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The Progress and Challenges of CRISPR-Cas System from Laboratory to Clinical Practice

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Abstract The CRISPR-Cas system, as an innovative and efficient gene editing technology, has provided new possibilities for genetic research and disease treatment. This technology draws inspiration from the natural defense mechanisms of microorganisms, enabling precise editing of genes by "cutting" and "pasting" DNA sequences. While it has found extensive applications in laboratory conditions and demonstrated promising results in clinical trials, its transition from the laboratory to clinical applications faces several key issues and challenges, including safety and side effects, ethical and moral considerations, as well as legal and regulatory issues. This review will focus on the progress of CRISPR-Cas system applications from the laboratory to clinical settings, particularly in the realms of gene therapy and disease research. It will extensively analyze and discuss challenges in aspects such as safety, precision, delivery efficiency, aiming to provide meaningful insights and guidance for future research and applications in this field.

Keywords CRISPR-Cas system; Gene editing; Gene therapy; Safety; Ethical considerations

With the rapid advancement of biotechnology, genome editing has become a prominent topic in contemporary scientific research. Among these techniques, the CRISPR-Cas system, owing to its distinctive efficiency and precision, has been widely employed in genetic editing studies. This method of altering the traits and functions of organisms through gene modification allows for a deeper understanding of the mysteries of life and, concurrently, also brings new possibilities for the treatment of diseases. However, the translation of CRISPR-Cas system from laboratory applications to clinical settings poses numerous challenges and issues in this process.

The technical principles of the CRISPR-Cas system are derived from the immune systems of bacteria and archaea (Koonin and Makarova, 2019). Scientists have leveraged this principle to modify and optimize the CRISPR-Cas system, transforming it into a powerful tool for gene editing. Over the past few years, significant progress has been made in the application of the CRISPR-Cas system, not only extensively in laboratory settings but also initiating its use in clinical applications, offering new hope for the treatment of various diseases. However, as its scope of application expands, we also encounter numerous challenges, such as ensuring the precision of its editing and avoiding unnecessary side effects.

The breakthrough applications of the CRISPR-Cas system in the laboratory have brought tremendous hope to the fields of gene editing and gene therapy. However, its use in clinical research still faces challenges related to safety and side effects, ethical and moral considerations, as well as legal and regulatory issues. Future directions for development include technological improvements and optimizations, interdisciplinary collaboration, knowledge sharing, and the application of the CRISPR-Cas system in other fields. By overcoming these challenges, the CRISPR-Cas system holds the potential to become a revolutionary gene therapy tool, bringing positive advancements to human health.

Therefore, this review elaborates on the progress of the CRISPR-Cas system from the laboratory to clinical applications, discussing its transformative impact on gene editing, gene therapy, and disease research. Simultaneously, it delves into the current challenges and issues that need resolution, aiming to provide insights and guidance for the future applications of the CRISPR-Cas system.

1 Progress of the CRISPR-Cas System in Basic Research

1.1 Genome editing and functional studies

The CRISPR-Cas system has made significant strides in basic research, particularly in the realms of genome editing and functional studies. Its application in basic research provides an efficient and precise tool for genome editing and functional studies. Through this technology, researchers can elucidate the functions and regulatory mechanisms of genes, laying crucial foundations for the understanding of biology and the treatment of diseases.

Genome editing is one of the most crucial applications of the CRISPR-Cas system. By introducing the CRISPR-Cas system, researchers can precisely edit target genes, including additions, deletions, and modifications. This precise genome editing technology provides a powerful tool for elucidating the roles of genes in organism development, physiological functions, and disease occurrence.

