The Application Progress, Challenges, and Future Directions of Gene Editing Technology in Immunotherapy

Jiayi Zou *

Dalian Royal School, Dalian, China

* Corresponding Author Email: Julie.zou.jy@student.dalianroyalschool.com

Abstract. In recent years, gene editing technologies, especially CRISPR-Cas9, have become key tools for enhancing the effectiveness of immunotherapy and are widely applied in the treatment of cancer, viral infections, and genetic diseases. By improving the specificity and durability of immune cells, gene editing boosts the efficacy of anti-tumor therapies, such as CAR-T cell therapy, and optimizes immune checkpoint inhibitors to enhance immune response and inhibit cancer immune evasion. Moreover, CRISPR demonstrates significant potential in antiviral immunity, particularly achieving breakthroughs in targeting HIV and other viral replication mechanisms. Despite the promising outlook of gene editing technology in clinical applications, it still faces challenges such as off-target effects, ethical concerns, and technical limitations. Researchers are actively exploring new editing tools (e.g., Cas12, Cas13) and delivery systems (e.g., lipid nanoparticles, adeno-associated viruses) to improve editing accuracy and safety. With the integration of gene editing with multiple therapies, the advancement of personalized medicine, and improvements in delivery technology, gene editing is expected to achieve wider clinical application in the future, drive the progress of precision medicine, and offer innovative treatment strategies for hard-to-treat cancers and genetic diseases.

Keywords: Gene Editing; CRISPR-Cas9; Immunotherapy; Cancer Treatment; Personalized Medicine.

1. Introduction

In recent years, immunotherapy has attracted increasing attention in the field of tumor treatment. Unlike traditional methods such as surgery, radiotherapy, and chemotherapy, immunotherapy primarily works by modulating the body's immune system to encourage immune cells to identify and eliminate tumor cells, thereby restoring the patient's antitumor capabilities. Specifically, immunotherapy can either cultivate immune cells in vivo to directly attack tumors or prepare antitumor immune cells ex vivo or then infuse them into the patient for treatment [1]. This therapy not only allows for more precise targeting and control of tumor cells but also activates immune memory, enabling continuous surveillance and elimination of recurring tumor cells. Consequently, immunotherapy is increasingly regarded as a frontline treatment option, especially displaying notable advantages in combating resistant and advanced tumors [2].

With the successful application of immune checkpoint inhibitors, such as anti-CTLA-4 and anti-PD-1 antibodies, the status of immunotherapy in tumor treatment has been solidified, marking the beginning of a new era in cancer treatment. Immune checkpoint inhibitors block the immune evasion mechanisms of tumor cells, enabling the immune system to persistently eliminate residual tumor cells and restore immune functions compromised by radiotherapy and chemotherapy. Extensive clinical research has shown that immunotherapy not only improves patient survival rates but also enhances quality of life, offering hope for long-term tumor suppression and even complete eradication of tumors [3].

In this context, gene editing technology has gradually become a key tool for enhancing the efficacy of immunotherapy. Gene editing not only modifies immune cell functions but also reduces side effects during treatment. For example, in chimeric antigen receptor T-cell (CAR-T) therapy, CRISPR gene editing is employed to knock out endogenous T-cell receptors and human leukocyte antigens (HLA) in donor T cells, thereby reducing the risks of graft-versus-host disease and immune rejection. Additionally, gene editing is widely applied in DNA vaccine development by inserting genes encoding tumor antigens into vectors and introducing them into host cells to induce a specific immune response [4].

However, despite the breakthrough gene editing has brought to immunotherapy, certain challenges remain in its practical application. Off-target effects and potential gene mutations may lead to unintended side effects, affecting the safety and efficacy of treatment. Additionally, immune evasion mechanisms in tumor cells could significantly undermine the effectiveness of gene editing. Therefore, while advancing the application of gene editing technology, it is essential to conduct further research into its potential risks and refine the technology to optimize its role in immunotherapy [5].

This review explores the application of gene editing technology, particularly CRISPR/Cas9, in immunotherapy, with a focus on its potential to enhance immune efficacy, reduce side effects, and enable personalized treatment. Furthermore, this paper discusses recent advancements and future potential applications of gene editing in antitumor, antiviral, and other disease treatments.

2. Basic Principles and Advances of Gene Editing Technology

2.1. Principles of the CRISPR-Cas9 System

The CRISPR-Cas9 system is one of the most prominent gene-editing technologies in recent years, originating from the immune systems of bacteria and archaea, initially used to defend against invading viruses and plasmids. The CRISPR locus consists of repetitive sequences and spacer sequences, with the former arranged uniformly in the host genome and the latter recording DNA fragments from foreign invaders. Cas genes encode Cas proteins, which work in conjunction with CRISPR sequences to form a powerful DNA-targeting tool. The working mechanism of the CRISPR-Cas9 system can be divided into three stages: spacer acquisition, CRISPR expression, and target interference [6, 7].

