

Applications of Gene Editing Technology: The Revolution of CRISPR-Cas Systems and Future Perspectives

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Abstract. Gene editing technologies are undergoing breakthrough advancements, with next-generation tools such as base editing and prime editing demonstrating significant advantages. Base editors, which combine a Cas9 nickase with a deaminase, enable precise single-base conversions (e.g., C→T or A→G) without inducing double-strand DNA breaks, showing great promise for treating diseases caused by point mutations. In terms of clinical applications, there are now over 300 global clinical trials in gene therapy, primarily focused on hematological disorders and solid tumors. Innovations in delivery systems—such as lipid nanoparticles (LNPs) and adeno-associated virus (AAV) vectors—have improved tissue targeting, although long-term safety monitoring remains to be further refined. Regulatory policies vary significantly across countries; therefore, future efforts should prioritize the establishment of unified safety standards, tiered risk assessment frameworks, and comprehensive, lifecycle-oriented ethical review mechanisms. Such measures are essential to facilitate the safe translation of these technologies and unlock their vast potential in precision medicine, agriculture, and beyond.

Keywords: CRISPR-Cas9, Gene editing, Gene therapy

1. Introduction

Gene editing refers to a suite of technologies that enable precise modification of an organism's genome through artificial means. Among these, the CRISPR-Cas9 system is representative: it utilizes a guide RNA (gRNA) to direct the Cas9 nuclease to a specific genomic locus, where it induces a double-strand break; subsequent cellular repair mechanisms—such as non-homologous end joining (NHEJ) or homology-directed repair (HDR)—then facilitate gene knockout, insertion, or base substitution. Other established platforms include TALENs and zinc finger nucleases (ZFNs), all sharing key characteristics of high specificity, programmability, and efficiency. In biomedicine, gene editing holds broad therapeutic potential, including the treatment of genetic disorders (β -thalassemia), cancer immunotherapy (via engineering of CAR-T cells), and antiviral strategies (targeting the latent HIV reservoir). In agriculture, the technology has enabled the development of disease- and pest-resistant crops (CRISPR-edited wheat resistant to powdery mildew) and nutritionally enhanced varieties (vitamin A-enriched Golden Rice). Furthermore, gene editing is widely applied in generating animal disease models (Alzheimer's disease mouse models) to

accelerate the study of pathogenic mechanisms, as well as in optimizing metabolic pathways in industrial microorganisms for the production of high-value compounds.

Despite significant technological advancements, existing reviews often focus on single application domains and lack an interdisciplinary, integrated analysis of the broader landscape of gene editing technologies, particularly with insufficient discussion on data lines, ethical regulations, and societal implications. This article aims to address the following core questions: first, the trade-offs among different editing tools (ZFNs, CRISPR, TALENs) in terms of efficiency, precision, and suitability for specific applications; second, the variations and emerging consensus in global regulatory frameworks governing gene editing; and third, how to balance the innovative potential of these technologies with ethical and societal considerations during their translation into real-world applications. Gene editing is reshaping the frontiers of life sciences; however, its comprehensive deployment still requires overcoming technical bottlenecks and achieving a robust balance between scientific progress and ethical responsibility.

2. Comparison of mainstream gene editing technologies

The development of gene editing technologies has achieved breakthrough progress over the past few decades, evolving from early-generation zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) to the now-dominant CRISPR-Cas systems. Each successive generation has brought significant improvements in precision, efficiency, and ease of use. Different gene editing tools exhibit distinct characteristics in terms of targeting mechanisms, design strategies, and applicable scenarios. The selection of an appropriate editing technology depends on specific research objectives and application contexts [1].

2.1. ZFN

Zinc finger nucleases (ZFNs) were among the first gene editing technologies to be widely adopted. They rely on the sequence-specific DNA recognition mechanism of zinc finger protein domains to achieve targeted genome editing. Zinc finger proteins are a common class of transcription factors in eukaryotes, with each zinc finger domain recognizing approximately three base pairs. By linking multiple zinc finger domains in tandem, binding modules specific to desired DNA sequences can be constructed. A ZFN consists of two functional components: a DNA-binding domain (composed of engineered zinc finger proteins) and a non-specific DNA cleavage domain derived from the FokI restriction endonuclease, which must dimerize to become active. Targeted DNA cleavage occurs only when two ZFN monomers bind to adjacent sites on opposite DNA strands, enabling FokI dimerization and subsequent double-strand break (DSB) induction. The DSB is then repaired by cellular repair pathways—non-homologous end joining (NHEJ) or homology-directed repair (HDR)—resulting in gene knockout, insertion, or correction [2].

