

The Application Prospects of CRISPR-Cas9 Technology in Gene Therapies

Wang Jianer ✉

Infinite (China) Co., Ltd., Zhuji, 31180, China

✉ Corresponding author email: 1136425476@qq.com

International Journal of Molecular Medical Science, 2024, Vol.14, No.1 doi: [10.5376/ijmms.2024.14.0002](https://doi.org/10.5376/ijmms.2024.14.0002)

Received: 01 Dec., 2024

Accepted: 07 Jan., 2024

Published: 19 Jan., 2024

Copyright © 2024 Wang. This is an open access article published under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Preferred citation for this article:

Wang J.E., 2024, The application prospects of CRISPR-Cas9 technology in gene therapy, International Journal of Molecular Medical Science, 14(1): 8-15 (doi: [10.5376/ijmms.2024.14.0002](https://doi.org/10.5376/ijmms.2024.14.0002))

Abstract The CRISPR-Cas9 technology, as a revolutionary gene-editing tool, has achieved remarkable breakthroughs, significantly impacting the field of gene therapy. This review provides an overview of the fundamental principles and technological developments of CRISPR-Cas9, tracing the progress in gene therapy and extensively discussing its applications in the treatment of monogenic inherited diseases and the prospective role in cancer therapy. Additionally, the review delves into the potential applications of CRISPR-Cas9 technology in organ transplantation and regenerative medicine, elucidating the challenges and ethical considerations it faces. While summarizing the research progress of CRISPR-Cas9 technology, the review also looks ahead to the future prospects of CRISPR-Cas9 in gene therapy. This comprehensive review serves as a valuable reference for the innovation and significance of CRISPR-Cas9 technology in the field of gene therapy, providing insights to propel further development and application in this prominent area.

Keywords CRISPR-Cas9 technology; Gene therapy; Genetic diseases; Cancer treatment; Organ transplantation

Gene therapy, as a revolutionary medical strategy, is offering new hope for human health. With the continuous development and breakthroughs of gene editing technology, CRISPR-Cas9 technology has attracted widespread attention and research as an emerging gene editing tool (Zhang, 2021). The advent of CRISPR-Cas9 technology has brought about revolutionary changes in gene therapy. Its efficient, precise, and customizable characteristics make it an ideal gene editing tool, opening up novel prospects for gene therapy.

Gene therapy aims to treat, prevent, or alleviate genetic and other diseases by directly repairing abnormal genes or inserting normal genes into the patient's body. However, gene therapy has long faced several challenges, such as the selection of effective vectors, the precision and safety of gene editing, among others. Traditional gene editing techniques, such as zinc finger nucleases and TALENs, have made significant progress, but they still have certain limitations in terms of precision, efficiency, and operability. In comparison, CRISPR-Cas9 technology has higher precision and manipulability, making gene therapy more feasible and practical.

The CRISPR-Cas9 technology has opened a new era in the field of gene editing by harnessing the CRISPR RNA from the natural immune system of bacteria to guide the Cas9 nuclease in gene cutting and editing. The core of this technology lies in the design and synthesis of a short nucleotide sequence, allowing the CRISPR-Cas9 system to accurately recognize and target specific loci in the genome for editing. Compared to traditional gene editing tools, CRISPR-Cas9 technology offers higher manipulability and efficiency, enabling the editing and repair of target genes within a relatively short timeframe.

This review aims to comprehensively explore the prospects of CRISPR-Cas9 technology in gene therapy. Challenges faced by CRISPR-Cas9 technology, such as issues related to technical accuracy, non-specific cleavage, and ethical concerns, are discussed. The paper summarizes current research progress and anticipates the future potential of CRISPR-Cas9 technology in gene therapy. It is believed that CRISPR-Cas9 technology will catalyze a revolution in the field of gene therapy, bringing about significant changes and hope for human health. Through this review, we hope to provide a deeper understanding of the importance and innovativeness of CRISPR-Cas9 technology in the field of gene therapy, serving as a reference to further drive the development and application of CRISPR-Cas9 technology.

