CRISPR/Cas9-Based Genome Editing in Precision Medicine: Current Trends and Recent Advancements

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ABSTRACT

The CRISPR/Cas9 system, known for its tremendous efficacy and accessibility, has revolutionized fundamental scientific research as a programmable genome-editing tool. The utilization of CRISPR/Cas9 system-based technologies has equipped researchers with potent instruments to explore the impact of genetics on the advancement of diseases. By genetically correcting a specific mutation unique to each patient, this approach treats human illnesses that traditional methods cannot effectively treat. This article highlights the importance of genetic factors in determining susceptibility to diseases and the need for personalized treatment methods. The main purpose of this review is to discuss how CRISPR/Cas9 is used to treat monogenic disorders and investigate its wider impact on medical research. The review also explores the collaboration between guide RNA and Cas9 nuclease in making accurate modifications to DNA, shedding light on the molecular processes of CRISPR/Cas9. Emphasis is placed on technological strides such as refinements in Cas9 and novel delivery techniques, which boost the accuracy and effectiveness of gene editing while reducing off-target impacts. The review also covers the ethical, legal, and societal impacts of genome editing, especially with regard to changes in the genetic line, and the importance of strong regulatory structures. CRISPR/Cas9 is highly esteemed as a cutting-edge technology in the healthcare sector, mostly due to its ability to accurately pinpoint genetic defects and facilitate a substantial revolution in cancer treatment. This review emphasizes the importance of collaboration between scientists, ethicists, and policymakers to ensure the ethical implementation of CRISPR/Cas9 technology in healthcare.

Keywords: CRISPR/Cas9, Gene therapy, Genome editing, Off-target effects, Precision medicine

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Introduction

The precise modification of DNA at the level of individual bases is known as genome editing, a form of genetic engineering. This process has revolutionized biological research and has the potential to tackle or preempt several human genetic disorders. For a genome-editing tool to be considered ideal, it should be able to modify a genetic sequence efficiently, exhibit a substantial degree of DNA sequence specificity, and have minimal unintended consequences in non-target areas. The initial development of genome-editing techniques took place in yeast and mammalian cells, where the natural repair mechanism called homologous recombination was used to substitute foreign DNA sequences for specific sections of the genome. In the late 1980s, researchers made a conscious decision to employ the method of natural homologous recombination in mouse embryonic stem cells to create animals with a certain genotype. The application of this technology has thus enabled the study of human diseases using mice and other animal models, greatly aiding the identification and development of new medications.[1]

To address these constraints, many organizations have created tools that enable the targeted creation of double-stranded breaks (DSBs) at particular locations within the genome using specialized enzymes known as "meganucleases."[2] This refers to endonucleases that possess a unique recognition site, which allows them to identify and cut certain DNA sequences to activate the homology-directed repair (HDR) mechanism.[3] It is necessary to introduce a DNA donor template that has ends that are comparable to the region of the DNA break to carry out this procedure.[4]

HDR is responsible for triggering non-homologous end joining (NHEJ) when DSBs are present. NHEJ has the potential to make it easier to successfully reunite the two ends of the break by inducing a random insertion or deletion of nucleotides.[5] Although the NHEJ repair pathway is very effective in producing functional gene knockouts, it also leads to the unintended occurrence of indels (insertions–deletions).[6] Thus, the current goal of the research is to generate site-specific DSBs that precisely induce HDR while suppressing NHEJ activity.[7,8]

Recent advancements have significantly improved the capability and precision of CRISPR/Cas9, reducing the likelihood of off-target consequences. Off-target consequences of genetic treatments have been identified as a significant risk due to their potential unintended impact on patients.[9] Utilizing CRISPR/Cas9 in precision medicine to treat genetic illnesses is a fascinating application. Certain genetic diseases such as muscular dystrophy, cystic fibrosis (CF), and sickle cell anemia can be cured by changing defective genes in a small number of cells.[10] To enhance the precision of editing, scientists have developed several methodologies including fundamental editors and high-fidelity Cas9 variants.

The CRISPR/Cas9 system has made gene editing more accessible. To cause a specific kind of DSB, CRISPR/Cas9 uses a small RNA molecule instead of a protein. Cas9 has complex but simple DNA target sequence alignment requirements: The target DNA and a 20-nucleotide "guide RNA" (gRNA) are needed to form base pairs, and the DNA must have a "protospacer-adjacent motif" (PAM). The Cas9 protein of every bacterial species has its unique PAM, which is a short DNA sequence located next to the complementary region. This two-step approach has replaced the laborious process of protein synthesis for ZFs and TALENs, in which sgRNA is responsible for directing the Cas9 nuclease to a specific sequence of DNA.[11]

It above approach was greatly aided by the simplicity of the CRISPR/Cas9 system, as well as its unique ability to cleave DNA and target multiple regions, and the existence of various type II CRISPR-Cas system variants. This has allowed for the advancement of using this technology, which is easily accessible and inexpensive, to modify the genomic DNA of various cells and organisms in an accurate and efficient manner.[12] Despite the fact that CRISPR/Cas9 has become the standard tool for editing genomes in research conducted all around the world, there has been a discernible increase in the number of exciting and unique studies that investigate its application in preclinical and clinical contexts.[13] This review article presents an in-depth evaluation of current research on the application of CRISPR/Cas9 system in the field of precision medicine. It also explores the several advantages this technology offers and the significant obstacles to its integration into wider healthcare management. Additionally, it sheds light on the ethical implications raised by the broader public regarding its use in disease management.

