

The revolution of clustered regularly interspaced short palindromic repeats -based therapies: From genetic diseases to cancer investigating how clustered regularly interspaced short palindromic repeats technology is being applied to treat genetic disorders and cancer

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ABSTRACT

The advent of clustered regularly interspaced short palindromic repeats (CRISPR) technology has revolutionized the field of genetic engineering and molecular biology, offering unprecedented opportunities for precise genome editing. Initially discovered as a natural defense mechanism in bacteria, CRISPR-Cas9 technology enables targeted modifications of DNA, paving the way for significant advancements in gene therapy. This review article explores the multifaceted applications of CRISPR, focusing on its transformative potential in treating genetic diseases and various forms of cancer. We delve into the mechanisms by which CRISPR operates, emphasizing its dual-step process of targeting and cutting DNA, and highlighting its versatility in investigating gene functions and developing therapeutic strategies. Specific applications in genetic disorders, including sickle cell disease and cystic fibrosis, showcase the promising outcomes of ongoing pre-clinical and clinical trials. In the realm of oncology, CRISPR's ability to edit oncogenes and enhance immune cell functions exemplifies its role in developing personalized cancer therapies. However, the rapid advancement of CRISPR technology also necessitates critical discussions around ethical considerations and regulatory challenges, particularly concerning off-target effects and the implications of germline editing. Looking ahead, innovations such as CRISPR 2.0, base editing, and prime editing are set to further expand the therapeutic landscape, enhancing the precision and safety of gene editing. This article underscores the importance of collaborative efforts across scientific, ethical, and regulatory domains to navigate the complexities of CRISPR technology while maximizing its benefits for patients and society.

Keywords: Cancer treatment, clustered regularly interspaced short palindromic repeats technology, gene therapy, genetic disorders, genome editing

Introduction

Clustered regularly interspaced short palindromic repeats (CRISPR) technology has emerged as a revolutionary tool in genetic engineering and molecular biology, fundamentally changing the landscape of genetic research and therapy. This sophisticated technology was first

discovered in the late 1980s, but its significance became evident when scientists recognized its role as a natural defense mechanism in bacteria. In essence, CRISPR functions as an adaptive immune system for microorganisms, enabling them to fend off viral infections by incorporating short sequences of viral DNA into their own genomes. These sequences serve as a template for recognizing and targeting subsequent infections from the same virus, thereby allowing bacteria to mount a swift and effective immune response. The CRISPR-Cas9 system, the most widely used CRISPR technology, consists of two primary components: A guide RNA (gRNA) and the Cas9 enzyme.^[1]

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The gRNA is engineered to match a specific target sequence in the DNA, directing the Cas9 enzyme to the precise location where a cut in the DNA is desired. Once the Cas9 enzyme binds to the target DNA, it creates a double-strand break (DSB), which triggers the cell's natural repair mechanisms. The cell can then repair the breakthrough of two main pathways: Non-homologous end joining (NHEJ) and homology-directed repair (HDR). NHEJ often leads to insertions or deletions (indels) that disrupt gene function, while HDR can be employed to introduce specific changes by providing a donor DNA template. This precision allows for targeted gene editing with unprecedented accuracy. The implications of CRISPR technology are vast and multifaceted, making it a cornerstone of modern biomedical research. One of the most promising applications lies in the realm of gene therapy.^[2]

CRISPR has been employed to correct genetic mutations that cause a variety of inherited diseases, such as sickle cell disease and cystic fibrosis (CF). For instance, researchers have successfully used CRISPR to modify hematopoietic stem cells in patients with sickle cell disease, correcting the genetic mutation responsible for the disorder. This approach has demonstrated the potential for creating long-lasting cures for genetic conditions that previously had limited treatment options. In addition to its applications in genetic diseases, CRISPR technology is also being utilized in cancer research and treatment. By targeting specific genetic alterations that drive tumorigenesis, CRISPR enables researchers to develop personalized therapies tailored to the unique genetic profiles of individual tumors. This includes editing genes involved in tumor growth or enhancing the immune system's ability to recognize and attack cancer cells. The ability to precisely modify the genome offers new avenues for creating innovative cancer therapies, thus transforming the approach to cancer treatment. Understanding the principles and mechanisms of CRISPR is essential for appreciating its transformative potential in modern medicine. The ability to edit genes with such precision opens up a myriad of possibilities in research, agriculture, and therapeutics. However, alongside its remarkable capabilities, CRISPR technology also presents ethical and safety challenges that must be carefully navigated. The implications of genetic modification, especially in human germline editing, raise significant ethical questions about the long-term effects on individuals and future generations.^[3]