The fundamental principle of the CRISPR-Cas system involves the pairing of guide RNA (gRNA) with the CRISPR characteristic sequences located near some CRISPR-associated genes that encode a series of Cas proteins, collectively known as the CRISPR-Cas system (Figure 1). This system directs Cas proteins to the target DNA sequence, and through the nucleolytic activity of Cas proteins, the target DNA is cleaved. During the cleavage process, the cell's self-repair mechanisms intervene, leading to genome editing. This genome editing technology can be employed to study the function of genes, such as investigating their role in organisms by knocking out specific genes (Manghwar et al., 2019).
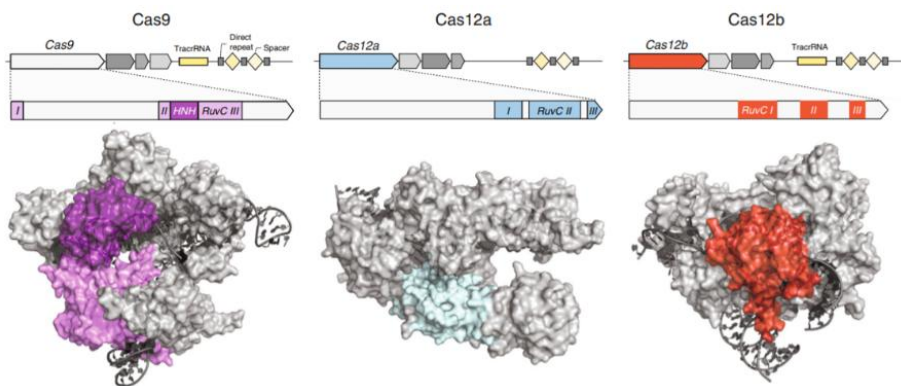

Figure 1 CRISPR-Cas system

In addition to gene knockout, the CRISPR-Cas system can also achieve gene addition and modification. By introducing exogenous DNA fragments and the CRISPR-Cas system, researchers can add specific genes to the target genome, thereby enabling functional studies of genes. Furthermore, the CRISPR-Cas system can facilitate gene modification, such as studying gene function and regulatory mechanisms through point mutations or insertions.

The advancement of genome editing technology provides a powerful tool for gene function studies. Through the CRISPR-Cas system, researchers can precisely edit target genes, elucidating the roles of genes in organism development, physiological functions, and disease occurrence. The application of this technology has made significant breakthroughs in various organisms, including model organisms such as mice, fruit flies, nematodes, and zebrafish.

1.2 Establishment and study of disease models

In addition to its applications in genome editing and functional studies, the CRISPR-Cas system has made significant strides in the establishment and study of disease models. Through this technology, researchers can simulate mutations associated with genetic diseases and investigate the impact of these mutations on the occurrence and development of diseases. Moreover, the CRISPR-Cas system can reveal the functions and regulatory mechanisms of disease-related genes, aiding in the screening and validation of potential therapeutic targets and drugs. These advancements provide new perspectives and approaches for the treatment and prevention of diseases.

The CRISPR-Cas system can be employed to establish disease models, particularly models for genetic diseases (Garcia-Robledo et al., 2020). By introducing the CRISPR-Cas system, researchers can simulate mutations

present in human genetic diseases and investigate the impact of these mutations on the occurrence and development of diseases in model organisms. The establishment of such disease models helps researchers gain a better understanding of the pathogenic mechanisms underlying diseases and provides new perspectives and methods for the treatment and prevention of diseases.

The CRISPR-Cas system can also be utilized to study the functions and regulatory mechanisms of disease-related genes. Through genome editing techniques, researchers can precisely knock out or modify genes associated with specific diseases, and then observe the impact of these genetic alterations on the occurrence and development of diseases. This research can unveil the functions and regulatory mechanisms of disease-related genes, providing new targets and strategies for the treatment and prevention of diseases.

Furthermore, the CRISPR-Cas system can be employed for the screening and validation of potential therapeutic targets and drugs. Using genome editing techniques, researchers can knock out or modify genes associated with diseases and observe the impact of these genetic alterations on the therapeutic efficacy in disease models. This screening and validation process can assist researchers in identifying potential therapeutic targets and drugs, expediting the development and clinical application of new medications.

1.3 Potential applications of gene therapy

Gene therapy is still in the research and development stage, with many technical and security issues still need to be addressed. Challenges related to ethics and legal aspects also confront the application of gene therapy. The CRISPR-Cas system presents new possibilities for gene therapy, but further research and exploration are needed for its practical application.

The CRISPR-Cas system can be used for genome editing, precisely modifying the genome of organisms. This technology enables researchers to study the functions and regulatory mechanisms of specific genes, as well as the effects of gene mutations on organisms. Through the CRISPR-Cas system, researchers can knock out or modify specific genes and observe the impact of these genetic changes on organisms, thereby revealing the functions and regulatory mechanisms of genes.

The CRISPR-Cas system can also be employed for genome-wide screening, involving the knockout or modification of a large number of genes to study their functions and interactions in organisms. This screening method helps researchers identify genes associated with specific biological processes or diseases and reveals the mechanisms of action of these genes in organisms.