1 The Basic Principles and Technological Innovations of CRISPR-Cas9 Technology

1.1 Discovery and evolution of CRISPR-Cas9 technology

CRISPR-Cas9 technology was initially discovered in bacterial genomes (Zhu et al., 2019). In the 1990s, researchers identified a class of repetitive sequences called CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), the function of which remained unknown for a long time. Subsequent studies revealed that CRISPR sequences were highly similar to viral sequences in bacteriophage or plasmid genomes. Further research has found that the CRISPR immune system is a natural defense mechanism of bacteria against viral invasions. The CRISPR-Cas system consists of CRISPR sequences and associated Cas proteins, with Cas proteins being crucial factors capable of recognizing and cleaving foreign DNA.

In 2012, Emmanuelle Charpentier, along with a research team from Stanford University, discovered a type II CRISPR-Cas system, which includes a protein called Cas9. The Cas9 protein exhibits highly effective gene editing capabilities. The CRISPR-Cas9 technology has garnered significant attention and widespread application due to its simplified design, convenient operation, precise gene editing, broad applicability, and relatively low cost and time consumption.

The further development and improvement of CRISPR-Cas9 technology are ongoing. Scientists through in-depth studies of the structure and function of the Cas9 protein, have developed various Cas9 variants, such as nuclease-dead Cas9 (dCas9) and high-fidelity Cas9. Additionally, the introduction of modified single-guide RNA (sgRNA) and chemically modified primers has further enhanced the efficiency and precision of gene editing.

1.2 Basic principles and working mechanism of CRISPR-Cas9 technology

CRISPR sequences consist of a set of repetitive DNA sequences and interspersed viral or plasmid DNA segments derived from bacterial or archaeal genomes. These sequences serve as the immune system of bacteria, used to store previously encountered foreign DNA segments to defend against future viral attacks. Through a series of DNA modifications and RNA processing steps, the CRISPR sequences are transcribed into a type of RNA molecule called CRISPR RNA (crRNA).

In the CRISPR-Cas9 system, scientists design and construct an artificially synthesized RNA molecule called single-guide RNA (sgRNA). It is formed by the fusion of crRNA and a transcribed RNA segment known as tracrRNA. This sgRNA can bind to the Cas9 protein, forming a complex with the ability to guide Cas9 to recognize and bind to specific DNA sequences (Wang et al., 2019).

The Cas9 protein is a central component of the CRISPR-Cas9 system, possessing DNA binding and cleavage functions. Once the sgRNA binds to the Cas9 protein, the complex can recognize a specific region of the target DNA sequence that pairs with the sgRNA. Upon binding to the specific DNA sequence, the Cas9 protein induces a double-strand break, resulting in alterations to the genomic DNA sequence. Following the formation of the double-strand break, the cell initiates its own DNA repair mechanism, attempting to mend the break. Through different repair mechanisms, such as non-homologous end joining or homologous recombination, precise editing of the target gene can occur, leading to insertions, deletions, or replacements in the genome (Figure 1).

1.3 Comparison between CRISPR-Cas9 and traditional gene editing technologies

CRISPR-Cas9 exhibits significant advantages compared to traditional gene editing technologies. The CRISPR-Cas9 technology allows for highly precise gene editing. Different from traditional methods, Cas9 protein can accurately locate and cleave the target DNA sequence by designing appropriate sgRNA. CRISPR-Cas9 technology is highly flexible, as changing the target gene simply involves replacing the sgRNA sequence, whereas traditional methods often require multiple optimizations. Additionally, CRISPR-Cas9 technology is cost-effective and time-saving, utilizing sgRNA and Cas9 protein as the main tools without the need for expensive recombinant enzymes and reagents. Lastly, CRISPR-Cas9 technology is applicable to a variety of organisms, including cells and whole organisms, while traditional methods are typically limited to specific cell types or species (Cao et al., 2020).