CRISPR/Cas9 System

In 1987, Ishino et al. observed a repetitive DNA sequence cluster interspersed with spacer sections in *Escherichia coli*. Mojica et al. discovered the presence of similar recurring sequences across a wide range of bacteria and archaea, which they designated clustered regularly interspaced palindromic repeats, abbreviated as CRISPR.[14] Bacteria first developed CRISPR/Cas9 as a defense mechanism against viruses, but now it can edit the genomes of many animals thanks to certain modifications (Figure 1).[15] A gRNA is used in this method to direct the Cas9 enzyme to a specific area of DNA where it starts a DSB. The cell's natural DNA repair mechanisms, including HDR or NHEJ, are activated in response to this cellular disturbance. Some genes may be mutated, or new genomic material may be inserted due to this process. CRISPR/Cas9 has transformed the area of biological research because of its user-friendliness, great efficiency, and impressive precision. The potential for this technology to propel advancements in biotechnology, agriculture, and health is enormous.[7,16]

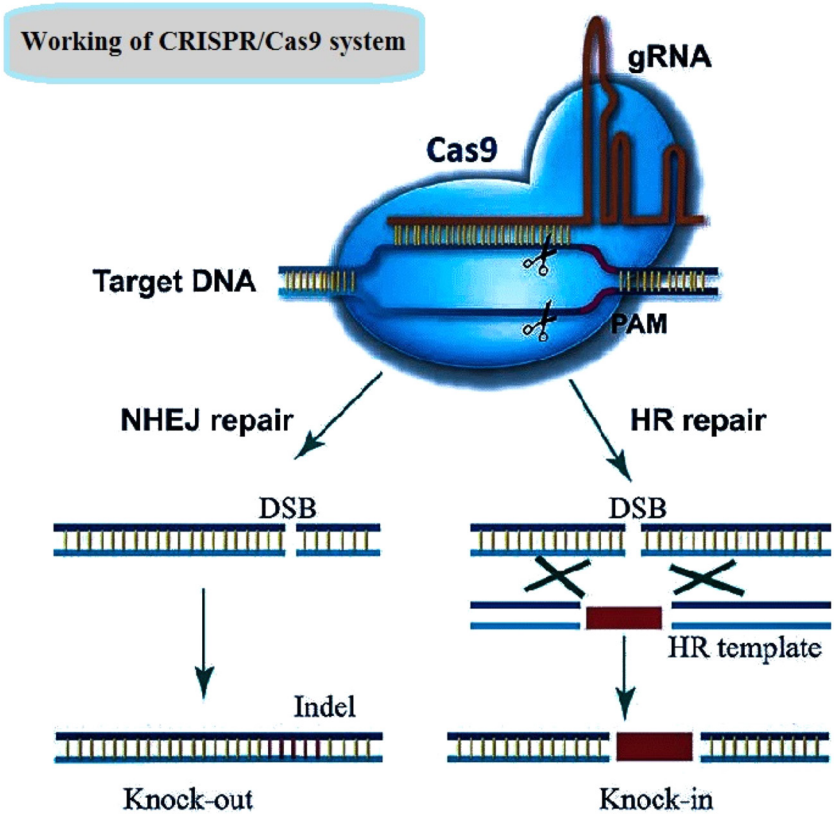

Fig 1 | Working of CRISPR/Cas9

Mechanism of Action

The CRISPR systems are broadly divided into two main classes, class 1 and class 2, based on the structural composition and organization of their effector protein complexes. These systems exhibit a significant amount of variety. Category I, category III, and category IV are the three separate groups that the first class distinguishes. These categories are then further divided into 15 subtypes, as described by Makarova et al..[17] Compared to class 1, class 2 can be summarized by the existence of an effector module, a single protein instead of an effector complex of multiple proteins. Category II, category V, and category VI are the subcategories that differentiate this class. Previous studies have conducted exhaustive research on a variety of other CRISPR systems.[18]

gRNA and Targeting

The CRISPR/Cas9 system functions via a constituent called the gRNA. The gRNA is an artificially created RNA molecule designed to possess a sequence complementary to a certain target DNA sequence inside the genome. The sequence specificity of the gRNA enables it to accurately attach to the specific targeted site, guaranteeing the Cas9 protein, which is an endonuclease, is guided to the proper spot in the DNA.[19] A CRISPR RNA (crRNA) that is complementary to the target DNA sequence and a trans-activating crRNA that attaches to the Cas9 protein to form a complex that is capable of cleaving DNA are the two basic components that make up the gRNA. Together, these two components form the gRNA.[20]

DNA Cleavage by Cas9

A DSB is introduced by the Cas9 protein after the gRNA–Cas9 complex has been directed to the target DNA location by the complementary base pairing that exists between the gRNA and the DNA. This break takes place at a particular location that is close to a PAM sequence, which is necessary for Cas9 binding and activity.[21] As a result of its ability to discriminate between the target and non-target DNA sites, the inclusion of the PAM sequence is essential because it prevents the unintentional editing of sequences that are similar to the target site.

DNA Repair Mechanisms

Since Cas9 is responsible for introducing the DSB, the natural repair systems of the cell are triggered to heal

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the break.[22] Two major routes are involved in the DNA repair process.Non-Homologous End JoiningNHEJ is the predominant repair mechanism in many cell types, particularly non-dividing cells. Due to the fact that this process immediately rejoins the broken DNA ends, it frequently results in indels at the location where the break occurred.[22] Indels like this have the potential to cause a frameshift mutation, which can result in the premature cessation of protein synthesis or the generation of a nonfunctional protein. This can cause the target gene to become dysfunctional. This feature is utilized in gene knockout experiments, which inhibit the function of a gene.[23]

Homology-Directed Repair

In order to ensure that the HDR mechanism is accurate, it is necessary to have a homologous template to direct the repair process. When it comes to genetic modification, this template can either be an endogenous homologous chromosome or an exogenously given DNA sequence that contains the desired alteration. When it comes to precise genome editing, such as fixing a point mutation or inserting a new gene, high-data-resolution (HDR) is a useful tool.[23] On the other hand, HDR is less effective in cells that are not proliferating, and it often necessitates the presence of the repair template in close proximity to the DSB. In experimental conditions, the efficiency of high-dynamic range (HDR) can be improved by synchronizing cells in phases of the cell cycle (S and G2), which is the period of the cell cycle in which HDR activity is naturally higher.[24]