Furthermore, the CRISPR-Cas system can be utilized for genome regulation studies. Through the CRISPR-Cas system, researchers can precisely regulate the expression levels of genes, thereby studying gene regulatory networks and signaling pathways. This technology helps researchers understand the mechanisms of gene regulation and the relationship between abnormal gene expression and diseases.

Regarding the potential applications of gene therapy, the CRISPR-Cas system offers a precise method for gene editing, providing new possibilities for gene therapy. Gene therapy is a strategy for treating diseases by repairing or replacing defective genes within a patient. The CRISPR-Cas system can be used to repair mutated genes within patients or restore normal gene function by knocking out or modifying abnormal genes. This ability for precise gene editing makes gene therapy more accurate and effective, offering new hope for the treatment of many genetic diseases.

2 Progress of CRISPR-Cas System in Clinical Research

2.1 Clinical trials in gene therapy

The CRISPR-Cas system has made significant strides in clinical research, particularly in the field of gene therapy. Although still in the early stages, there are ongoing clinical trials to assess the safety and efficacy of the CRISPR-Cas system in treating human diseases. With continuous technological development and refinement, gene therapy holds the potential to become a crucial treatment method, bringing new hope to patients with various genetic diseases.

One of the most famous clinical trials is aimed at β-mediterranean anemia treatment (Khiabani et al., 2023). Ninety percent of β-thalassemia is caused by point mutations in the β-globin gene, with a minority resulting from larger segment deletions or duplications (Figure 2). In this trial, researchers use the CRISPR-Cas system to edit patients' hematopoietic stem cells to correct the mutated genes within the patients. The aim of this trial is to provide a potential therapeutic approach for patients with β-thalassemia, aiming to alleviate or eliminate their symptoms.
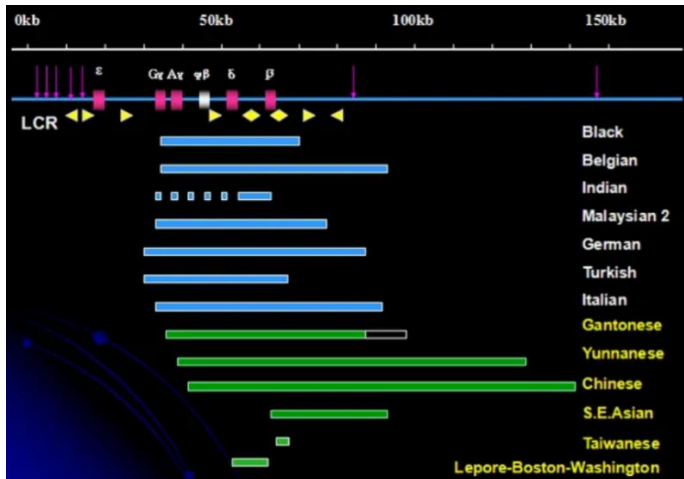

Figure 2 Causes β- Gene deletion in thalassemia

Additionally, there are several other ongoing clinical trials involving the use of the CRISPR-Cas system to treat other genetic diseases, such as cystic fibrosis, hereditary deafness, and hereditary retinal disorders. These trials aim to assess the safety and efficacy of the CRISPR-Cas system in treating these diseases, providing new therapeutic options for gene therapy.

However, it is important to note that there are still challenges and limitations in clinical applications. The precision and efficiency of the CRISPR-Cas system need further improvement to ensure accurate editing of target genes and avoid adverse effects on other genes. Furthermore, safety is a crucial consideration, requiring assurance that the use of the CRISPR-Cas system does not trigger adverse reactions or potential side effects. Additionally, ethical and legal issues need careful consideration to ensure that the application of gene therapy is rational and ethical.

2.2 Clinical applications of genome editing

Clinical applications of the CRISPR-Cas system in genome editing have made progress, yet further research and clinical trials are necessary to assess its safety and efficacy. Genome editing involves precise modifications and repairs of the human genome using the CRISPR-Cas system, aiming to treat genetic diseases or enhance human health.

In clinical research, the CRISPR-Cas system has been employed for the treatment of certain genetic diseases. For instance, as mentioned earlier, it has been applied in the treatment of β-thalassemia. Additionally, the CRISPR-Cas system has been utilized in the treatment of other genetic disorders. By editing and repairing the patient's genome, researchers aim to address the root causes of these diseases, ultimately achieving therapeutic outcomes.