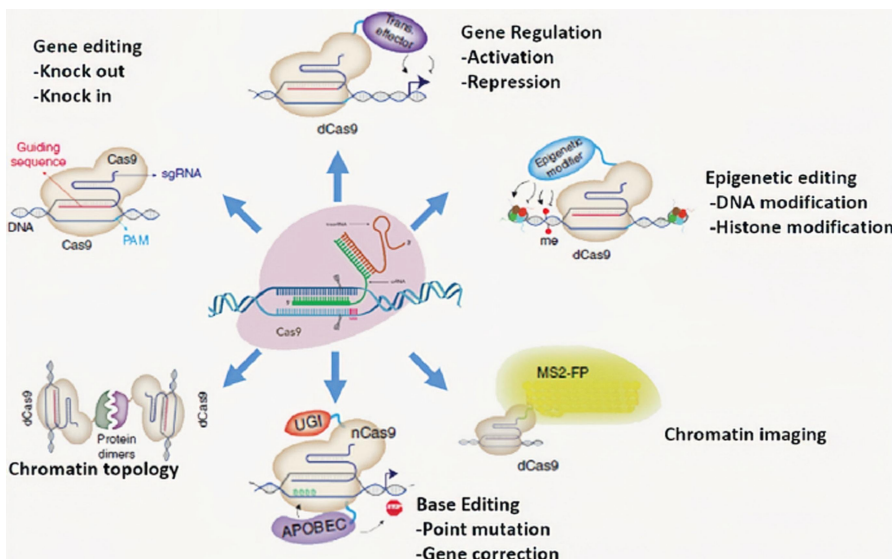


Figure 1 Application of CRISPR/Cas9

Despite the numerous advantages of CRISPR-Cas9 technology, it also faces several challenges. For instance, unintended cutting or editing events may occur, leading to unexpected genetic alterations. Additionally, the application of CRISPR-Cas9 technology in specific tissues or cell types poses challenges. However, with ongoing technological improvements, it is foreseeable that CRISPR-Cas9 will become a powerful and widely utilized gene editing tool. In summary, CRISPR-Cas9 offers higher precision, flexibility, cost-effectiveness, and shorter working times compared to traditional gene editing techniques.

2 The History and Development of Gene Therapy

2.1 Overview of traditional gene therapy approaches

Traditional gene therapy methods refer to non-CRISPR-related techniques used to repair or regulate human genes. These approaches hold potential in the treatment of certain diseases, particularly monogenic disorders. However, they also face challenges such as low delivery efficiency, cell specificity, immune responses, and long-term stability. Traditional gene therapy methods include gene replacement, gene correction, and gene suppression, among others.

Gene replacement involves introducing a normal gene into a patient's body to replace a defective gene. This can be achieved by delivering the normal gene into the patient's cells using vectors, such as viral vectors or plasmid DNA. Once the normal gene is introduced and expressed, it can correct the genetic defect and restore normal function.

Gene correction refers to the correction or modification of erroneous gene sequences to rectify genetic defects. This can be achieved by using DNA modifying enzymes such as zinc finger nucleases and transcription active factors (Belete, 2021).

Gene suppression involves inhibiting the expression of a defective gene through the use of interfering RNA (siRNA) or antisense RNA. Interfering RNA is a short RNA molecule that can interact with specific gene sequences in patient cells, selectively reducing or inhibiting the expression of the targeted gene. Antisense RNA is an RNA molecule that prevents its translation into a protein by interacting with the mRNA molecule of the target gene.

2.2 Progress and limitations of gene therapy

Gene therapy is an emerging therapeutic approach that has made significant strides over the past few decades. In terms of clinical applications, gene therapy has successfully been used for some genetic diseases, such as severe combined immunodeficiency and familial hypercholesterolemia (Godbout and Tremblay, 2023). Furthermore, the genetic engineering of patient T cells to express chimeric antigen receptors (CARs) has been successfully applied

in the treatment of leukemia. Gene therapy has also shown improvement in ocular diseases like retinal pigmentosa and hereditary optic neuropathy.

However, gene therapy also faces limitations and challenges. Delivery efficiency remains a challenge, and improvements are needed to effectively deliver therapeutic genes to target cells and tissues. Safety is another crucial consideration, with attention required for immune reactions, inflammatory responses, or potential adverse events. Ensuring stable expression and persistence of the therapeutic gene, as well as addressing individual variability, are also issues that need resolution.