Recent Advancements

Enhancing Specificity and Reducing Off-Target Effects

The possibility for off-target consequences is one of the key issues that are associated with the CRISPR/Cas9 technique, unintended cuts at sites other than the target sequence. Off-target effects can lead to unintended genetic mutations, which may have significant implications, especially in clinical settings. To address this, several high-fidelity Cas9 variants have been developed. For instance, the SpCas9-HF1 variant features modifications that reduce off-target activity by altering the protein's interactions with DNA.[25] Similarly, the eSpCas9 variant has been engineered to minimize off-target effects by improving the enzyme's specificity.[26]

Base Editing and Prime Editing

Base editing is an innovative CRISPR-based approach that directly converts one DNA base into another without creating DSBs. This method uses a catalytically impaired Cas9 fused to a base-modifying enzyme, such as cytidine deaminase, to achieve (G to A) or C to T conversions, common mutations in many genetic diseases.[27] This capability is expanded by the use of a pegRNA (prime editing gRNA), also known as a primary editing gRNA, in conjunction with reverse transcriptase. This allows for the insertion, deletion, or substitution of nucleotides at the target spot. It is possible that the risk of off-target effects and other difficulties connected with DSBs could be reduced with the help of this technology, which offers a versatile tool for conducting precise genome editing (Figure 2).[28]

Delivery Methods

One of the most important factors that determines the efficacy of genome-editing therapy is the efficient distribution of CRISPR/Cas9 components into cells. Lipid nanoparticles (LNPs) and viral vectors, such as adeno-associated viruses (AAVs), are examples of delivery systems that are frequently utilized. LNPs can encapsulate Cas9 mRNA and gRNAs, protecting them from degradation and facilitating cell uptake. AAV vectors, capable of delivering CRISPR components directly to specific tissues, are particularly useful in targeting

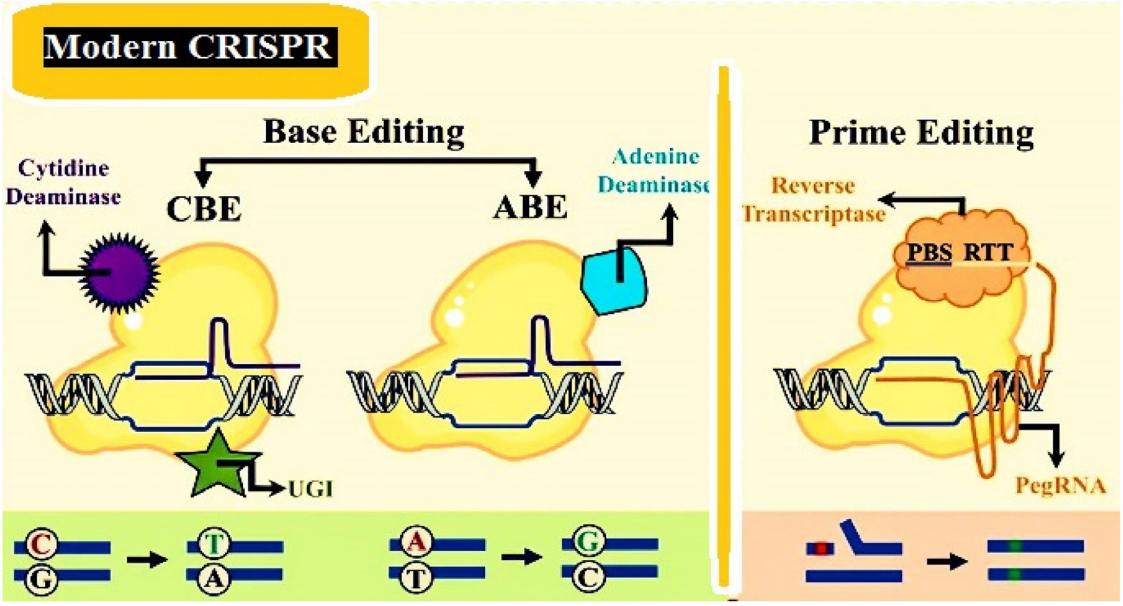

Fig 2 | Base editing and prime editing of modern CRISPR

the liver and muscle tissues.[29] These advancements in delivery methods enhance the efficiency and specificity of CRISPR-mediated gene editing.

Applications in Precision Medicine

Correction of Genetic Disorders

Cystic Fibrosis: Mutations in the CFTR gene cause severe respiratory and digestive issues in CF. Recent gene therapy advances may correct these mutations. Case studies and clinical trials show that CRISPR-Cas9 can target and repair the CFTR gene in patient-derived cells. In one study, using a viral vector to deliver CRISPR components directly to lung cells restored CFTR function.[30] These promising results suggest that gene therapy might cure CF and reduce the need for lifelong symptomatic treatments.

Sickle Cell Anemia: Because of a single point mutation in the HBB gene, sickle cell anemia is a hereditary condition that can be quite severe. This mutation results in aberrant hemoglobin distribution. Gene editing strategies, mainly those utilizing CRISPR-Cas9, have significantly addressed this condition.[31] Researchers have developed methods to correct the HBB mutation or induce fetal hemoglobin production, which can compensate for the defective adult hemoglobin. Recent clinical trials have demonstrated successful editing of hematopoietic stem cells, which, when reinfused into patients, produce healthy red blood cells.[32] Figure 3 illustrates various applications of CRISPR/Cas9 technologies in gene editing, including functional gene identification, drug targeting libraries, and RNA-targeting applications.