First, in the spacer acquisition stage, bacteria integrate a small segment of invading viral DNA into the CRISPR locus, forming a new spacer sequence. This process is primarily mediated by Cas1 and Cas2 proteins, which identify and cut the PAM sequence of foreign DNA and integrate it near the leader region of the CRISPR locus.

The next stage is CRISPR expression, where the CRISPR locus is transcribed to produce pre-crRNA, which pairs with tracrRNA through complementary base pairing to form a double-stranded RNA complex and then binds with the Cas9 protein. RNase III cleaves pre-crRNA, ultimately producing a mature crRNA complex, which prepares for targeted DNA cleavage.

The final stage, target interference, involves the mature crRNA complex pairing with corresponding spacer sequences in foreign DNA via complementary base pairing and relying on the nuclease activity of Cas9 protein to cut the DNA near the PAM sequence. The two domains of Cas9 protein, HNH and RuvC, respectively cleave the complementary and non-complementary DNA strands, causing a double-strand to break in the foreign DNA and completing the gene-editing process [2].

2.2. Other Gene Editing Technologies

In addition to CRISPR-Cas9, zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) are also important gene-editing tools. ZFNs function by binding specific DNA sequences through a zinc finger DNA-binding domain and then utilizing the DNA cleavage domain of a restriction endonuclease to achieve targeted DNA breakage. Although ZFN technology is relatively mature, its high off-target rate and complex design process limit its application.

TALENs, on the other hand, are based on the invasion mechanism of plant pathogens, utilizing effector proteins secreted by bacteria that specifically bind to promoter regions in the host genome to regulate gene expression. TALENs achieve efficient gene editing by linking FokI nucleases with customized TALEs and are widely used in plant and animal cells. However, the complex modular assembly and high cost of TALENs make them challenging to use extensively.

Although CRISPR-Cas9, ZFN, and TALEN have important applications in the field of gene editing, CRISPR-Cas9 has gradually become the mainstream tool due to its simplicity, efficiency, and low cost. While ZFN and TALEN technologies still have specific applications, their complex design and high off-target rates make them less competitive [1].

2.3. Recent Technological Advances

In recent years, gene editing technology has achieved significant advancements, particularly in the further optimization of the CRISPR system. For instance, CRISPR-Cas12 and Cas13 systems have been developed for DNA and RNA editing, respectively. Cas12 has higher cutting precision and is suitable for specific DNA modification needs, while Cas13 focuses on RNA targeting, opening new possibilities for treating RNA viral diseases. The emergence of these new CRISPR tools has significantly improved the flexibility and efficiency of gene editing [8, 9].

Furthermore, improvements to CRISPR technology have focused on reducing off-target effects and increasing specificity. In recent years, developments such as high-fidelity Cas9 (HiFi Cas9) and enhanced Cas9 variants have substantially lowered the occurrence of unintended mutations during editing. The advent of these new tools has further unlocked the potential of gene editing technology in biomedicine, driving the rapid advancement of precision medicine [10].

3. Applications of Gene Editing Technology in Immunotherapy

3.1. CAR-T Cell Therapy

CAR-T cell therapy is one of the most significant applications of gene editing technology in immunotherapy. By using CRISPR-Cas9 technology to modify T cells, they can be engineered to express chimeric antigen receptors (CAR), enabling these T cells to recognize and attack specific cancer cells. This technology has shown remarkable efficacy in treating hematological cancers, such as acute lymphoblastic leukemia.

The application of CRISPR-Cas9 in CAR-T therapy primarily involves editing T cell genes to enhance their targeting specificity and antitumor effects. For example, researchers have knocked out the PD-1 receptor on T cells to block the immune evasion mechanism of tumor cells, thereby boosting the killing ability of CAR-T cells. Additionally, CRISPR can be used to increase the persistence of CAR-T cells, reducing the risk of autoimmune attacks. These technical advancements have greatly improved the effectiveness of CAR-T cell therapy, presenting promising prospects for cancer treatment [7, 11].

3.2. Optimization of Immune Checkpoint Inhibitors

Immune checkpoint inhibitors represent another important application of gene editing technology in immunotherapy. PD-1/PD-L1 inhibitors work by reversing the suppression of immune responses by tumor cells, reactivating T cells to effectively attack cancer cells. CRISPR technology can optimize this treatment strategy by precisely editing immune checkpoint molecules [4].

In recent years, researchers have used CRISPR to knock out the PD-1 gene in T cells, significantly enhancing their antitumor functionality. This genetic modification not only improves the efficacy of immunotherapy but also reduces the side effects associated with the prolonged use of immune checkpoint inhibitors. Additionally, gene editing technology can be used to investigate the functions of other immune checkpoint molecules, such as LAG-3, thereby further expanding the scope of immunotherapy applications [7].