Additionally, the high cost of gene therapy restricts its widespread application, and accessibility is further limited by technical requirements and healthcare resource constraints. Moreover, gene therapy involves ethical and moral considerations, such as privacy protection and fair distribution. Despite the challenges and limitations, gene therapy is a promising field that provides new options for the treatment of many diseases. With advances in scientific technology and accumulated experience, it is believed that gene therapy will continue to evolve in the future, bringing benefits to more patients.

2.3 Breakthrough of CRISPR-Cas9 technology and comparison with traditional gene therapy approaches

CRISPR-Cas9 technology is a revolutionary gene editing tool that offers numerous breakthrough advantages compared to traditional gene therapy methods. CRISPR-Cas9 enables precise gene editing in the genome, allowing for gene knockout, repair, and insertion operations. In contrast, traditional gene therapy typically involves gene addition or replacement. CRISPR-Cas9 is characterized by its flexibility and versatility, making it widely applicable in both research and treatment. This technology achieves targeted editing of specific DNA sequences by designing guide RNA, enhancing treatment specificity—a feature often lacking in traditional gene therapy methods. CRISPR-Cas9 technology can directly perform gene editing *in vivo*, enabling patients to receive treatment without the need for *ex vivo* cells or gene delivery vectors, thereby enhancing treatment efficiency and convenience (Chen et al., 2019).

While CRISPR-Cas9 technology faces some challenges and safety risks, it is considered to have groundbreaking potential, opening up new possibilities in the field of gene therapy. It provides a more precise, flexible, and efficient approach to gene therapy, offering promising prospects for the treatment of genetic diseases and advancements in biological research. As this technology continues to develop and improve, it is believed to bring forth further innovations and progress in the field of gene therapy.

3 CRISPR-Cas9 Technology in the Prospects of Gene Therapy

3.1 Application in the treatment of monogenic genetic diseases

CRISPR-Cas9 technology has shown promising progress in the treatment of monogenic genetic diseases (Xu, 2020). Cystic fibrosis, a common genetic disease, can be corrected using CRISPR-Cas9 technology to rectify mutations in the human CFTR gene, restoring its function, which has been successful in both *in vitro* and *in vivo* studies. Similarly, for certain genetic blood disorders such as sickle cell anemia and β -thalassemia, CRISPR-Cas9 technology can be applied to correct patients' hematopoietic stem cells, preventing or alleviating disease symptoms. Additionally, immune deficiency disorders like severe combined immunodeficiency can be addressed by using CRISPR-Cas9 technology for gene editing to restore patients' immune function. For neurological disorders such as Huntington's disease, amyotrophic lateral sclerosis, and hereditary retinal dystrophy (Figure 2), CRISPR-Cas9 is also being researched for potential therapeutic applications. While the application in the treatment of monogenic genetic diseases faces some challenges, CRISPR-Cas9-based therapies are still regarded as a highly promising field, offering the potential for more effective and personalized treatment options for patients.