Despite demonstrating robust gene editing capability in early studies, the application of ZFNs has been constrained by several key limitations. First, the design and construction of ZFNs involve complex protein engineering, requiring highly optimized zinc finger modules for precise targeting. Due to context-dependent effects—where adjacent zinc fingers influence each other's DNA-binding specificity—designing efficient and specific ZFNs is extremely challenging, often resulting in low success rates. Second, ZFN development is costly, typically requiring custom synthesis, with the optimization process for each target site taking several months. Furthermore, off-target effects are a significant concern, as imperfectly specific zinc finger modules may lead to unintended DNA

cleavage, potentially causing genomic instability and cytotoxicity. These drawbacks have limited the broad adoption of ZFNs in large-scale research, clinical applications, and commercial deployment.

2.2. TALENs

The advent of transcription activator-like effector nucleases (TALENs) addressed many of the limitations associated with ZFNs and established TALENs as the second-generation mainstream gene editing tool. Structurally similar to ZFNs, TALENs also employ a modular DNA-binding domain fused to the non-specific FokI nuclease cleavage domain, but their DNA recognition mechanism is more flexible and predictable. The DNA-binding domain of TALENs is derived from transcription activator-like effector (TALE) proteins secreted by plant pathogenic bacteria, such as *Xanthomonas*. Each TALE repeat unit specifically recognizes a single DNA base (A, T, C, or G), and the binding specificity is determined by amino acid residues at positions 12 and 13 within the repeat—known as the "TALE code." This one-to-one recognition code enables researchers to rationally design and assemble DNA-binding modules for virtually any desired target sequence.

TALENs offer several advantages over ZFNs. First, their DNA recognition modules are simpler and more intuitive to design; because each TALE unit recognizes only one base, the context-dependent interference observed in ZFNs is largely avoided, allowing for more efficient and reliable targeting. Second, TALENs exhibit higher success rates and, in many studies, demonstrate superior editing efficiency and reduced off-target activity compared to ZFNs [3]. Furthermore, TALENs can target a broader range of genomic regions, including sequences with high GC content or high levels of DNA methylation—regions that often pose challenges for ZFN binding.

Nevertheless, TALENs are not without limitations. One major drawback is their large protein size (typically exceeding 100 kDa), which hampers delivery efficiency, particularly in applications requiring packaging into viral vectors with limited cargo capacity, such as adeno-associated viruses (AAVs). Additionally, although TALEN design and construction are simpler than those of ZFNs, they remain relatively complex and costly, limiting their widespread adoption in low-resource or high-throughput settings.

2.3. CRISPR-Cas systems

The CRISPR-Cas system represents the most revolutionary breakthrough in the field of gene editing in recent years, with CRISPR-Cas9 emerging as the most widely used tool. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) were originally identified as part of an adaptive immune system in bacteria and archaea, evolved to defend against viral infections. The function of CRISPR-Cas9 relies on two core components: the Cas9 nuclease and a guide RNA (gRNA). The gRNA consists of two parts: the CRISPR RNA (crRNA), which provides sequence complementarity to the target DNA (typically 20 nucleotides), and the trans-activating CRISPR RNA (tracrRNA), which stabilizes the RNA structure and mediates binding to Cas9. Target DNA recognition requires the presence of a protospacer adjacent motif (PAM), typically NGG (where N is any nucleotide), located immediately downstream of the target sequence. Guided by the gRNA, Cas9 binds to the target site and induces a double-strand break (DSB) in the DNA.

CRISPR-Cas9 has rapidly become the preferred gene editing platform due to several key advantages. First, unlike ZFNs and TALENs, CRISPR system design is based entirely on RNA-DNA base pairing, eliminating the need for complex protein engineering. Researchers can target virtually any genomic locus simply by designing and synthesizing a specific gRNA. This simplicity dramatically reduces technical barriers and costs, enabling broad adoption across diverse research

laboratories. Second, CRISPR-Cas9 exhibits significantly higher editing efficiency than previous technologies, enabling high-frequency gene modifications in a wide range of cell types, including mammalian cells, plants, and microorganisms. Moreover, CRISPR-Cas9 supports multiplexed editing—simultaneous targeting of multiple genes—by introducing several gRNAs, which is essential for studying gene regulatory networks and the mechanisms of complex diseases.