3.3. Applications in HIV and Other Viral Infections

Gene editing technology has also shown immense potential in antiviral immunotherapy. Specifically in the field of HIV treatment, the CRISPR-Cas9 system has been used to modify the CCR5 receptor gene, thereby preventing the HIV virus from entering host cells. This strategy, which edits critical receptors on the surface of immune cells, equips these cells with resistance to viral infections, marking a groundbreaking advancement in HIV treatment [12].

Beyond HIV, CRISPR technology has been applied to treat other viral infections as well. For instance, CRISPR-Cas13 can be used to target viral RNA, enabling precise inactivation of RNA viruses, which opens up new avenues for treating viral infections such as influenza and hepatitis B. These applications broaden the potential of gene editing technology in infectious disease treatment, making it a powerful tool for addressing global health challenges [11, 13].

4. Challenges of Gene Editing Technology in Immunotherapy

4.1. Risks of Off-Target Effects

Gene editing technologies such as CRISPR-Cas9, TALENs, and ZFNs, while powerful tools in modern biomedicine, still face significant challenges with off-target effects. Off-target effects refer to unintended modifications in DNA sequences outside the target site by gene-editing tools. Such unintended alterations can lead to mutations, posing serious safety concerns. For instance, certain critical genes might be inadvertently disrupted, resulting in cellular dysfunction, cancerous changes, or other adverse reactions. During drug development, off-target effects may impact the drug's effectiveness and increase toxicity. Therefore, early identification and reduction of off-target effects are essential to ensure the safety of gene-editing therapies [3].

While recent technological advancements, such as high-fidelity CRISPR-Cas9 variants, have significantly reduced off-target effects, completely eliminating this risk remains an unresolved challenge. For example, the Cas9 protein recognizes sequences with approximately 20 base pairs, making it susceptible to misrecognition within the genome’s many similar sequences. Additionally, DNA cleavage in the CRISPR-Cas system often relies on the cell's own repair mechanisms, which may not always accurately repair the damaged DNA, potentially resulting in unpredictable editing outcomes [11].

4.2. Ethical and Legal Challenges

The rapid development of gene-editing technology has raised numerous ethical and legal issues, particularly regarding human genome editing. For instance, germline gene editing, which alters the genes of reproductive cells or embryos, has sparked intense debates concerning the rights of future generations. Opponents argue that such editing could infringe upon an individual’s right to their own genetic characteristics and impact their identity. Additionally, questions around the boundary between "natural" and "man-made" interventions in religious and philosophical contexts have led to widespread societal skepticism toward gene-editing technology [11].

The ethical dilemma is particularly evident in CRISPR-Cas system human trials. The 2018 human embryo gene-editing experiment by Chinese scientists caused a global outcry due to its unpredictable consequences and potential safety risks. Although gene editing may offer treatments for various genetic diseases, its impact on individual moral foundations and possible implications for social fairness and justice create substantial challenges for relevant regulation and policy development [11].

4.3. Technical Limitations

Despite the significant progress of gene-editing technologies like CRISPR-Cas9 in biomedicine, technical limitations still exist. First, although CRISPR-Cas9 can effectively target specific genes, its precision in controlling editing sites still requires improvement. Many experiments have found that the DNA cleavage sites created by CRISPR-Cas9 do not always align with expectations, leading to unintended frameshift mutations, base deletions, or substitutions. Second, the efficiency of exogenous DNA repair remains low; many experiments indicate that although the editing tool can successfully cut DNA, the newly introduced DNA fragment does not always integrate into the target location as anticipated [14].

These limitations not only affect the accuracy of experimental results but also restrict the clinical applications of gene-editing technology. In particular, in the complex mammalian genome, off-target effects and incomplete editing may result in suboptimal therapeutic outcomes or even additional health risks [11].

5. Future Directions

5.1. Development of New Gene Editing Tools

With continuous optimization of gene editing technology, many new tools have emerged. For example, novel systems such as CRISPR-Cas12 and Cas13 offer higher editing precision and broader applications compared to traditional Cas9 proteins. Cas12a, in particular, has a smaller size than Cas9, the ability to recognize different sequence regions, and reduced off-target effects, making it suitable for a wider range of genome editing needs. Furthermore, the Cas13 system enables targeted RNA modifications in addition to DNA editing, creating new possibilities for treating RNA viral infections [15].

The CPE (circular guide editor) system, a new generation of tools based on Cas12a, not only precisely identifies specific regions within the genome but also enables multiplex editing, thereby enhancing the efficiency and accuracy of gene editing. The development of these novel tools expands the application range of gene editing and offers new hope for overcoming current technological limitations [8, 9, and 16].