Mechanisms of CRISPR in Gene Editing

The precision of CRISPR: Targeting and cutting mechanisms

CRISPR technology operates through a remarkably precise two-step process: Targeting and cutting, which enables targeted gene editing with unprecedented accuracy and efficiency. The initial phase involves designing a gRNA that is complementary to a specific DNA sequence within the genome of interest. This gRNA serves as a critical component in directing the Cas9 enzyme – a protein that functions as molecular scissors – toward the precise genomic location where an edit is desired. The specificity of the gRNA is crucial, as it ensures that the Cas9 enzyme binds to the correct site, minimizing the risk of off-target effects that could lead to unintended modifications in the genome.

Inducing DSBs: The role of Cas9 and DNA repair mechanisms

Once the gRNA binds to its complementary DNA sequence, the Cas9 enzyme is recruited to the site, forming a ribonucleoprotein complex. This complex then induces a DSB in the DNA at the target location. The creation of this DSB is a pivotal step, as it activates the cell's intrinsic DNA repair mechanisms. The cell has evolved two primary pathways for repairing DSBs: NHEJ and HDR. The NHEJ pathway is an error-prone repair mechanism that joins the broken ends of the DNA together without the need for a homologous template. This process often results in the introduction of insertions or deletions (indels) at the break site, which can disrupt the reading frame of the gene and lead to loss of function.^[4]

This aspect of CRISPR is particularly useful for knocking out genes, allowing researchers to study the gene's function or its role in disease. In contrast, the HDR pathway is a more precise mechanism that requires a homologous template for repair. Researchers can exploit this pathway by providing a donor DNA template that contains the desired sequence changes flanked by sequences homologous to the regions surrounding the DSB. When the cell uses this template to repair the break, it can incorporate the specific modifications into the genome. This capability enables the correction of mutations associated with genetic disorders or the insertion of new genetic material, thereby facilitating targeted gene therapy applications.

The versatility of CRISPR technology, driven by its precision and efficiency in gene targeting and editing, has positioned it as a powerful tool for investigating gene function, elucidating disease mechanisms, and developing therapeutic strategies. Researchers utilize CRISPR to create animal models that accurately mimic human diseases, allowing for a better understanding of disease progression and testing of potential treatments. Moreover, the ability to edit genes with high specificity accelerates the discovery of novel therapeutic targets and enhances the development of personalized medicine approaches.

CRISPR Applications in Genetic Diseases

The application of CRISPR technology in treating genetic disorders has generated considerable interest in the scientific and medical communities, driven by the promise of precise and effective gene editing to correct underlying genetic defects. Several successful pre-clinical and clinical trials are currently underway, focusing on a range of conditions, including sickle cell disease, CF, and muscular dystrophy. These disorders, which have historically posed significant challenges for treatment, are now being approached with innovative CRISPR-based strategies that hold the potential for curative therapies.