Despite its transformative potential, CRISPR-Cas9 has certain limitations, the most prominent being off-target effects. Because the gRNA may bind to genomic sequences with partial homology, unintended genetic modifications can occur—this is particularly critical in clinical applications requiring high precision, such as gene therapy. In recent years, multiple strategies have been developed to mitigate off-target risks, including high-fidelity Cas9 variants (e.g., Cas9-HF1, eSpCas9), improved gRNA design algorithms, and dual-gRNA screening approaches to enhance target specificity [4]. In addition, the requirement for a specific protospacer adjacent motif (PAM) sequence limits the targeting scope of CRISPR-Cas9, particularly in genomic regions lacking the canonical NGG PAM. However, this constraint is being progressively addressed by the discovery and engineering of novel Cas variants—such as Cas12a (also known as Cpf1), which recognizes a TTTN PAM—thereby expanding the range of accessible genomic sites.

In summary, ZFNs, TALENs, and CRISPR-Cas systems represent three pivotal stages in the evolution of gene editing technologies, each characterized by distinct features in terms of efficiency, ease of use, and application breadth. As the first-generation tool, ZFNs played a crucial role in validating the concept of targeted genome editing, but their high development cost and design complexity have led to their gradual replacement by TALENs. TALENs improved design flexibility through a modular DNA recognition system, yet they still face challenges related to protein engineering complexity and delivery limitations. The emergence of CRISPR-Cas9 has revolutionized the field, as its RNA-guided mechanism enables unprecedented efficiency, scalability, and cost-effectiveness, accelerating the translation of basic research into clinical and agricultural applications.

Despite current challenges such as off-target effects and delivery efficiency, ongoing optimization and innovation—such as base editing, prime editing, and epigenome editing—are continuously expanding the capabilities and applications of CRISPR-based technologies. Overall, researchers can select the most suitable platform based on experimental requirements and available resources, but CRISPR-Cas systems are undoubtedly the most versatile and promising choice at present. Looking ahead, further advancements in gene editing will focus on enhancing precision, broadening targeting scope, and minimizing cellular toxicity, thereby enabling transformative progress in personalized medicine, synthetic biology, and sustainable agriculture.

3. Core application domains of gene editing

Since its emergence, gene editing technology has demonstrated transformative potential across fundamental scientific research, medical therapeutics, and agricultural improvement.

3.1. Medical applications

Gene editing holds revolutionary promise for the treatment of genetic disorders. For example, in sickle cell disease (SCD), researchers have employed CRISPR-Cas9 to genetically modify patients' hematopoietic stem cells, precisely correcting or compensating for the mutated β -globin gene, thereby enabling the production of functional hemoglobin. In 2023, the U.S. FDA approved Casgevy, the world's first CRISPR-based gene therapy, for the treatment of both sickle cell disease

and β -thalassemia, marking a milestone in the clinical translation of gene editing technologies. Similarly, therapeutic strategies for cystic fibrosis (CF) are being developed using gene editing to correct common mutations in the CFTR gene or to introduce functional gene copies into respiratory epithelial cells, aiming to restore normal chloride ion channel activity. According to a 2022 review published in *Cellular & Molecular Biology Letters*, multiple practical applications of CRISPR/Cas9 in cancer therapy have been documented [5].

Studies have shown that CRISPR/Cas9-mediated knockout of the multidrug resistance gene ABCB1 (also known as MDR1) significantly increases the intracellular accumulation of doxorubicin in breast, ovarian, and colon cancer cells, effectively reversing the drug-resistant phenotype. In HPV-associated cervical cancer, targeted disruption of the viral oncogenes E6 and E7 not only suppresses cancer cell proliferation and induces apoptosis but also markedly enhances sensitivity to cisplatin chemotherapy. Furthermore, genome-wide CRISPR/Cas9 screening has successfully identified novel genes associated with drug resistance, such as SLFN11, APE1, RSF1, and CDK5 [6]. These findings collectively demonstrate the powerful potential of CRISPR/Cas9 in overcoming tumor resistance, targeting oncogenic drivers, and discovering functional cancer genes, laying a solid foundation for precision oncology.