5.2. Combining Gene Editing with Multiple Therapies

In the future, gene editing technology may be combined with other therapies (such as chemotherapy, targeted therapy, and immunotherapy) to further enhance its therapeutic effects. For example, CRISPR technology can improve the efficacy of CAR-T cell therapy by editing genes to enhance the targeting specificity and durability of CAR-T cells against cancer. Additionally, gene editing technology can be used in conjunction with immune checkpoint inhibitors to optimize immune responses in patients and counteract immune evasion mechanisms in tumor cells [3, 17, and 18].

As CRISPR technology advances, researchers are exploring its application across various disease models. For instance, gene editing can be used to generate animal models for specific diseases, facilitating a deeper understanding of disease pathogenesis and providing new targets for future treatments. The combination of gene editing technology with other therapies holds the potential to achieve breakthrough progress in treating refractory cancers and genetic diseases [15].

5.3. The Future of Personalized Immunotherapy

Gene editing technology has immense potential in personalized medicine, especially within the field of immunotherapy. Researchers can use gene editing technology to modify a patient’s immune cells to specifically recognize and attack cancer cells with unique mutation profiles, thus achieving truly personalized treatment. For example, by sequencing the genomes of a patient’s tumor and healthy tissues, scientists can identify specific mutations and design T cell receptors through gene editing that can recognize these mutations. These engineered T cells are then reinfused into the patient to precisely target tumor cells [6].

Moreover, gene editing technology can optimize a patient’s immune response and reduce side effects during treatment. For instance, editing immune checkpoint genes in T cells can enhance their anticancer effects while reducing the risk of autoimmune diseases caused by excessive immune responses. This personalized immunotherapy not only improves treatment efficacy but also significantly reduces the treatment burden on patients [11, 19].

6. Conclusion

The development of gene editing technology has introduced several mature tools, such as CRISPR-Cas9, ZFN, and TALEN, advancing the deeper application of gene editing in biomedicine, particularly in immunotherapy. In cancer treatment, CRISPR gene editing technology has facilitated the progress of CAR-T cell therapy by modifying T cells to recognize and attack cancer cells. For instance, knocking out the PD-1 receptor in T cells with CRISPR/Cas9 has significantly enhanced their anticancer capacity, and such candidate therapies have already entered clinical trials. Additionally, CRISPR technology has been employed to target and correct oncogenic mutations, such as selectively cutting tumor-specific gene fusion sites to inhibit tumor growth. In glioblastoma mouse models, CRISPR systems delivered via lipid nanoparticles (LNPs) have achieved a 70% gene-editing efficiency, demonstrating notable antitumor effects and the potential for extended survival.

In treating viral infections, the CRISPR-Cas9 system also shows considerable promise. By directly cutting viral genomes or disrupting essential host factors for viral replication, CRISPR technology offers new possibilities for combating various viral infections, including HIV and HBV. Gene-editing strategies targeting viral genes, such as inhibiting virus-specific antigen expression or disrupting viral replication mechanisms, can effectively reduce viral spread within host cells.

Gene editing also holds significant potential in treating genetic diseases. For example, in the treatment of sickle cell disease, CRISPR can correct mutations in the hemoglobin gene or activate alternative hemoglobin expression to improve patient conditions. In cases of genetic disorders like Duchenne muscular dystrophy, CRISPR can repair or replace mutated genes, thereby restoring muscle function in patients. While gene editing offers new treatment avenues for genetic diseases, challenges regarding safety, specificity, and delivery systems remain to be addressed.

Future developments in gene editing will focus on enhancing specificity, reducing off-target effects, and improving delivery technologies. To this end, researchers will explore new immune-compatible delivery vectors and introduce more precise gene-editing control technologies to ensure safety. Furthermore, novel CRISPR variants and editing methods, such as base editing and prime editing, avoid DNA double-strand breaks by directly replacing bases or inserting specific sequences, thus reducing off-target risks. With early successes of CRISPR technology in clinical applications for diseases like sickle cell disease and β-thalassemia, future research will aim to expand its therapeutic scope and further validate the safety and efficacy of these techniques through clinical trials.

The development of personalized medicine has also benefited from advancements in gene editing technology. By combining patients’ genetic information with gene-editing techniques, personalized treatment plans can be designed based on individual genetic mutations, enhancing treatment precision. Future research will continue to explore ways to leverage gene-editing technology to address individual genetic variations, driving the advancement of precision medicine and meeting patients’ individualized treatment needs.

In summary, gene editing technology has a broad outlook in immunotherapy applications. As the technology continues to mature and challenges are gradually overcome, gene editing is expected to play an increasingly vital role in cancer, viral infections, and genetic diseases in the coming years, steering medicine toward greater precision, efficacy, and personalization. Advances in gene-editing technology not only hold promise for improving disease treatment outcomes but also lay the groundwork for the future of personalized medicine.

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