Cardiovascular Disease: According to the World Health Organization, cardiovascular illnesses, also known as cardiovascular diseases (CVDs), are the leading cause of death across the entire world. The advancements in CRISPR/Cas9 technology have significantly influenced the creation of novel in vivo instruments that enable more comprehensive processes behind CVD. Significantly, the proliferation of CRISPR/Cas9 technology expedited the development of innovative medicines that can treat CVDs.[33] Consequently, during the course of the past year, there has been a substantial rise in the number of published studies that provide evidence of the application of CRISPR/Cas9 in the treatment of CVDs.[34]

Duchenne Muscular Dystrophy: The Duchenne muscular dystrophy (DMD) gene, which is the most critical factor in gradual muscle degradation, a severe genetic illness, is caused by mutations in the gene. Recent breakthroughs in gene therapy have focused on using CRISPR-Cas9 to correct these mutations.[35] One approach involves excising the mutated exons to re-establish the reading frame of the DMD gene, thereby enabling the creation of a functional, albeit shorter, dystrophin protein.[36]

CRISPR/Cas9 Applications in Cancer Treatment

There has been a significant advancement in the research of cancer genetics with the advent of the CRISPR system, which makes it possible to rapidly create mutations in tumor suppressor genes, oncogenes, or other critical factors in the development of cancer. These mutations can be classified as loss-of-function (LOF) or gain-of-function (GOF) genes. An example of this is research conducted by Matano et al., which showcased the potential of CRISPR in enhancing comprehension of the formation and advancement of human colorectal cancer (CRC).[37] According to the findings of the study, human intestinal organoids that had not experienced any transformation were given a set of mutations known as LOF and GOF. These mutations are frequently associated with CRC, and they were introduced into the organoids. It is interesting to note that the researchers found that they were unable to accurately simulate the tumorigenic and metastatic features of this particular human sickness.[15] Taking this into consideration, it would appear that additional genetic and epigenetic pathways are required for the invasive behavior of CRC.[38,39]

CAR-T Cell Therapy

The treatment of hematologic malignancy has been completely transformed by CAR-T therapy. The use of CRISPR technology has made it entirely possible to achieve significant improvements in both the efficacy and CAR-T cell safety. Researchers use CRISPR-Cas9 to alter T cells to obliterate tumors, stay in the body, and decrease side effects.[40] CRISPR may knock out genes that reduce T cells or bring in novel receptors that target cancer cells. These modifications might make CAR-T cell treatments more effective and long-lasting, humanizing cancer therapy results.[41]

Clinical experiments using CRISPR-engineered CAR-T cells have shown promise. In one critical work, CRISPR was used to adapt the PD-1 gene in T cells, which cancer tumors upregulate to evade immune detection.[42] In advanced intractable cancer patients, adapted CAR-T cells had amplified anti-tumor activity and extensive durability.[43] One more clinical experiment employed CRISPR to knock off immune rejection genes in allogeneic CAR-T cells, making the treatment easier to get and scalable.[42] Additionally, CRISPR technology has been used to target oncogenes and tumor suppressor genes directly. For instance, CRISPR has been employed to knock out the oncogene MYC, implicated in various cancers, resulting in reduced tumor growth and improved survival in preclinical models.[38, 39]

CRISPR/Cas9 for HIV and HBV

The use of CRISPR/Cas9 in developing antiviral strategies has shown great promise, particularly for chronic infections such as HIV and HBV. For HIV, CRISPR/Cas9 has been employed to target and excise the integrated viral DNA from the host genome, a critical step toward potentially curing the infection.[44] By designing gRNAs that specifically target conserved regions of the HIV genome, researchers have successfully demonstrated the ability to disrupt viral replication and significantly reduce the viral load in infected cells.[45] In the case of HBV, targeting the cccDNA (covalently closed circular DNA) with CRISPR/Cas9 results in disruption of

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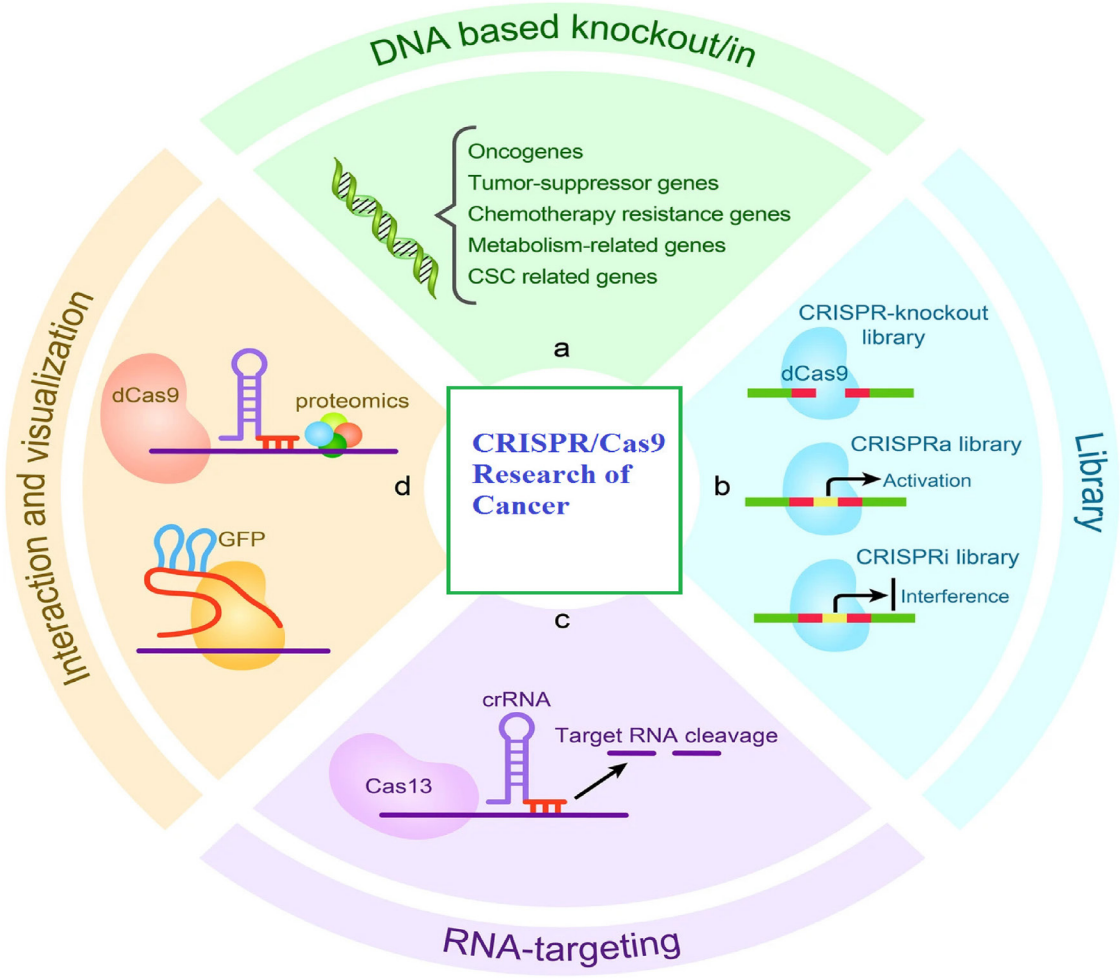

