effect of long-term gene repair. The symptoms that always need blood transfusion, the attack of blood vessel blockage, etc. have all improved, and some of them have been cured functionally. By combining high-precision Cas mutant and optimized delivery system, the risk of editing outside the target position and immune reaction is reduced, and the editing work becomes safer and easier to control. With the maturity of precision tools such as prime editing and base editing, the focus of research has gradually shifted from “blocking a gene” to “precisely correcting point mutation” for more rare genetic metabolic diseases and neuromuscular diseases. With the development of direct editing technology in vivo, the treatment steps will become simpler, and the risk of cell damage and rejection caused by in vitro work will also be reduced. These new clinical advances not only prove that gene editing can be used as a fundamental treatment, but also bring unprecedented treatment hope to patients with rare disease, making precision medicine a “long-term treatment suitable for everyone.”

3.2 Exploration of Gene Editing Strategies in Precision Cancer Treatment

In the precise treatment of cancer, CRISPR-Cas technology is changing from passive treatment to active adjustment using many strategies. In hematological cancers, researchers use gene editing to remodel T-cell receptors or eliminate immune check point molecules. In this way, chimeric antigen receptor T cells will work for a long time, which will not only strengthen the ability to kill cancer, but also reduce the risk of side effects such as cytokine storms. In solid-state cancer, the combination of delivery system into the body and multiple edit has been studied more deeply. By targeting the genes that increase cancer and adjusting the surrounding environment, we have achieved two effects: precise killing and active immunity. With the advent of the new edit tool, researchers can accurately insert or reinsert specific mutations, thus overcoming the problem of drug failure and making the treatment suitable for everyone. These strategic studies not only improve the accuracy and safety of editing to the organization, but also expand the application scope of gene editing in tumor immunotherapy. It opens up a new way of intervention for incurable cancer, from improving technology to updating clinical strategy, showing logical progress.

3.3 Innovative Applications in Complex Diseases and Regenerative Medicine

The fields of complex disease and regenerative medicine are using the new usage of CRISPR-Cas to develop from a goal-oriented approach to a holistic approach. In the complex disease involving diabetes and cardiovascular disease, the function of cells is changed and metabolic disorder is corrected by editing multiple gene and epigenetic regulation. In regenerative medicine, Stem cell and progenitor cell edited by gene can be transformed into functional tissues by correctly correcting the mutation that causes the disease, which is helpful for organ repair and preparation for transplantation. The success rate of transplantation and long-term adaptation to the body are great. Combining the organoid model and the high-throughput screening platform, the speed of confirming and improving new therapies is also faster. These new usages rely on the continuous improvement of the efficiency and accuracy of the initial editing, changing the treatment of complex disease from “relieving symptoms” to “lobbying reasons”, and at the same time mass-producing on regenerative medicine.

4. CONCLUSION

CRISPR-Cas gene editing system has developed from bacterial adaptive immune mechanism to accurate genome editing platform. Due to the structural analysis, the evolution of protein family diversity, the continuous progress of high fidelity variant and optimization strategy, the editing efficiency and specificity have been greatly improved, which has laid a strong technical foundation for clinical application. In the treatment of genetic diseases and rare diseases, the symptoms of patients have improved for a long time and their physical functions have recovered through the treatment methods edited in vitro. In addition, the study of direct editing technology in vivo has further expanded the scope of application. In the precise treatment of cancer, a number of strategies to edit and reshape immune cells have produced a new approach of immunotherapy suitable for individuals. In the field of complex diseases and regenerative medicine, the correct changes of stem cells and the application of organoid model show the broad possibility of systemic adjustment and tissue repair. These advances show the logical evolution from the basic mechanism to the new research on disease treatment, and highlight the core value of this technology in precision medicine.

In the future, important breakthroughs will be needed in the areas of in vivo delivery efficiency, long-term safety monitoring and ethical regulation mechanism. At the same time, it is important to deepen the integration of cutting-edge technologies such as multiplexing, epigenetic Control and AI-aided design, and accelerate its application in more chronic diseases and regeneration scenarios. CRISPR-Cas system is expected to be a revolutionary tool to change human health. Through continuous improvement, we can expand the scope of clinical use, provide us with safer and more efficient fundamental treatment of hereditary diseases, accurate treatment of tumors and tissue reconstruction, and help us achieve medical goals suitable for individuals and everyone.

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