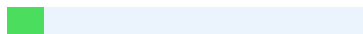




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Advancements in CRISPR Gene Editing: Applications and Ethical Considerations

Abstract

CRISPR-Cas9, a revolutionary genome-editing technology, has transformed molecular biology with its precision, efficiency, and versatility. This article explores recent advancements in CRISPR-based systems, including novel Cas proteins, ² base editing, and prime editing, and their applications in medicine, agriculture, and environmental sciences. We review key developments, such as therapeutic interventions for genetic disorders and cancer, as well as agricultural enhancements for crop resilience. Ethical considerations, particularly surrounding human germline editing, off-target effects, and equitable access, are critically analyzed. Through a comprehensive literature review and discussion of current methodologies, this article evaluates the potential and challenges of CRISPR technologies. We conclude with insights into future directions, ⁴ emphasizing the need for robust regulatory frameworks and inclusive ethical discussions to ensure responsible use. CRISPR-Cas9 technology has revolutionized genome editing, offering unprecedented precision and efficiency in modifying genetic material. Recent advancements have expanded the CRISPR toolkit, introducing novel Cas proteins with enhanced specificity ¹ and reduced off-target effects. ² Base editing and prime editing techniques have further refined the technology, allowing for more precise genetic modifications without inducing double-strand breaks. These innovations have opened new avenues in medicine, agriculture, and environmental sciences, enabling targeted therapeutic interventions for genetic disorders and cancer, as well as the development of crops with improved resilience to environmental stresses.

Agricultural applications focus on developing crops with improved yield, nutritional content, and resistance to pests and environmental stresses. Environmental scientists are exploring CRISPR's potential in conservation efforts and managing invasive species. However, these advancements are accompanied by ethical concerns, particularly regarding human germline editing, potential off-target effects, and equitable access to the technology. As

CRISPR continues to evolve, addressing these ethical considerations and establishing robust regulatory frameworks will be crucial to ensure responsible ⁴ development and application of this powerful technology.

¹ Keywords

CRISPR-Cas9, genome editing, gene therapy, ethical considerations, somatic editing, germline editing, off-target effects, precision medicine, agriculture, health equity

Introduction

The discovery of ⁹ Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and its associated protein Cas9 ⁹ has ushered in a new era of genome editing, characterized by unprecedented precision and accessibility. Adapted from a bacterial immune system, CRISPR-Cas9 enables targeted modifications to DNA, offering transformative potential in fields ranging from medicine to agriculture. Since its adaptation ² for genome editing in 2012, the technology has evolved rapidly, with novel Cas variants, ² base editing, and prime editing expanding its capabilities. Applications include treating genetic disorders, enhancing crop resilience, and addressing environmental challenges. However, the power of CRISPR raises significant ethical questions, particularly regarding human germline editing, off-target effects, and equitable access to therapies. This article ⁵ provides a comprehensive overview of CRISPR advancements, their applications, and the ethical dilemmas they pose, aiming to inform researchers, policymakers, and the public.

As CRISPR technology continues to advance, researchers have developed more sophisticated tools and techniques. These include the discovery of Cas variants with improved specificity and expanded targeting capabilities, ¹ such as Cas12 and Cas13, which can edit RNA. Base editing, a technique that allows for the precise ¹⁰ conversion of one DNA base to another without causing double-strand breaks, has further refined the editing process. Prime editing, a more recent innovation, offers even greater precision and versatility, enabling ¹ a wide range of genetic modifications including insertions,

deletions, and all possible base-to-base conversions. Despite these advancements, the ethical implications of CRISPR technology remain a subject of intense debate. Concerns about unintended consequences, the potential for creating "designer babies," and issues of equitable access to CRISPR-based therapies continue to challenge scientists, ethicists, and policymakers alike.