Gene editing has also accelerated advances in immunotherapy. By engineering chimeric antigen receptor (CAR)-T cells, gene editing enables the knockout of immune checkpoint molecules (e.g., PD-1) to enhance anti-tumor activity or the insertion of multiple antigen-targeting receptors to improve efficacy against solid tumors. In 2022, *Nature* reported a groundbreaking study in which scientists used base editing technology to engineer CAR-T cells capable of simultaneously targeting multiple surface markers on leukemia cells while resisting immunosuppressive signals in the tumor microenvironment, resulting in significantly improved long-term remission rates in patients [7]. Additionally, gene editing facilitates the development of "off-the-shelf" allogeneic CAR-T cells by disrupting HLA molecules to reduce the risk of immune rejection, thereby improving the scalability, affordability, and accessibility of this promising therapeutic approach.

3.2. Agricultural applications

CRISPR/Cas9 enables precise, efficient, and predictable genome modifications in agriculture, facilitating the rapid development of crop varieties with improved traits without introducing foreign genes. This technology provides a powerful scientific foundation for addressing global challenges such as food security, nutritional health, and environmental sustainability. Multiple reviews have systematically outlined its broad applications in agriculture, spanning crop improvement, nutritional enhancement, and stress resistance.

In terms of improving crop quality and palatability, CRISPR/Cas9 has demonstrated significant potential. For instance, to meet the growing demand for healthier diets, researchers have successfully developed low-gluten wheat using this technology. By targeting and knocking out gene clusters encoding gliadin proteins, the content of immunogenic peptides that trigger celiac disease and other gluten-related disorders was substantially reduced, offering a safer staple food option for gluten-sensitive populations [8]. Additionally, to enhance the flavor of fruits and vegetables, CRISPR-mediated gene editing has been applied to modulate sugar metabolism pathways in tomatoes, resulting in new varieties with increased sweetness without compromising yield—achieving a synergistic optimization of taste and productivity. In biofortification, CRISPR/Cas9 offers a novel approach to combat "hidden hunger." By precisely editing key genes involved in nutrient biosynthesis and transport, the nutritional value of crops can be significantly enhanced. For example, recent studies indicate that overexpression of metal transporter genes (e.g., OsIRT1,

OsZIP1) and storage protein genes (e.g., ferritin) can boost the uptake and accumulation of essential micronutrients such as iron and zinc in rice [9]. Simultaneously, engineering key enzymes in the carotenoid biosynthesis pathway—such as phytoene synthase (psy) and carotene desaturase (crtl)—can elevate β -carotene (a precursor of vitamin A) levels in staple crops, aligning with the concept of "Golden Rice" but with greater precision and efficiency.

In breeding for stress resilience and disease resistance, CRISPR/Cas9 enables targeted improvements in crops like rice and wheat, leading to new lines with enhanced resistance to specific pathogens. For example, by editing genes that regulate responses to biotic and abiotic stresses, crops can better withstand pests, drought, or salinity, thereby reducing reliance on chemical pesticides and fertilizers and promoting more sustainable agricultural practices. Furthermore, gene stacking strategies allow the integration of multiple favorable traits—such as insect resistance and herbicide tolerance—into a single cultivar. In cotton, for instance, stacking Cry1Ac and Cry2Ab genes via CRISPR has conferred broad-spectrum insect resistance, significantly improving overall crop resilience and yield stability [10]. Collectively, CRISPR/Cas9 is driving agricultural breeding into a new era of precision and efficiency.

3.3. Basic research

Gene editing has revolutionized the generation of animal models. Traditional methods for creating transgenic animals are often inefficient and costly, whereas CRISPR technology enables the rapid and precise construction of complex genetic models in a wide range of model organisms, including mice, zebrafish, and *Drosophila*. In neuroscience, this advancement has been particularly transformative: researchers have employed brain-region-specific gene editing to develop sophisticated animal models that recapitulate complex neurodevelopmental and neurodegenerative disorders, such as autism spectrum disorder and Alzheimer's disease, thereby accelerating the mechanistic understanding of these conditions.

Recently, a novel "gene drive" system based on CRISPR principles has been developed, capable of rapidly spreading specific genetic modifications through wild populations. While this technology holds significant potential for applications such as controlling disease vectors—including malaria-transmitting mosquitoes—it has also sparked widespread debate regarding ecological safety and unintended consequences on ecosystems. Additionally, the emergence of epigenome editing tools—such as dCas9 (nuclease-deficient Cas9) fused to epigenetic effector domains—has enabled researchers to modulate gene expression without altering the underlying DNA sequence. These tools allow targeted manipulation of chromatin modifications (e.g., methylation, acetylation), opening new avenues for investigating the functional role of epigenetic regulation in development, disease, and cellular identity [11].