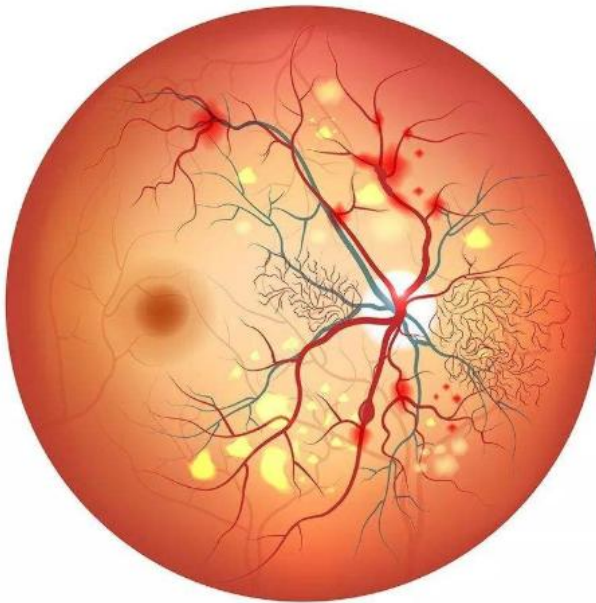


Figure 2 Retinitis Pigmentosa

3.2 Application in cancer treatment

CRISPR-Cas9 technology holds potential breakthrough applications in cancer treatment. Among them, It can be used to suppress the growth and division of cancer cells through gene editing, disrupting the survival and proliferation capabilities of malignant tumor cells by precisely editing cancer-related genes. Additionally, CRISPR-Cas9 can enhance immunotherapy by editing and modifying patients' immune cells to improve their anti-tumor effects, including knocking out immune checkpoint genes and enhancing CAR-T cell therapies. Furthermore, CRISPR-Cas9 can be employed to restore or correct inactivated or mutated tumor suppressor genes, impeding the development and proliferation of tumors. While the application in cancer treatment still faces challenges, CRISPR-Cas9 technology is poised to become a more effective and personalized therapeutic strategy, offering improved treatment outcomes and survival opportunities for cancer patients. However, further research and clinical trials are still needed to assess the safety and long-term efficacy of CRISPR-Cas9 in cancer treatment.

3.3 Potential applications in organ transplantation and regenerative medicine

CRISPR-Cas9 technology demonstrates significant potential applications in the fields of organ transplantation and regenerative medicine (Yu et al., 2021). It can reduce the risk of rejection during organ transplantation and enhance the success rate by gene editing. The technology can precisely edit specific genes in donor and recipient cells, such as HLA genes, thereby increasing the compatibility of transplanted organs. CRISPR-Cas9 plays a crucial role in promoting organ regeneration and repair. By editing and activating specific genes in stem cells, it can accelerate and improve their ability to differentiate into specific cell types, thus facilitating the process of organ regeneration and repair. Moreover, this technology can be applied to organ engineering and in vitro organ cultivation, producing customized organ substitutes that meet the specific needs of individual patients by editing and modifying donor cells or regenerated stem cells.

Despite facing technical and ethical challenges in this field, CRISPR-Cas9 holds tremendous potential. This technology is poised to offer new solutions to alleviate organ shortage issues and provide better treatment options for patients. Future research and clinical experiments will further propel the practical application of CRISPR-Cas9 technology in the fields of organ transplantation and regenerative medicine, assessing its safety and feasibility.

4 Potential Applications and Challenges of CRISPR-Cas9 Technology

4.1 Accuracy issues

The accuracy of CRISPR-Cas9 technology in gene editing is a crucial topic of discussion. Despite its widespread application and remarkable achievements, there are challenges related to its accuracy. The specificity of the

CRISPR-Cas9 editing system may be problematic, as it can lead to non-specific editing due to the presence of similar sequences or homologous genes in the genome. Non-specific edits have the potential to impact the function of other genes, resulting in potential adverse effects. There are also limitations in the selection of target sequences for CRISPR-Cas9 technology. Designing appropriate single-guide RNA (sgRNA) is a crucial factor in ensuring the correct editing of the target gene. However, due to the complexity and diversity of the genome, identifying an accurate and specific sgRNA is not always straightforward. Moreover, incorrect binding during the repair process and repair errors can lead to inaccurate gene editing. The CRISPR-Cas9 system typically relies on the natural repair mechanisms within cells to mend DNA breaks. However, this repair process may introduce unpredictable insertions, deletions, or other erroneous modifications, resulting in unexpected outcomes. In terms of clinical applications, gene editing and gene therapy have made significant strides. Clinical trials for some genetic diseases, such as cystic fibrosis and hereditary retinal disorders, have been conducted using gene editing or gene therapy, yielding promising results. These studies have laid the groundwork for further exploration of the clinical applications of gene editing and gene therapy.

To address the accuracy issues of CRISPR-Cas9 technology, researchers are actively pursuing various improvements and innovations. For instance, the introduction of modified Cas9 protein variants or strategies, such as the use of dual RNA forms, aims to enhance specificity. Additionally, the development of better sgRNA design and specificity analysis methods contributes to improved accuracy. Despite the challenges faced by CRISPR-Cas9 technology in terms of accuracy, ongoing efforts within the scientific community are focused on refining and optimizing the technique. With advancements in technology and deeper research, it is believed that the accuracy of CRISPR-Cas9 technology will continue to improve, presenting more opportunities and prospects for gene editing research and applications.