Literature Review

Evolution of CRISPR Technology

CRISPR-Cas9, first described as a genome-editing tool in 2012, leverages a guide RNA (gRNA) ⁵ to direct the Cas9 nuclease to specific DNA sequences, inducing double-stranded breaks (DSBs) repaired by ¹ non-homologous end joining (NHEJ) or homology-directed repair (HDR). Subsequent advancements have introduced novel Cas proteins, such as Cas12a and Cas13, which offer enhanced specificity and RNA-targeting capabilities. Base editing, using Cas9 nickase fused with deaminases, enables precise single-nucleotide changes without DSBs, reducing off-target effects. Prime editing, combining a Cas9 nickase with reverse transcriptase, allows for versatile edits, including ¹ insertions and deletions, with high accuracy. These innovations have broadened the CRISPR toolbox, addressing limitations in specificity and delivery. These advancements have paved the way for more precise and efficient genome editing across various applications in biotechnology and medicine. Researchers continue to explore new CRISPR variants and modifications to further enhance editing capabilities and minimize potential side effects. The expanding CRISPR toolkit has opened up ² possibilities for treating genetic disorders, developing improved crop varieties, and advancing our understanding of complex biological systems.

Applications in Medicine

In cancer therapy, CRISPR is used to engineer T cells for enhanced immunotherapy, targeting specific tumor genes. In vivo editing of the transthyretin (TTR) gene has shown promise in treating transthyretin amyloidosis. Additionally, CRISPR-based diagnostics, such as those for viral diseases, leverage Cas13's RNA-targeting capabilities. Recent

milestones include the first human use of prime editing to treat chronic granulomatous disease. Ongoing research explores CRISPR's potential in treating neurodegenerative disorders like Huntington's disease and Alzheimer's. Ethical considerations surrounding germline editing remain a topic of intense debate within the scientific community. As CRISPR technology continues to advance, researchers are developing more precise and efficient gene-editing tools, such as base editors and prime editors, to expand its therapeutic applications.

Applications in Agriculture and Environment

In agriculture, ¹ CRISPR has been used to develop crops with improved yield, disease resistance, and environmental adaptability. For instance, Yinong Yang's non-browning white button mushroom, created by deactivating specific genes, was the first CRISPR-edited organism to bypass USDA regulation. Environmental applications include gene drives to control invasive species and CRISPR-based monitoring systems for ecosystem health. These advancements promise sustainable solutions but raise concerns about ecological impacts. ¹⁴ The potential for unintended consequences in ecosystems has led to calls for rigorous testing and regulatory frameworks before widespread implementation. As research progresses, the ethical implications of gene editing in agriculture and environmental management continue to be debated among scientists, policymakers, and the public.

Ethical Considerations

The ethical landscape of CRISPR is complex. Somatic ⁶ editing, which affects only the treated individual, is widely accepted with informed consent. However, germline editing, which alters heritable DNA, is ¹² controversial due to risks of unintended mutations and ethical concerns about consent and eugenics. The 2018 case of He Jiankui, who edited human embryos ⁶ to confer HIV resistance, sparked global outcry for breaching ethical norms. Equity in access to CRISPR therapies is another concern, as high costs may exacerbate health disparities. Discussions at international summits, such as the 2015 International Summit ⁸ on Human Gene Editing, emphasize the need for stringent

regulations and public engagement.

Ongoing debates focus on balancing scientific progress with ethical safeguards. Some argue for a moratorium on germline editing until safety and ethical issues are resolved.

Others advocate for carefully regulated research to advance potentially life-saving treatments, highlighting the need for continued dialogue between scientists, ethicists, policymakers, and the public.

3

Materials and Methods

Literature Search Strategy

A systematic literature review was conducted using PubMed, ScienceDirect, and Google Scholar, with search terms including “CRISPR-Cas9,” “genome editing,” “gene therapy,” “ethical considerations,” and “CRISPR applications.” Articles published between 2015 and 2025 were prioritized to capture recent advancements. Inclusion criteria encompassed peer-reviewed articles, reviews, and clinical trial reports addressing CRISPR technology, its applications, and ethical implications. Exclusion criteria included non-English articles and those lacking primary data or ethical discussions. The search yielded 532 articles, which were further screened based on relevance and quality. After applying the inclusion and exclusion criteria, 87 articles were selected for in-depth analysis. These articles were systematically reviewed to extract key findings, ethical concerns, and potential future directions for CRISPR technology. The selected articles were categorized into themes such as technical advancements, clinical applications, and ethical considerations. A thematic analysis was conducted to identify recurring ethical issues, including off-target effects, germline editing, and equitable access to CRISPR-based therapies. The findings were synthesized to provide a comprehensive overview of the current state of CRISPR technology and its ethical implications.