2.3 Prospects of personalized medicine and precision gene editing

The advancements of the CRISPR-Cas system in clinical research have opened significant prospects for personalized medicine and precision gene editing. Personalized medicine involves tailoring medical treatments to individual patients based on their genomic information and other relevant factors. The emergence of the CRISPR-Cas system has enhanced the precision and efficiency of genome editing, providing a robust tool for personalized medicine. With continuous technological development, personalized medicine and precision gene editing are poised to become routine therapeutic approaches, offering improved treatment outcomes and quality of life for patients with various diseases.

Through the CRISPR-Cas system, doctors can precisely edit and repair specific gene mutations in patients,

thereby treating genetic diseases. This personalized treatment approach can significantly enhance therapeutic efficacy, reduce unnecessary side effects, and improve patients' overall quality of life. Furthermore, the CRISPR-Cas system can be utilized for precision gene editing, targeting specific genes to achieve desired therapeutic effects. For instance, researchers can use the CRISPR-Cas system to enhance the anti-tumor capabilities of immune cells for cancer treatment (Das et al., 2022). This precise gene editing method holds the potential for groundbreaking advancements in the treatment of many diseases.

However, personalized medicine and precision gene editing face several challenges. There is a need for a deeper understanding of the relationship between genes and diseases to identify which genes can be edited to achieve therapeutic effects. Further improvement in the precision and efficiency of the CRISPR-Cas system is required to ensure accurate editing of target genes and avoid adverse effects on other genes. Additionally, ethical and legal considerations need thorough attention to ensure the rational and ethical application of personalized medicine and precision gene editing.

3 Challenges Faced by the CRISPR-Cas System

3.1 Safety and side effects

The clinical application of the CRISPR-Cas system indeed presents several challenges, including safety and side effects (Liu et al., 2019). Therefore, corresponding regulatory and safety measures need to be established. Only with full consideration of ethical, legal, and safety issues can the CRISPR-Cas system truly unleash its potential, providing better treatment outcomes and quality of life for patients.

Precision is a crucial concern with the CRISPR-Cas system. Despite its ability to precisely edit target genes, there is still a certain error rate, leading to unintended gene mutations or other adverse effects. Further refinement of the precision of the CRISPR-Cas system is essential to ensure accurate editing of target genes and to avoid adverse effects on other genes.

The CRISPR-Cas system may also induce unpredictable side effects. Genome editing may result in unknown impacts, including effects on other genes, alterations in cellular functions, and potential immune reactions. These side effects could have negative implications for the health of patients. Therefore, more research is needed to understand the long-term safety of the CRISPR-Cas system and to establish corresponding regulatory and safety measures.

Additionally, ethical and legal issues require thorough consideration. The application of the CRISPR-Cas system involves the modification of the human genome, raising a host of ethical and moral concerns. Questions arise regarding the determination of which genes can be edited to treat diseases and how to ensure that edited genes are not misused or employed for non-therapeutic purposes. Furthermore, the development of relevant legal and regulatory frameworks is necessary to ensure that the application of the CRISPR-Cas system is rational and ethical.

3.2 Ethical and moral considerations

The development and application of the CRISPR-Cas system have raised a myriad of ethical and moral considerations (Gonzalez-Avila et al., 2021). When addressing these ethical and moral issues, extensive public discourse and engagement are necessary to ensure that decisions are transparent, inclusive, and aligned with societal consensus. Additionally, the establishment of relevant legal and regulatory frameworks is crucial to ensure that the application of the CRISPR-Cas system occurs within ethical and moral boundaries. Only through comprehensive consideration of ethical and moral issues can the CRISPR-Cas system truly fulfill its potential and benefit humanity.

The CRISPR-Cas system allows for direct editing of the human genome, posing ethical questions. For instance, should gene editing be conducted to correct genetic diseases or enhance human traits? How to determine which genes can be edited, and what are the scope and limitations of editing? These questions involve considerations of respect for human life and dignity, as well as the impact on individuals and society.

While the CRISPR-Cas system enables gene editing in individuals, these changes may also be passed on to future

generations. This raises ethical issues, such as whether germline gene editing should be conducted to transmit changes to the next generation, involving considerations of the impact and responsibility for future generations.