4.2 Non-specific cleavage and safety concerns

The non-specific cleavage and safety issues of CRISPR-Cas9 technology are crucial considerations in its application. Non-specific cleavage refers to the potential cutting action of the CRISPR-Cas9 system at locations outside the target DNA sequence. This may result in unintended genome editing, potentially affecting the functionality of other genes. The side effects of non-specific cleavage may lead to unexpected genetic mutations or other chromosomal abnormalities, potentially causing harm to the normal functioning of cells or organisms.

The repair processes introduced by CRISPR-Cas9 technology in genome editing can lead to unexpected outcomes. After the Cas9 protein cuts the target DNA, cellular repair mechanisms come into play to mend the DNA breaks. However, errors may occur during the repair process, resulting in insertions, deletions, or other changes in genome structure. These changes can have unforeseen effects, including the introduction of unstable genomic alterations, activation of undesirable gene expression, or potential pathogenic mutations.

CRISPR-Cas9 technology also faces safety considerations. Ensuring the accuracy and specificity of the CRISPR-Cas9 system is crucial before direct application in the human body. As CRISPR-Cas9 technology is still in development, precise control and monitoring of the effects of target gene editing are essential to minimize potential adverse consequences and unexpected outcomes.

To address these issues, researchers are making efforts to enhance the specificity and safety of CRISPR-Cas9 technology. For instance, reducing the occurrence of non-specific cleavage events can be achieved by introducing plasmid restriction enzymes or using specific Cas9 variants. Furthermore, a more comprehensive evaluation and preclinical studies of the CRISPR-Cas9 system to assess potential side effects and long-term safety are crucial steps in ensuring the safety of its application.

4.3 Ethical and moral issues and public acceptance

CRISPR-Cas9 technology has raised numerous ethical and moral concerns, impacting on its public acceptance. The gene-editing capability of CRISPR-Cas9 technology has sparked ethical and moral controversies regarding human genetic modification. The genetic alteration of human embryos or reproductive cells, resulting in modified and improved genes transmitted through the individual's genetic lineage, has triggered concerns about the

long-term effects and ethical consequences of human genetic changes (Wang, 2018). The potential for such genetic modifications has ignited ethical debates involving fundamental values such as human dignity, autonomy, social fairness, and justice.

The widespread application of CRISPR-Cas9 technology has also raised concerns among the public. The public is concerned that this technology may be abused or misused, such as through unnecessary genetic modification or selective gene editing. These concerns are linked to the feasibility, safety, grey-area applications, and potential issues of inequality associated with the technology. Public acceptance of gene editing technology may be influenced by these concerns, necessitating broad public education and engagement to foster understanding and acceptance of the technology. Additionally, CRISPR-Cas9 technology involves issues related to intellectual property, commercialization, and drug regulation, which also impact ethical considerations and public acceptance. Protecting the use of innovative and inclusive technologies, ensuring a balance of public interest, is an important ethical challenge.

To address the ethical concerns of CRISPR-Cas9 technology and enhance public acceptance, collaborative efforts from various stakeholders are essential. The scientific community, government bodies, legal institutions, ethics committees, and the public should engage in robust discussions and collaborate to formulate relevant regulations, ethical guidelines, and policies to govern the research and application of the technology. Simultaneously, enhancing scientific communication and public education is crucial to enable the public to understand and participate in discussions surrounding gene editing technology. This ensures that the development and application of the technology align with the expectations and values of the broader public.

5 Summary and Outlook

CRISPR-Cas9 technology holds vast prospects and significance in gene therapy. With its efficient, precise, and customizable features, this technology provides a revolutionary tool for gene therapy. By harnessing the "scissors" function of the CRISPR-Cas9 system, scientists can accurately edit and repair defects in the genome, offering the potential to treat or even cure numerous genetic diseases. This implies that CRISPR-Cas9 technology may bring hope to patients with conditions such as blood disorders, genetic blindness, cystic fibrosis, and more, opening up new possibilities for gene therapy.