Fig 3 | Applications of gene editing technologies based on CRISPR/Cas9 are being proposed. (a) CRISPR/Cas9 was utilized to confirm and identify the specific functional gene, (b) library for drug targeting and functional genes, (c) RNA targeting of CRISPR application, and (d) dCas9 was used for interaction research and other work's GFP, proteomics

the viral replication cycle, as cccDNA is essential for producing new viral particles.[46] Preclinical studies have shown that these approaches can lead to significant reductions in viral load and enhanced immune responses in animal models.[47]

Rare, Undiagnosed Diseases, and Personalized Medicine

Case Studies of Individualized CRISPR Treatments

CRISPR technology has shown great promise in personalized medicine, particularly for rare and undiagnosed diseases where conventional treatments are often unavailable or ineffective. A notable case study involves a young patient with a severe, undiagnosed genetic disorder causing debilitating symptoms. Through whole-genome sequencing, researchers identified a unique mutation responsible for the condition. They used CRISPR-Cas9 to design a personalized gene therapy to correct the mutation in the patient's cells.[48]

Examples of Rare Genetic Disorder Treatments

Several rare genetic disorders have been targeted using CRISPR technology, showcasing its versatility and efficacy. An exemplary instance is the management of Leber congenital amaurosis (LCA), a rare hereditary retinal condition that results in profound visual impairment. Scientists used CRISPR technology to rectify the CEP290 gene mutation, which is accountable for LCA, in retinal cells produced from patients. This intervention successfully reinstated the normal functioning of the gene, providing a promising prospect for the restoration of eyesight in afflicted people.[49] Another illustration pertains to the therapy of spinal muscular atrophy, which is a neuromuscular illness that is extremely uncommon and is brought on by mutations in the gene named SMN1. By utilizing CRISPR to upregulate the expression of the SMN2 protein, which can partially compensate for the loss of SMN1 function, researchers achieved significant improvements in motor function in animal models, paving the way for potential therapeutic applications in humans.[50]

Multiplexed Genome Editing: Methods and Techniques

Simultaneous Targeting of Multiple Genes

Multiplexed genome-editing targets many genes or genomic regions in one experiment. This technique

uses CRISPR-Cas systems to drive the Cas protein to different genomic locations using frequent gRNAs. Researchers may simultaneously adjust many genes by creating and delivering gRNAs that target more than a few genes.[51] This move is valuable for researching gene associations and pathways because it permits systematic disturbance or alteration of several genes to evaluate combinatorial effects.[52–55]

CRISPR-Based RNA Modification

CRISPR-based RNA editing is a growing pasture that can modify RNA molecules without altering the underlying DNA. REPAIR, which stands for "RNA Editing for Programmable A to I Replacement," and RESCUE, which stands for "RNA Editing for Specific C to U Exchange," are two notable methods that have been developed to edit RNA. REPAIR utilizes a catalytically still Cas13 enzyme compound to an adenosine deaminase, which specifically converts adenosine to inosine.[12,54] However, RESCUE involves a fusion of Cas13 with a cytidine delaminate to change cytosine to uridine. These systems pressure the programmable nature of CRISPR to target specific RNA sequences for alteration, offering a versatile and passing move toward gene directive and correction of genetic defects at the RNA level.[56]

Therapeutic Potential

RNA editing holds significant therapeutic potential for treating diseases caused by point mutations or aberrant splicing. By targeting and correcting RNA transcripts, these systems can restore standard protein reason without enduringly altering the DNA.[56] For occurrence, RNA editing can be used to correct mutations in mRNA transcripts associated with genetic disarray, such as CF, well-built dystrophy, and certain neurological situations.[11]

Technological Innovations in CRISPR/Cas9 Delivery

Lipid Nanoparticles

Because of their capacity to encapsulate and safeguard nucleic acids, LNPs have emerged as a potentially useful delivery vehicle for CRISPR components. This is because LNPs make it easier for nucleic acids to be transported into cells. As stated by Zhang et al., LNPs are composed of ionizable lipids, phospholipids, cholesterol, and polyethylene glycol lipids, which come together to form a structure that is both stable and easily biocompatible.[57] Upon administration, LNPs can either fuse with cell membranes or be endocytosed, which causes their payload to be released into the cytoplasm. The delivery of CRISPR-Cas9 components for gene editing has been accomplished with great success using LNPs in a variety of organs, including the liver, where they have demonstrated a high level of efficiency in editing target genes.[58]

Viral Vectors

Due to the remarkable efficacy with which viral vectors, such as lentiviruses, and adenoviruses, can transduce a wide variety of cell types, they are frequently utilized for the delivery of CRISPR.[59] When it comes to delivering genetic material to cells that are not dividing, AAV vectors are particularly appreciated due to their low immunogenicity. Stable and long-term production of CRISPR components can be achieved through the integration of lentiviral vectors produced from HIV into the genome of the host.[60]