The application of the CRISPR-Cas system may exacerbate social inequalities. For instance, gene editing may become prevalent only among the affluent, leading to increased societal inequality. Additionally, gene editing may result in inequalities between races or ethnic groups. These issues necessitate considerations of fairness, justice, and societal impacts.

The application of the CRISPR-Cas system may also be prone to misuse or non-therapeutic purposes. For example, gene editing may be used for enhancing human traits or creating designer babies, raising ethical questions on how to ensure the ethical and moral application of gene editing and how to establish corresponding regulatory and legal frameworks to prevent misuse.

3.3 Legal and regulatory issues

The development and application of the CRISPR-Cas system face challenges, including legal and regulatory issues. Therefore, international collaboration is essential to establish clear legal and regulatory frameworks, ensuring that the application of the CRISPR-Cas system occurs within safe, reliable, and ethical boundaries. Scientists, doctors, and the public must actively engage in discussions and decision-making processes to ensure that the application of gene editing aligns with societal consensus and values.

Currently, many countries lack explicit legal frameworks for gene editing and the application of the CRISPR-Cas system. This results in legal uncertainty and ambiguity, posing confusion and risks for scientists and doctors engaging in gene editing. Therefore, the establishment of clear legal frameworks is necessary to ensure that the application of gene editing occurs within legal and regulated boundaries.

The application of the CRISPR-Cas system involves the direct editing of the human genome, raising safety and risk concerns. For example, gene editing may lead to unforeseen side effects or unpredictable consequences. Therefore, comprehensive safety and risk assessments are required to ensure that the application of gene editing is safe and reliable.

The application of the CRISPR-Cas system is a global challenge requiring international cooperation and standardization. Different countries and regions may have varying legal and regulatory requirements, creating difficulties for multinational research and applications. Therefore, the establishment of international cooperation mechanisms and the development of common standards are necessary to facilitate the development and application of the CRISPR-Cas system.

Gene editing involves a range of ethical and moral issues, and determining appropriate regulatory and ethical frameworks is a challenge. Regulatory bodies need to formulate clear rules and guidelines to ensure that the application of gene editing occurs within ethical and moral boundaries. Additionally, public engagement and opinions should be incorporated into the regulatory and decision-making processes.

4 Future Directions and Prospects

4.1 Technological improvements and optimization

The CRISPR-Cas system, as a revolutionary gene-editing technology, holds vast prospects and potential, promising transformative changes in fields such as medicine, agriculture, and the environment. With continuous technological improvements and optimization, the CRISPR-Cas system is poised to become a powerful and precise gene-editing tool, making significant contributions to human health and sustainable development.

Currently, the editing efficiency and precision of the CRISPR-Cas system still face certain limitations. Future research and technological improvements will focus on enhancing editing efficiency and precision, aiming to reduce unnecessary side effects and errors. In addition to the commonly used CRISPR-Cas9 system, scientists are developing and refining other types of CRISPR tools and technologies, such as CRISPR-Cas12, CRISPR-Cas13, and more. These new tools and technologies may offer higher editing efficiency, broader genome coverage, and

diversified functionalities.

The CRISPR-Cas system holds tremendous potential in gene therapy and disease treatment. In the future, scientists will continue to explore and develop applications of the CRISPR-Cas system in various disease treatments, including cancer, genetic disorders, and immune system diseases. Currently, the CRISPR-Cas system is primarily used for genome knockout, insertion, and repair. Future research will strive to achieve more precise and intricate genome editing, such as single-base editing and genome rearrangements.

4.2 Interdisciplinary collaboration and knowledge sharing

In the future development of the CRISPR-Cas system, interdisciplinary collaboration and knowledge sharing will play a crucial role. Such collaboration and sharing will drive the advancement of the CRISPR-Cas system, accelerating its applications in medicine, agriculture, environmental sciences, and other fields. Simultaneously, emphasis should be placed on legal and ethical considerations to ensure the application of the CRISPR-Cas system within a rational and sustainable framework. Education and training are also pivotal, cultivating a diverse pool of professionals to promote widespread application and development of the technology.