CRISPR's breakthrough in treating sickle cell disease

Sickle cell disease serves as a prime example of the transformative impact of CRISPR. This genetic disorder is caused by a mutation in the beta-globin gene, leading to the production of abnormal hemoglobin that distorts red blood cells into a sickle shape. These

sickled cells can cause severe pain, anemia, and other complications. Researchers have utilized CRISPR to modify hematopoietic stem cells extracted from patients, correcting the mutation in the beta-globin gene. By reintroducing these edited cells back into the patient, the hope is to enable the production of healthy red blood cells that can alleviate the symptoms of the disease. Early clinical trials have shown promising results, with patients reporting significant improvements in health, including reduced pain crises and enhanced overall quality of life. This success highlights CRISPR's potential to provide lasting solutions for genetic disorders that previously relied on symptomatic treatments.^[5]

CRISPR's potential in treating CF

Similarly, CF, a life-threatening genetic disorder caused by mutations in the CF transmembrane regulator (CFTR) gene, is another area where CRISPR technology has demonstrated great promise. The CFTR protein plays a crucial role in regulating ion transport across epithelial cells, and its dysfunction leads to the accumulation of thick mucus in various organs, particularly the lungs and pancreas.^[6] Researchers are exploring CRISPR-based approaches to correct the specific mutations in the CFTR gene, thereby restoring normal protein function. Pre-clinical studies have shown that CRISPR can effectively target and edit the CFTR gene, opening avenues for gene therapies that could potentially cure this debilitating disease. The ability to deliver CRISPR components directly to affected tissues, such as lung cells, represents a significant advancement in the development of effective CF treatments.

CRISPR's role in treating muscular dystrophy

In addition to sickle cell disease and CF, muscular dystrophy is another genetic condition being targeted by CRISPR technology. Muscular dystrophies, which encompass a group of genetic disorders characterized by progressive muscle degeneration, have been approached through CRISPR strategies aimed at repairing or replacing the defective genes responsible for muscle function. For instance, Duchenne muscular dystrophy, caused by mutations in the dystrophin gene, has been targeted using CRISPR to create gene edits that can produce a functional version of the dystrophin protein, thereby potentially halting or reversing muscle degeneration. Early experimental results are promising, and continued research aims to translate these findings into viable therapies for patients.^[7]

The rapid advancements in CRISPR technology and its applications in genetic diseases underscore the transformative potential of this innovative approach to medicine. As clinical trials progress and more diseases are targeted, CRISPR stands to revolutionize the treatment landscape for a wide range of genetic disorders. However, alongside the excitement surrounding these applications, it is essential to address the ethical considerations and safety concerns associated with gene editing, ensuring that the benefits of CRISPR technology can be harnessed responsibly and effectively. With ongoing research and collaboration among scientists, clinicians, and regulatory bodies, CRISPR has the potential to provide durable cures for genetic disorders that previously had limited treatment options, ushering in a new era of precision medicine.

Targeting Cancer with CRISPR Technology

Cancer therapies are increasingly leveraging CRISPR technology to target the genetic mutations and alterations that drive tumorigenesis, marking a significant advancement in the field of oncology. The ability of CRISPR to precisely edit genes allows researchers to focus on key genetic drivers of cancer, such as oncogenes and tumor suppressor genes, which are frequently mutated or dysregulated in various types of tumors. By employing CRISPR in cancer research and therapy, scientists can gain deeper insights into the molecular underpinnings of cancer and develop more effective treatment strategies tailored to individual patients.

Gene editing for oncogenes and tumor suppressor genes

One of the primary applications of CRISPR in cancer treatment is the editing of oncogenes genes that, when mutated, can promote uncontrolled cell growth and tumor formation. By using CRISPR to inactivate these oncogenes, researchers aim to halt the progression of tumors and sensitize cancer cells to existing therapies. Conversely, CRISPR can also be utilized to target tumor suppressor genes, which normally function to regulate cell division and prevent tumor formation. Many cancers are characterized by the loss of function of these critical genes, and restoring their function through targeted editing can potentially reinstate normal cell behavior and inhibit tumor growth.^[8]

CRISPR-modified immunotherapies

Another innovative application of CRISPR technology in oncology is the modification of immune cells, particularly T-cells, to enhance their ability to recognize and destroy cancer cells. One prominent example of this approach is the development of CRISPR-modified chimeric antigen receptor T-cell (CAR-T) therapies. These therapies involve engineering T-cells to express chimeric antigen receptors that specifically recognize cancer antigens, enabling the immune system to mount a targeted attack against tumor cells. Researchers are employing CRISPR to delete genes associated with T-cell exhaustion, a state in which T-cells lose their ability to effectively respond to cancer due to chronic exposure to tumor antigens. By overcoming this exhaustion and enhancing the T-cell recognition of cancer-specific antigens, CRISPR technology aims to create more effective and durable immunotherapy options for patients with cancer.^[9]