Furthermore, CRISPR-Cas9 technology holds significant promise in the fields of cancer treatment and reproductive health. In the realm of cancer, CRISPR-Cas9 technology can be employed for the specific targeting of cancer cells to inhibit tumor growth, induce cell apoptosis, or enhance the immune system's attack on tumors. This provides a potential avenue for the development of novel cancer treatment approaches. Additionally, CRISPR-Cas9 technology can be applied in the field of reproductive health to repair embryos or reproductive cells carrying genetic mutations, thereby preventing the transmission of hereditary diseases. This offers possibilities for the prevention and treatment of hereditary diseases within families.

However, realizing the potential of CRISPR-Cas9 technology requires further research and collaboration. Firstly, scientists need to continuously improve the specificity and precision of the technology to reduce non-specific cleavage and enhance editing accuracy, thereby mitigating potential safety risks. Secondly, interdisciplinary collaboration is crucial, involving scientists, government agencies, ethics committees, and the public, to collectively explore and establish relevant policies, ethical guidelines, and standards. This ensures that CRISPR-Cas9 technology aligns with ethical standards in medical practice and garners public support and acceptance. Additionally, ongoing efforts to strengthen scientific communication, public education, and ethical societal discussions are imperative. These efforts aim to increase public understanding and sustained interest in CRISPR-Cas9 technology while simultaneously establishing corresponding safety and regulatory mechanisms to ensure its safe, effective, and sustainable application.

Acknowledgments

Special thanks to Ms. Xuan Jia and Ms. Lingfei Jin for their guidance and review of the paper. Many of their constructive suggestions have greatly contributed to the refinement of this research.

References

- Belete T.M., 2021, The Current Status of Gene Therapy for the Treatment of Cancer, *Biologics: Targets and Therapy*, 15: 67-77.
<https://doi.org/10.2147/BTT.S302095>
PMid:33776419 PMCID:PMC7987258
- Cao J.X., Wang Y.L., and Wang Z.X., 2020, Advances in precise regulation of CRISPR/Cas9 gene editing technology, *Yichuan (Hereditas)*, 42(12): 1168-1177.
- Chen X., Chen L., and Li D.L., 2019, Research progress of gene therapy in clinical application, *Shengwu Gongcheng Xuebao (Chinese Journal of Biotechnology)*, 35(12): 2295-2307.
- Godbout K., and Tremblay J.P., 2023, Prime editing for human gene therapy: where are we now? *Cells*, 12(4), 536.
<https://doi.org/10.3390/cells12040536>
PMid:36831203 PMCID:PMC9954691
- Wang C.P., 2018, Clinical ethics of human embryo gene editing mediated by CRISPR/Cas9 technology, *Ziran Bianzhengfa Tongxun (Journal of Dialectics of Nature)*, 40(11), 105-112.
- Wang H.Y., Yang X.F., Hu C.F., and Li F.R., CRISPR/Cas9 gene editing in diabetes cell therapy: recent advances, *Shengming Kexue (Chinese Bulletin of Life Sciences)*, 31(7): 723-730.
- Xu Y. Y., 2020, Current status and challenges of gene therapy products, *Zhongguo Shengwu Gongcheng Zazhi (China Biotechnology)*, 40(12): 95-103.
- Yu D.H., Cao H., and Wang X.R., 2021, Advances and applications of organoids: a review, *Shengwu Gongcheng Xuebao (Chinese Journal of Biotechnology)*, 37(11): 3961-3974.
- Zhang B.H., 2020, CRISPR/Cas gene therapy, *Journal of Cellular Physiology*, 236(4): 2459-2481.
<https://doi.org/10.1002/jcp.30064>
PMid:32959897
- Zhu P.Q., Jiang W.D., and Zhou N., 2019, CRISPR/ Cas9 system and its application in medical research, *Zhongguo Bijiao Yixue Zazhi (Chinese Journal Of Comparative Medicine)*, 29(2): 116-123.