Targeting Specific Tissues and Cells

Innovations in tissue-specific delivery aim to enhance the accuracy of CRISPR gene editing by targeting the delivery of CRISPR components to specific cells or tissues. This can be achieved by designing delivery vehicles that recognize and bind to cell surface markers unique to the target tissue. For example, it is possible to accomplish the functionalization of LNPs by utilizing ligands or antibodies that precisely bind to receptors on the surface of hepatocytes or tumor cells.[61] This ensures that the CRISPR components are delivered directly to the intended cells. Strategies for improving specificity and reducing off-target effects include using high-fidelity Cas9 variants, dual-gRNAs targeting adjacent sequences, and transient delivery methods such as mRNA or ribonucleoprotein complexes for CRISPR delivery, which can reduce the duration of exposure to CRISPR components, minimizing off-target effects.[62]

Ethical, Legal, and Social Implications

Ethical Considerations

Germline editing engages in making genetic modifications that are heritable, moving not only the individual but also their offspring. This area of CRISPR technology is highly controversial due to several ethical concerns. The primary risk is the possibility of any accidental penalty, such as off-target effects, that could bring harmful mutations into the human gene pool. Ethical concerns also turn around the potential for creating "designer babies," where genetic characters are selected for non-medical reasons, potentially exacerbating social inequality and leading to new forms of discrimination.[63] Off-target effects, where CRISPR induces accidental genetic changes, pose significant ethical and protection anxiety. These unintentional edits can potentially lead to harmful mutations, raising the risk of diseases such as cancer. The impulsiveness and potential to cause damage elevate questions about the ethicality of using CRISPR in clinical settings without addressing these risks.[64] Furthermore, off-target effects challenge the "do no harm" principle in medical ethics and necessitate exact preclinical testing and nonstop monitoring in clinical applications. Addressing this ethical apprehension requires advances in CRISPR correctness and the expansion of high-fidelity variants to decrease off-target activity.[65]

International perspectives on CRISPR vary significantly, reflecting cultural, ethical, and legal differences. For example, China has been more lenient in its regulatory approach, allowing clinical trials involving CRISPR-based gene editing for cancer treatment. In contrast, many Western countries take a more careful stance, with strict regulations on germline editing

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and a focus on ethical considerations.[66] The lack of a global consensus regulating CRISPR technology leads to dissimilarity in research and clinical applications. International collaboration and harmonization of rigid values are necessary to address these differences and ensure CRISPR technology's accountable and fair use worldwide.

Public Opinion on Gene Editing

Public insight into gene editing is varied, prejudiced by ethical concerns, potential profit, and media depiction. Surveys identify that while many people are familiar with the potential of CRISPR to treat genetic diseases, there is essential anxiety about its ethical implications, mostly concerning germline editing. Propaganda and sensationalism in media coverage can create inferior fears and misconceptions. Public opinion is also formed by educational and religious beliefs, which can either support or oppose genetic interventions.[67,68]

Future Applications in Precision Medicine

Future advancements in CRISPR technology are unsurprising in enhancing the modified medicine field. By tailoring gene editing therapies to the specific genetic outline of individual patients, CRISPR might allow highly customized treatments that address restricted genetic mutations and variations. This adapted approach could improve treatments' efficacy and minimize unfavorable effects, leading to more successful outcomes for patients with genetic.[7]

The potential for CRISPR technology to understand widespread clinical use is promising. As the technology becomes more advanced and reliable, it is likely to be included in routine clinical practice for various applications, from gene therapy to cancer treatment. Advances in delivery methods, reduced off-target effects, and enhanced safety profiles will facilitate the broader adoption of CRISPR-based therapies.[69] In addition, the development of rigorous authoritarian frameworks and ethical norms will be very important in order to guarantee the deployment of CRISPR in therapeutic settings in a manner that is both accountable and successful.[12]

Conclusion

The introduction of the CRISPR/Cas9 technology has ushered in a new era of change in the field of genetic engineering, presenting prospects for precision medicine that have never been seen before. This powerful tool can potentially revolutionize the treatment of genetic disorders by providing targeted, efficient, and customizable interventions. As we continue to explore its capabilities, it is crucial to balance scientific advancement with ethical responsibility. The promise of CRISPR/Cas9 lies in its technical prowess and its potential to address complex genetic diseases and improve the quality of life for countless individuals. Our ability to ensure that the benefits of CRISPR/Cas9 are achieved in a manner that is safe, egalitarian, and in line with our shared values may be ensured by cultivating a culture that encourages open conversation and rigorous inspection. In a world where genetic disorders are treated and cured, those afflicted will have hope and healing thanks to the careful incorporation of this technology into clinical practice. To make sure the advantages are worth it and to lessen the dangers, it is vital to set ethical and regulatory standards. The discovery of the CRISPR/Cas9 system allowed for the expansion of the variety of possible applications in the medical field. There is a good chance that it will bring about a substantial change in the way that we deal with genetic diseases in the years to come.