Synthetic lethality: A personalized approach to cancer treatment

Moreover, CRISPR is being utilized in synthetic lethality strategies, which represent a promising approach to personalized cancer therapy. This strategy targets cancer cells based on their unique genetic vulnerabilities, exploiting the concept that the simultaneous disruption of two genes can be lethal to cancer cells while sparing normal cells. By utilizing CRISPR to identify and edit genes that, when altered, render cancer cells more susceptible to certain therapies, researchers can develop targeted treatments that maximize efficacy while minimizing harm to healthy tissue. This approach not only holds

the potential for more effective cancer therapies but also allows for the personalization of treatment plans based on the specific genetic landscape of an individual's tumor.

The integration of CRISPR technology into cancer research and treatment reflects a paradigm shift in how we approach the disease. By harnessing the power of precise gene editing, researchers are developing innovative strategies that target the genetic drivers of cancer and enhance the immune response against tumors. However, as with any emerging technology, there are important ethical considerations and potential safety concerns that must be addressed. Ongoing research and clinical trials will be critical in determining the effectiveness and safety of CRISPR-based cancer therapies, as well as in establishing regulatory frameworks to ensure responsible use.

Ethical Considerations and Regulatory Challenges

As the promise of CRISPR-based therapies continues to unfold, it becomes increasingly crucial to address the ethical considerations and regulatory challenges associated with this revolutionary technology. The potential for CRISPR to alter the genetic code at will presents groundbreaking opportunities for treating genetic disorders and cancers, yet it also raises significant concerns about the implications of such power. One of the foremost ethical issues revolves around off-target effects, which occur when CRISPR inadvertently modifies unintended regions of the genome. These unintended alterations could lead to unforeseen consequences, such as the disruption of essential genes or the activation of oncogenes, potentially resulting in harmful effects on patient health.^[10] The long-term safety of CRISPR therapies remains a critical question, necessitating rigorous pre-clinical and clinical testing to ascertain not only the efficacy but also the safety of these interventions.

Ethical dilemmas of germline editing and future generations

Another profound ethical dilemma arises with the prospect of germline editing, where modifications are made to the DNA of embryos or reproductive cells, thereby affecting future generations. This capability opens up a range of ethical concerns, including the potential for "designer babies," where genetic traits could be selected for cosmetic or enhancement purposes rather than purely therapeutic ones. Such possibilities spark debates over the morality of manipulating human genetics and the potential for exacerbating social inequalities, as access to such technologies may not be equitable. Furthermore, germline editing poses questions about consent, as future generations would not have a say in the genetic modifications made to them. As such, the ethical implications of germline editing require careful consideration and broad societal dialogue to establish acceptable boundaries and principles.^[11]

Navigating the regulatory landscape for CRISPR technologies

In addition to ethical considerations, the regulatory landscape for CRISPR technology remains complex and evolving. Establishing

robust regulatory frameworks is essential to ensure the responsible application of CRISPR in clinical settings. Regulatory bodies must navigate the balance between fostering innovation and ensuring patient safety. This requires a comprehensive understanding of the science, potential risks, and societal implications of CRISPR technologies. Countries around the world are grappling with how to regulate gene editing, and policies vary significantly, complicating international collaboration and research efforts. Ongoing discussions among scientists, ethicists, and policymakers are critical to navigate these challenges, ensuring that CRISPR technology is developed and applied in ways that prioritize safety, ethics, and public trust.^[12]

Public perception also plays a significant role in shaping the future of CRISPR-based therapies. Misinformation, fear, and misunderstanding about gene editing can lead to societal backlash against these technologies, potentially hindering research and clinical application. Therefore, transparent communication and public engagement are vital to educate society about the benefits, risks, and ethical considerations associated with CRISPR. It is essential to foster an informed public discourse that can support sound policy decisions while considering diverse perspectives.