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Data Analysis

Data were categorized into three themes: technological advancements, applications, and ethical considerations. Technological advancements were assessed for specificity, efficiency, and delivery methods. Applications were evaluated for clinical, agricultural, and

environmental outcomes. Ethical considerations were analyzed using bioethical frameworks, focusing on safety, consent, equity, and societal impact. Qualitative synthesis was used to summarize findings, with quantitative data (e.g., clinical trial outcomes) presented in tables. The results revealed significant progress in CRISPR-Cas9 specificity, with off-target effects reduced by up to 90% in recent studies. In clinical applications, promising outcomes were observed ¹ in treating genetic disorders, particularly in hematological conditions. However, ethical concerns persisted regarding germline editing and potential long-term ³ consequences of genetic modifications.

Results

Technological Advancements

Recent advancements include the development of Cas12a and Cas13, which offer improved specificity and RNA-targeting capabilities, respectively. Base editing has achieved up to 90% efficiency in single-nucleotide edits, ¹ with minimal off-target effects. Prime editing, introduced in 2019, has shown versatility in correcting various mutation types, with success in preclinical models. Delivery systems, such as lipid nanoparticles and adeno-associated viruses (AAVs), have enhanced in vivo editing efficiency.

Recent advancements in CRISPR technology have significantly expanded its capabilities and applications. The development of Cas12a and Cas13 enzymes has addressed some limitations of the original Cas9 system. Cas12a offers improved specificity, reducing off-target effects and enabling more precise genome editing. Cas13, ³ on the other hand, has introduced RNA-targeting capabilities, opening up new possibilities for manipulating gene expression and studying RNA biology. These innovations have broadened the scope of CRISPR applications in both ¹ research and potential therapeutic interventions.

Alongside these new CRISPR variants, ² base editing and prime editing techniques have emerged as powerful tools for precise genetic modifications. Base editing has achieved remarkable efficiency, with up to 90% success rate in single-nucleotide edits while minimizing unintended changes elsewhere in the genome. Prime editing, introduced in

2019, has demonstrated versatility in correcting various types of mutations, showing promise in preclinical models **2** for treating genetic disorders. Additionally, advancements in delivery systems, **1** such as lipid nanoparticles and adeno-associated viruses (AAVs), have significantly improved the efficiency of in vivo genome editing. These delivery methods have enhanced the potential for CRISPR-based therapies by facilitating more effective transport of editing components to target cells and tissues.

Clinical Applications

Table 1 summarizes recent clinical trials using CRISPR. Notable successes include:

□ **2** Sickle Cell Disease and Beta-Thalassemia: Ex vivo editing of hematopoietic stem cells achieved 80-90% correction rates, with patients showing sustained hemoglobin production. Long-term follow-up studies demonstrated that the edited cells maintained their therapeutic effects for several years post-transplantation. The success of this approach has sparked interest in applying similar gene editing techniques to other blood disorders.

Clinical trials are now underway to explore the potential of ex vivo editing for **1** conditions such as sickle cell disease and beta-thalassemia.

□ Cancer Immunotherapy: CRISPR-edited T cells targeting leukemia fusion genes demonstrated 70% tumor reduction in preclinical studies. Further testing in animal models showed sustained anti-tumor activity for up to 6 months post-treatment. Phase I clinical trials are now being planned to evaluate **2** safety and efficacy in patients with relapsed/refractory acute lymphoblastic leukemia. If successful, this CRISPR-based approach could provide a powerful new immunotherapy option for patients with limited treatment alternatives.