4. Future directions

4.1. Development of novel editing tools

From medical therapeutics to agricultural production and fundamental scientific research, gene editing technologies are profoundly transforming research paradigms and practical approaches across multiple disciplines. As the technology continues to evolve and regulatory frameworks are progressively established, the scope and depth of gene editing applications will expand further. In medicine, next-generation editing tools such as prime editing hold promise for enabling safer and more precise genome modifications, potentially extending therapeutic applications to more complex

polygenic disorders [12]. In agriculture, a key challenge will be balancing the transformative potential of gene editing with ecological risks and public acceptance. In basic research, the integration of gene editing with emerging single-cell multi-omics technologies will empower unprecedented systematic dissection of biological processes. Although ethical and safety considerations must be carefully addressed, it is undeniable that precise genome editing has become one of the core drivers of the 21st-century biotechnology revolution. Its future trajectory will continue to attract significant attention from the scientific community, industry, and the general public.

4.2. Clinical translation pathways and regulatory framework development

Globally, there are now over 300 ongoing gene therapy clinical trials, primarily focused on hematological disorders, ocular diseases, and certain solid tumors. Translating these therapies from the laboratory to clinical practice requires overcoming multiple challenges. First, optimization of delivery systems is critical; advances in novel lipid nanoparticles (LNPs) and adeno-associated virus (AAV) vectors are improving the targeting specificity and delivery efficiency of gene editing tools to specific tissues [13,14]. Second, robust long-term assessment of safety and efficacy necessitates the establishment of comprehensive monitoring systems, particularly for systematic evaluation of potential off-target effects and genomic instability.

From a regulatory perspective, significant differences exist across national policies. Future efforts toward global regulatory harmonization may focus on establishing standardized methods for off-target effect detection, developing risk assessment frameworks tailored to diverse application contexts, and strengthening ethical review mechanisms that span the entire research and development lifecycle. As these key challenges are progressively addressed, gene editing technologies are poised to achieve broader societal impact, particularly in the fields of precision medicine and sustainable agriculture.

5. Conclusion

This study presents a systematic literature review on the development, core application domains, and future directions of gene editing technologies, with a particular focus on the applications and potential of CRISPR/Cas9 in medicine and agriculture. The findings indicate that CRISPR/Cas9 has rapidly replaced ZFNs and TALENs as the dominant genome editing platform, owing to its simple design, low cost, high efficiency, and capacity for multiplexed editing. In the medical field, CRISPR/Cas9 demonstrates significant promise for precision cancer therapy, particularly in overcoming tumor chemoresistance. For instance, knocking out the multidrug resistance gene ABCB1/MDR1 effectively enhances the intracellular accumulation of chemotherapeutic agents such as doxorubicin, thereby reversing the drug-resistant phenotype. Additionally, CRISPR/Cas9 enables precise targeting of viral oncogenes; in HPV-associated cervical cancer, disruption of the E6 and E7 genes not only suppresses tumor cell proliferation and induces apoptosis but also significantly improves sensitivity to cisplatin chemotherapy. In agriculture, CRISPR/Cas9 enables targeted modification of crop genomes without introducing foreign DNA, facilitating the rapid development of improved varieties with desirable traits. Notable advances have been achieved in enhancing crop quality and nutritional content, such as engineering low-gluten wheat for improved food safety and increasing β -carotene levels in staple crops to combat micronutrient deficiencies.

Nevertheless, this study has certain limitations, particularly regarding clinical applications, where more systematic monitoring and long-term follow-up data are still needed. Looking ahead, future

research should focus on three key directions: First, strengthening clinical surveillance by establishing comprehensive long-term safety and efficacy evaluation systems. Second, integrating gene editing with dynamic modeling approaches to more accurately predict editing outcomes and optimize intervention strategies. Third, promoting interdisciplinary convergence, such as combining gene editing with synthetic biology. Breakthroughs in these areas will not only deepen the theoretical understanding of gene editing technologies but also provide robust scientific foundations and powerful technical drivers for advancements in healthcare, modern agriculture, and even artificial intelligence.

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