References

1 Li T, Huang S, Zhao X, Wright DA, Carpenter S, Spalding MH, et al. Modularly assembled designer TAL effector nucleases for targeted gene knockout and gene replacement in eukaryotes. Nucl Acids Res. 2011;39(14):6315–25.
2 Eid A, Mahfouz MM. Genome editing: the road of CRISPR/Cas9 from bench to clinic. Exp Mol Med. 2016;48(10).
3 Jasin M, Rothstein R. Repair of strand breaks by homologous recombination. Cold Spring Harb Perspect Biol. 2013;5(11).
4 Chapman JR, Taylor MR, Boulton SJ. Playing the end game: DNA double-strand break repair pathway choice. Mol Cell. 2012;47(4):497–510.
5 Mardis ER. A decade's perspective on DNA sequencing technology. Nature. 2011;470(7333):198–203.
6 Komor AC, Badran AH, Liu DR. CRISPR-based technologies for the manipulation of eukaryotic genomes. Cell. 2017;168(1):20–36.
7 Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. 2012;337(6096):816–21.
8 Ashley EA. Towards precision medicine. Nat Rev Genet. 2016;17(9):507–22.
9 Ledford H. CRISPR, the disruptor. Nature. 2015;522(7554):20–4.
10 Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. Science. 2014;346(6213):1258096.
11 Abudayyeh OO, Gootenberg JS, Essletzbichler P, Han S, Joung J, Belanto JJ, et al. RNA targeting with CRISPR–Cas13. Nature. 2017;550(7675):280–4.
12 Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. Cell. 2014;157(6):1262–78.
13 Lanphier E, Urnov F, Haecker SE, Werner M, Smolenski J. Don't edit the human germ line. Nature. 2015;519(7544):410–1.
14 Mojica FJ, Díez-Villaseñor C, Soria E, Juez G. Biological significance of a family of regularly spaced repeats in the genomes of Archaea, Bacteria and mitochondria. Mol Microbiol. 2000;36(1):244–6.
15 Matano M, Date S, Shimokawa M, Takano A, Fujii M, Ohta Y, et al. Modeling colorectal cancer using CRISPR-Cas9–mediated engineering of human intestinal organoids. Nat Med. 2015;21(3):256–62.
16 Nishimasu H, Ran FA, Hsu PD, Konermann S, Shehata SI, Dohmae N, et al. Crystal structure of Cas9 in complex with guide RNA and target DNA. Cell. 2014;156(5):935–49.
17 Makarova KS, Wolf YI, Alkhnbashi OS, Costa F, Shah SA, Saunders SJ, et al. An updated evolutionary classification of CRISPR–Cas systems. Nat Rev Microbiol. 2015;13(11):722–36.
18 Koonin EV, Makarova KS, Zhang F. Diversity, classification and evolution of CRISPR-Cas systems. Curr Opin Microbiol. 2017;37:67–78.
19 Sternberg SH, Redding S, Jinek M, Greene EC, Doudna JA. DNA interrogation by the CRISPR RNA-guided endonuclease Cas9. Biophys J. 2014;106(2):695a.
20 Torres RA, Martin MC, Garcia A, Cigudosa JC, Ramirez JC, Rodriguez-Perales S. Engineering human tumour-associated chromosomal translocations with the RNA-guided CRISPR–Cas9 system. Nat Commun. 2014;5(1):3964.
21 Razzouk S. A cornerstone for the evolution of precision medicine. Ann Hum Genet. 2018;82(6):331–57.
22 Gillyard T, Davis J. DNA double-strand break repair in cancer: a path to achieving precision medicine. Int Rev Cell Mol Biol. 2021;364:111–37.
23 Pawelczak KS, Gavande NS, VanderVere-Carozza PS, Turchi JJ. Modulating DNA repair pathways to improve precision genome engineering. ACS Chem Biol. 2018;13(2):389–96.