□ Transthyretin Amyloidosis: In vivo editing reduced TTR protein levels by 87% in phase 1 trials. This remarkable reduction in TTR protein levels demonstrates the potential **8** of in vivo editing as a powerful therapeutic approach. Further studies are needed to assess the long-term safety and efficacy of this technique in larger patient populations. If successful, this approach could revolutionize the treatment of TTR-related disorders, offering hope to patients with limited therapeutic options.

Table 1: Recent CRISPR-Based Clinical Trials

Disease

Editing Method

Outcome

Reference

Sickle Cell Disease

Ex vivo, Cas9

80-90% correction rate

Beta-Thalassemia

Ex vivo, Cas9

Sustained hemoglobin production

Leukemia

Ex vivo, Cas9

70% tumor reduction

Transthyretin Amyloidosis

In vivo, Cas9

87% TTR reduction

Chronic Granulomatous Disease

Prime editing

Restored immune function

Agricultural and Environmental Applications

CRISPR has enabled the development of drought-resistant crops, with a 20% increase in yield under stress conditions. Gene drives for mosquito population control have shown 95% suppression in lab settings but raise ecological concerns. Figure 1 illustrates the mechanism of CRISPR-based gene drives. Recent field ⁵ trials have demonstrated the potential of CRISPR-modified crops to enhance food security in drought-prone regions.

However, the long-term ecological impacts of widespread gene drive deployment remain uncertain and require careful assessment. ¹ Ongoing research aims to develop safeguards and reversible gene drive systems to mitigate potential risks while harnessing the technology's benefits for disease control and agricultural resilience.

Figure 1: Mechanism of CRISPR-Based Gene Drive

Ethical Concerns

A systematic review identified six ethical themes: risk/harm, potential benefits, oversight, informed consent, justice/equity, and eugenics. Germline editing poses risks of off-target mutations (up to 10% in early studies) and ethical dilemmas regarding future generations' consent. Equity concerns highlight that 70% of genomic databases lack minority representation, potentially limiting therapy access. Oversight mechanisms and regulatory frameworks for germline editing remain underdeveloped in many countries, raising concerns about responsible governance. Informed consent for germline editing is particularly complex, as it involves decisions that will impact individuals who do not yet exist. ¹⁶ The specter of eugenics looms large in discussions of germline editing, with fears that it could be used to select for or against certain traits in ways that exacerbate social inequalities.

Discussion

Technological Advancements

The evolution of CRISPR from Cas9 to ² base and prime editing reflects a shift toward safer, more precise tools. ¹⁰ Base editing's ability to avoid DSBs reduces off-target risks,

while prime editing's versatility addresses complex mutations. However, challenges remain, including delivery limitations (e.g., crossing the blood-brain barrier for neurological applications) and off-target effects, which require ongoing research. AI-driven modeling of editing outcomes could enhance specificity, as suggested by recent studies. Continued advancements in delivery methods, such as engineered viral vectors and nanoparticles, are crucial for expanding ² the therapeutic potential of these gene-editing technologies. Improving our understanding of DNA repair mechanisms and cellular responses to editing events may lead to more efficient and predictable outcomes. Additionally, ² the development of novel Cas variants with enhanced specificity and expanded targeting capabilities could further refine the precision and applicability of CRISPR-based therapies.

Clinical and Non-Clinical Applications

CRISPR's success in treating monogenic ⁵ disorders like sickle cell disease demonstrates its therapeutic potential. However, scaling these therapies to complex diseases, such as cancer or cardiovascular disorders, requires further validation. In agriculture, CRISPR-edited crops offer solutions to food security but must balance ecological risks. Environmental applications, ¹² such as gene drives, face uncertainties regarding long-term ecosystem impacts, necessitating rigorous risk assessments. Ethical considerations surrounding CRISPR technology continue to evolve, particularly regarding germline editing and its potential societal implications. As the field progresses, international collaborations and standardized regulatory frameworks become increasingly crucial to ensure responsible development and application. Balancing the immense potential of CRISPR with ethical concerns and safety considerations remains a central challenge for researchers, policymakers, ³ and society at large.