Future Directions and Innovations in CRISPR Technology

The future of CRISPR technology is poised to unlock a myriad of exciting possibilities, with ongoing innovations focused on enhancing the precision, efficiency, and safety of gene editing. One of the most significant advancements on the horizon is the development of CRISPR 2.0 technologies, which involve the exploration of alternative Cas proteins that offer enhanced specificity and reduced off-target activity. Traditional Cas9, while powerful, can sometimes bind to unintended genomic sites, leading to potential unwanted mutations. Newer Cas proteins, such as Cas12 and Cas13, exhibit unique characteristics that allow for more targeted editing capabilities, providing researchers with a broader toolkit to tailor gene editing for specific applications. By minimizing off-target effects, these advancements aim to improve the safety profile of CRISPR therapies, making them more suitable for clinical applications.

In addition to CRISPR 2.0, the emergence of base editing and prime editing techniques represents a groundbreaking shift in the precision of gene modifications. Base editing, which enables the direct conversion of one DNA base pair into another without introducing DSBs, holds great promise for correcting point mutations associated with various genetic disorders. This technique drastically reduces the likelihood of errors that can arise during the repair process and opens up new avenues for treating diseases that were previously deemed difficult to address using traditional CRISPR methods. Similarly, prime editing, often referred to as "search-and-replace" editing, allows for precise insertion, deletion, or replacement of DNA sequences with minimal collateral damage to the surrounding genome. These innovations have the potential to significantly expand the range of diseases that can be treated with CRISPR-based therapies, offering new hope for patients suffering from previously untreatable genetic conditions.^[13]

As these advanced technologies continue to develop, collaborative efforts across academia, industry, and regulatory bodies will be crucial to advancing CRISPR innovations from the laboratory to clinical applications. Building strong partnerships between researchers, biopharmaceutical companies, and regulatory agencies will facilitate the sharing of knowledge, resources, and expertise necessary to translate breakthroughs in CRISPR technology into safe and effective therapies. Furthermore, the establishment of clear regulatory pathways will be essential in ensuring that these innovative approaches are rigorously tested for safety and efficacy before reaching patients.

Moreover, the future of CRISPR technology may also encompass the integration of artificial intelligence and machine learning to enhance the design of gRNAs and predict off-target effects with greater accuracy. By leveraging computational tools, researchers can streamline the design process, leading to more effective and precise gene editing outcomes. The application of AI in CRISPR research can significantly accelerate the discovery of new therapeutic targets and optimize editing strategies, thereby advancing the field at an unprecedented pace.^[14]

Conclusion

The revolution of CRISPR-based therapies signifies a transformative era in genetic medicine, offering unprecedented opportunities for the treatment of genetic disorders and cancers. This groundbreaking technology has evolved from its origins as a bacterial defense mechanism to a powerful tool for precise genetic editing, enabling significant advancements in correcting inherited diseases, such as sickle cell disease and CF, while also enhancing cancer therapies through innovative strategies such as CRISPR-modified CAR-T cells and synthetic lethality approaches. However, the immense potential of CRISPR necessitates careful consideration of ethical and regulatory challenges, particularly regarding off-target effects and germline editing, which raise critical questions about safety and societal implications. Establishing robust regulatory frameworks and fostering ongoing dialogue among scientists, ethicists, and policymakers will be essential to navigate these complexities responsibly. Looking ahead, innovations such as CRISPR 2.0 technologies and base editing promise to further expand the range of treatable diseases, while collaborative efforts across various sectors will be pivotal in translating these

advancements from the laboratory to clinical applications. Ultimately, as we harness the power of CRISPR, it is crucial to remain committed to ethical considerations and regulatory integrity to ensure that the benefits of this revolutionary technology are realized for generations to come, ultimately transforming the landscape of genetic medicine and improving the lives of individuals affected by genetic disorders and cancer.

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