The application of the CRISPR-Cas system involves various disciplines such as biology, medicine, bioengineering, computer science, among others. In the future, scientists and researchers from different disciplines will strengthen collaboration to collectively address challenges and issues faced by the CRISPR-Cas system across various fields. For instance, biologists can contribute knowledge about genome structure and function, medical experts can provide insights into disease treatment needs and clinical practices, and engineers can offer support in technical improvements and equipment design.

Research and application of the CRISPR-Cas system require extensive data and knowledge support. In the future, scientists will enhance the sharing of data and knowledge related to the CRISPR-Cas system to facilitate research progress and technological innovation. This will contribute to accelerating the development of the CRISPR-Cas system, avoiding redundant efforts, and improving research efficiency.

The widespread application of the CRISPR-Cas system also raises a series of legal and ethical issues. In the future, there is a need to strengthen research and discussions in the legal and ethical aspects, formulate relevant policies and standards, and ensure that the application of the CRISPR-Cas system occurs within a reasonable and sustainable framework. As the CRISPR-Cas system advances, the importance of cultivating scientists and researchers with relevant knowledge and skills becomes paramount. In the future, there is a need to enhance education and training on the CRISPR-Cas system, fostering a greater number of professionals to drive widespread application and development of the technology.

4.3 Application of CRISPR-Cas system in other fields

In addition to the medical field, the CRISPR-Cas system holds vast application prospects in agriculture, bioenergy, environmental protection, biopharmaceuticals, medical technology, and biological research. With continuous technological development and refinement, the CRISPR-Cas system is poised to bring revolutionary changes and innovations to these diverse fields.

The CRISPR-Cas system can be employed to enhance crops by improving their disease resistance, drought tolerance, and yield (Zhu et al., 2020). By editing the genomes of plants, it is possible to create healthier and more nutritionally rich crop varieties. Moreover, the CRISPR-Cas system can also be applied to improve the genes of livestock and poultry, enhancing their disease resistance and productivity, thereby improving agricultural production efficiency and food safety.

The CRISPR-Cas system can be utilized to enhance microorganisms (Javed et al., 2018), enabling more effective production of biofuels and other useful chemicals. Additionally, the CRISPR-Cas system can be applied to modify harmful organisms in the environment, controlling the spread of pests and pathogens, consequently reducing the use of pesticides and environmental pollution.

The CRISPR-Cas system can be used for drug production and disease treatment. Through editing the cell genome, more efficient cell lines for drug production can be created, enhancing the yield and quality of pharmaceuticals. Furthermore, the CRISPR-Cas system can be used in gene therapy, repairing defective genes within patients to treat genetic diseases and conditions such as cancer.

In the field of biological research, the CRISPR-Cas system is instrumental in studying gene function and regulatory mechanisms. By editing the cell genome, researchers can investigate the function and impact of specific genes, unraveling the relationships between genes and biological processes. Additionally, the CRISPR-Cas system can be employed to study cutting-edge areas such as the three-dimensional structure of the genome and epigenetics.

5 Conclusion and Prospects

The CRISPR-Cas system has achieved remarkable progress in both laboratory and clinical settings, showcasing enormous potential. As a technology with tremendous promise, the CRISPR-Cas system is poised to revolutionize fields such as genome editing and gene therapy. It has found widespread applications in genome editing, gene therapy, and disease research. However, further research and efforts are still needed to address the challenges and issues it faces, aiming to realize its goal of widespread application.

The strength of the CRISPR-Cas system lies in its efficient, precise, and flexible gene-editing capabilities, enabling scientists to better understand gene function and disease mechanisms, and develop more effective treatment methods. In laboratory settings, the CRISPR-Cas system has been employed to edit the genomes of various organisms, including cells, animals, and plants. In clinical settings, the CRISPR-Cas system has begun to be applied in gene therapy, providing new treatment options for certain genetic diseases.

The CRISPR-Cas system still encounters challenges and issues that need resolution. One of them is ensuring the accuracy and safety of edits to avoid unnecessary side effects and unintended genetic alterations. Additionally, improvements in delivery efficiency and specificity of the CRISPR-Cas system are necessary to ensure its feasibility and reliability in clinical applications.

Looking ahead, the development trajectory and application prospects of the CRISPR-Cas system are vast. Beyond the medical field, it can play a crucial role in agriculture, bioenergy, environmental protection, and biological research. With continuous technological development and refinement, we can anticipate greater breakthroughs and applications of the CRISPR-Cas system in genome editing, gene therapy, and disease research.

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