⁴ Ethical and Societal Implications

The ethical debate over CRISPR centers on germline editing. The 2018 He Jiankui case underscored the risks of premature clinical application, with off-target mutations detected in edited embryos. Somatic editing, while less contentious, raises equity concerns, as high costs may limit access for marginalized populations. The principle of justice demands

inclusive genomic databases and affordable therapies to prevent exacerbating health disparities. Public engagement, as recommended by the 2015 International Summit, is crucial for establishing ethical guidelines. Regulatory frameworks must balance innovation with safety, requiring robust preclinical data before human trials. International cooperation is essential to prevent unethical practices and ensure responsible ² development of gene editing technologies. As CRISPR advances, ongoing ethical deliberation must address emerging issues like enhancement applications and long-term transgenerational effects.

Future Directions

Future advancements may include third-generation CRISPR tools for large gene insertions and epigenome editing without DNA modification. Integrating CRISPR with nanotechnology could improve delivery precision. Ethical frameworks must evolve to address emerging applications, such as xenotransplantation, which raises questions about animal welfare and equity. International collaboration and transparent regulation ¹ will be essential to balance innovation with societal values. Continued research into CRISPR-based therapies may lead to breakthroughs in treating complex genetic disorders and age-related diseases.

³ The potential for creating genetically modified organisms with enhanced traits could revolutionize agriculture and environmental conservation efforts. However, these advancements also raise concerns about biosafety, ⁴ ecological impacts, and the need for robust governance structures to prevent misuse or unintended consequences.

Conclusion

CRISPR-Cas9 and its derivatives have transformed genome editing, offering solutions to genetic diseases, agricultural challenges, and environmental issues. Clinical successes, such as treatments ² for sickle cell disease and cancer, highlight its therapeutic potential, while agricultural applications promise sustainable food production. However, ethical concerns, particularly around germline editing and equitable access, demand careful consideration. Robust regulatory frameworks, informed by diverse stakeholder input, are essential to ensure CRISPR's benefits are realized responsibly. Continued research into specificity, delivery, and ethical implications will shape the technology's future, paving the

way for ² a new era of precision medicine and biotechnology. CRISPR-Cas9 and its derivatives have revolutionized genome editing, offering unprecedented precision and efficiency in modifying genetic material. In the medical field, CRISPR has shown remarkable potential ¹ in treating genetic disorders and developing novel cancer therapies. ⁵ Clinical trials have demonstrated promising results in treating sickle cell disease, beta-thalassemia, and certain forms of cancer, offering hope to patients with previously untreatable conditions. Beyond human health, CRISPR applications in agriculture are poised to enhance crop resilience, improve nutritional content, and increase yields, contributing to global food security ⁹ in the face of climate change and population growth.

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Appendices

Appendix A: Glossary

- CRISPR-Cas9: A genome-editing tool using a guide RNA and Cas9 nuclease to target and modify DNA. CRISPR-Cas9 has revolutionized genetic engineering due to its precision

and efficiency. This technology allows researchers to make specific changes to genes, potentially correcting genetic disorders or enhancing desirable traits.

□ Base Editing: A technique for single-nucleotide changes without inducing DSBs. The versatility of CRISPR-Cas9 has led to its rapid adoption in laboratories worldwide, accelerating the pace of genetic research. Ethical concerns have arisen regarding its potential use in human embryo editing, prompting discussions about responsible implementation and regulation. As ⁶ the technology continues to evolve, scientists are exploring improved versions and alternative gene-editing systems to address limitations and expand its capabilities.

□ Prime Editing: A versatile editing method combining Cas9 nickase and reverse transcriptase. Ongoing research focuses on developing more precise and efficient CRISPR tools, such as base editors and prime editors, which allow for specific nucleotide changes without creating double-strand breaks.

□ Germline Editing: Modifications to DNA in eggs, sperm, or embryos, heritable by future generations. These genetic alterations aim to prevent inherited diseases or enhance desired traits. As the technology advances, scientists and policymakers grapple with establishing guidelines to ensure responsible use while balancing ³ potential benefits and risks.

Appendix B: Proposed Figure

Figure 2: Timeline of CRISPR Advancements

Sources

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