24 Smirnikhina SA, Zaynitdinova MI, Sergeeva VA, Lavrov AV. Improving homology-directed repair in genome editing experiments by influencing the cell cycle. Int J Mol Sci. 2022;23(11):5992.
25 Kleinstiver BP, Pattanayak V, Prew MS, Tsai SQ, Nguyen NT, Zheng Z, et al. High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. Nature. 2016;529(7587):490–5.
26 Slaymaker IM, Gao L, Zetsche B, Scott DA, Yan WX, Zhang F. Rationally engineered Cas9 nucleases with improved specificity. Science. 2016;351(6268):84–8.
27 Gaudelli NM, Komor AC, Rees HA, Packer MS, Badran AH, Bryson DI, et al. Programmable base editing of AT to GC in genomic DNA without DNA cleavage. Nature. 2017;551(7681):464–71.
28 Anzalone AV, Randolph PB, Davis JR, Sousa AA, Koblan LW, Levy JM, et al. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature. 2019;576(7785):149–57.
29 Liu C, Zhang L, Liu H, Cheng K. Delivery strategies of the CRISPR-Cas9 gene-editing system for therapeutic applications. J Control Release. 2017;266:17–26.
30 Bandara RA, Chen ZR, Hu J. Potential of helper-dependent Adenoviral vectors in CRISPR-cas9-mediated lung gene therapy. Cell Biosci. 2021;11(1):145.
31 Chen C, Liu Y, Rappaport AR, Kitzing T, Schultz N, Zhao Z, et al. MLL3 is a haploinsufficient 7q tumor suppressor in acute myeloid leukemia. Cancer Cell. 2014;25(5):652–65.
32 Koniali L, Lederer CW, Kleanthous M. Therapy development by genome editing of hematopoietic stem cells. Cells. 2021;10(6):1492.
33 Ma H, Marti-Gutierrez N, Park SW, Wu J, Lee Y, Suzuki K, et al. Correction of a pathogenic gene mutation in human embryos. Nature. 2017;548(7668):413–9.
34 German DM, Mitalipov S, Mishra A, Kaul S. Therapeutic genome editing in cardiovascular diseases. JACC Basic Transl Sci. 2019;4(1):122–31.
35 Viswanathan SK, Sanders HK, McNamara JW, Jagadeesan A, Jahangir A, Tajik AJ, et al. Hypertrophic cardiomyopathy clinical phenotype is independent of gene mutation and mutation dosage. PLoS One. 2017;12(11).
36 Mollanoori H, Rahmati Y, Hassani B, Mehr MH, Teimourian S. Promising therapeutic approaches using CRISPR/Cas9 genome editing technology in the treatment of Duchenne muscular dystrophy. Genes Dis. 2021;8(2):146–56.
37 Sánchez-Rivera FJ, Jacks T. Applications of the CRISPR–Cas9 system in cancer biology. Nat Rev Cancer. 2015;15(7):387–93.
38 Wang H, Xu X, Couturier J, Shao S, Wang X, Yue J, et al. Dual roles of the RNA-binding protein HuR in cancer. Mol Cancer Res. 2015;11(5):481–6.
39 Boutelle AM, Attardi LD. p53 and tumor suppression: it takes a network. Trends Cell Biol. 2021;31(4):298–310.
40 Wei W, Chen ZN, Wang K. CRISPR/Cas9: a powerful strategy to improve CAR-T cell persistence. Int J Mol Sci. 2023;24(15):12317.
41 Maus MV, Grupp SA, Porter DL, June CH. Antibody-modified T cells: CARs take the front seat for hematologic malignancies. Blood. 2014;123(17):2625–35.
42 Ren J, Zhang X, Liu X, Fang C, Jiang S, June CH, et al. A versatile system for rapid multiplex genome-edited CAR T cell generation. Oncotarget. 2021;8(10):17002–11.
43 Stadtmauer EA, Fraietta JA, Davis MM, Cohen AD, Weber KL, Lancaster E, et al. CRISPR-engineered T cells in patients with refractory cancer. Science. 2020;367(6481).
44 Lin H, Li G, Peng X, Deng A, Ye L, Shi L, et al. The use of CRISPR/Cas9 as a tool to study human infectious viruses. Front Cell Infect Microbiol. 2021;11:590989.
45 Kaminski R, Chen Y, Fischer T, Tedaldi E, Napoli A, Zhang Y, et al. Elimination of HIV-1 genomes from human T-lymphoid cells by CRISPR/Cas9 gene editing. Sci Rep. 2016;6:22555.
46 Mohammadzadeh I, Qujeq D, Yousefi T, Ferns GA, Maniati M, Vaghari-Tabari M. CRISPR/Cas9 gene editing: a new therapeutic approach in the treatment of infection and autoimmunity. IUBMB Life. 2020;72(8):1603–21.
47 Kostyushev D, Brezgin S, Kostyusheva A, Zarifyan D, Goptar I, Chulanov V. Orthologous CRISPR/Cas9 systems for specific and efficient degradation of covalently closed circular DNA of hepatitis B virus. Cell Mol Life Sci. 2019;76(9):1779–94.
48 Selvakumar SC, Preethi KA, Ross K, Tusubira D, Khan MWA, Mani P, et al. CRISPR/Cas9 and next generation sequencing in the personalized treatment of Cancer. Mol Cancer. 2022;21(1):83.
49 Maeder ML, Thibodeau-Beganny S, Osiak A, Wright DA, Anthony RM, Eichtinger M, et al. Rapid "open-source" engineering of customized zinc-finger nucleases for highly efficient gene modification. Mol Cell. 2019;31(2):294–301.
50 Martinez-Fernandez S, Osorio FG, Garcia-Ibanez Y, Sanchez MC, Luque-Gomez A, Rubio MA, et al. Regulation of progerin-associated nuclear envelope stiffness by TRAF2 and lamin A/C. Nat Commun. 2020;11(1):1–11.
51 Wright AV, Nuñez JK, Doudna JA. Biology and applications of CRISPR systems: harnessing nature's toolbox for genome engineering. Cell. 2016;164(1):29–44.
52 Bae S, Park J, Kim JS. Cas-OFFinder: a fast and versatile algorithm that searches for potential off-target sites of Cas9 RNA-guided endonucleases. Bioinformatics. 2014;30(10):1473–5.
53 Shen B, Zhang W, Zhang J, Zhou J, Wang J, Chen L, et al. Efficient genome modification by CRISPR-Cas9 nickase with minimal off-target effects. Nat Methods. 2014;11(4):399–402.
54 Wang H, La Russa M, Qi LS. CRISPR/Cas9 in genome editing and beyond. Annu Rev Biochem.. 2016;85(1):227–64.
55 Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, et al. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell. 2015;163(3):759–71.
56 Cox DBT, Gootenberg JS, Abudayyeh OO, Franklin B, Kellner MJ, Joung J, et al. RNA editing with CRISPR-Cas13. Science. 2017;358(6366):1019–27.
57 Zhang L, Wang P, Feng Q, Wang N, Chen Z, Huang Y, et al. Lipid nanoparticle-mediated efficient delivery of CRISPR/Cas9 for tumor therapy. NPG Asia Mater. 2017;9(10).
58 Yin H, Kauffman KJ, Anderson DG. Delivery technologies for genome editing. Nat Rev Drug Discov. 2014;16(6):387–99.
59 Yip BH. Recent advances in CRISPR/Cas9 delivery strategies. Biomolecules. 2020;10(6):839.
60 Naldini L. Gene therapy returns to centre stage. Nature. 2015;526(7573):351–60.
61 Duan L, Ouyang K, Xu X, Xu L, Wen C, Zhou X, et al. Nanoparticle delivery of CRISPR/Cas9 for genome editing. Front Genet. 2021;12:673286.
62 Chandrasekaran AP, Song M, Kim KS, Ramakrishna S. Different methods of delivering CRISPR/Cas9 into cells. Prog Mol Biol Transl Sci. 2018;159:157–76.
63 Baltimore D, Berg P, Botchan M, Carroll D, Charo RA, Church G, et al. A prudent path forward for genomic engineering and germline gene modification. Science. 2015;348(6230):36–8.
64 Hsu PD, Scott DA, Weinstein JA, Ran FA, Konermann S, Agarwala V, et al. DNA targeting specificity of RNA-guided Cas9 nucleases. Nat Biotechnol. 2013;31(9):827–32.
65 Sciences NA of. Engineering, and Medicine. Human Genome Editing: Science, Ethics, and Governance. The National Academies Press; 2017.
66 Cyranoski D. The CRISPR-baby scandal: what's next for human gene-editing. Nature. 2019;566(7745):440–2.
67 Gumer JM. The wisdom of germline editing: an ethical analysis of the use of CRISPR-Cas9 to edit human embryos. The New Bioethics. 2019;25(2):137–52.
68 Scheufele DA, Xenos MA, Howell EL, Rose KM, Brossard D, Hardy BW. U. S. attitudes on human genome editing. Science. 2017;357(6351):553–4.
69 O'Keefe M, Perrault S, Halpern J, Ikemoto L, Yarborough M, Ormond KE. "Editing" genes: a case study about how language matters in bioethics. Am J Bioeth. 2015;15(12):3–10.