

CRISPR - CAS MEDIATED GENOME EDITING FOR HUMAN DISEASE THERAPY: EMERGING APPLICATIONS, ADVANCEMENTS AND FUTURE PERSPECTIVES

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ABSTRACT

CRISPR - Cas - mediated genome editing has emerged as a transformative technology with considerable potential for the treatment of human diseases. The ability of CRISPR-Cas systems to recognize specific nucleic acid sequences and modify targeted genetic regions has expanded the possibilities of precision medicine and gene therapy. This review provides an overview of the recent advancements in CRISPR - Cas - mediated genome editing and its emerging therapeutic applications in human diseases. Particular attention is given to conventional Cas 9 - based editing, and other programmable editing strategies that can enable more precise genetic modifications. The therapeutic potential of CRISPR - Cas technology is being investigated across a broad range of conditions, including inherited genetic disorders, hematological diseases, cancer, infectious diseases, metabolic disorders, and other complex diseases. The review also discusses important development in ex vivo and in vivo genome - editing approaches and the role of delivery systems in including therapeutic efficiency and tissues and specificity. Despite rapid progress, factors such as unintended genetic alterations, limited delivery efficiency, immune responses, editing variability, long term safety, and ethical considerations remain important areas for continued investigations. Future developments are expected to focus on improving editing precision, developing safer and more effective delivery platforms, expanding clinical applications, establishing robust approaches for long-terms monitoring. Overall CRISPR - Cas - mediated genome editing represents a promising platform for advancing targeted and personalized therapies for human disease.

KEYWORDS: CRISPR-Cas Genome Editing, Gene Therapy, Precision Medicine, Therapeutic Applications.

INTRODUCTION

Clustered Regularly inter-spaced short palindromic Repeats (CRISPR) and CRISPR - associated (Cas) proteins have revolutionized genome editing by providing a precise, programmable, and relatively efficient approach for modifying genetic material.^[5,7,34] Originally discovered as part of the immune system of bacteria and archaea CRISPR - Cas technology has rapidly evolved into a powerful platform for biomedical research, disease modelling, and therapeutic genome engineering.^[16,18,34] Among the various CRISPR systems, CRISPR - Cas9 has been most extensively investigated because of its ability to recognize specific DNA sequences through a guide RNA and introduce targeted genetic modifications.^[5,20,23] The therapeutic potential of CRISPR - Cas - mediated genome editing extends across a broad range of human diseases, including inherited genetic disorders, cancer, infectious diseases, cardiovascular diseases, neurological disorders, and autoimmune conditions.^[28,29,32] The technology enables correction, disruption, or regulation of disease-associated genes and has also facilitated the development of more accurate cellular and animal disease models for drug discovery and personalized medicine.^[21,22,28]

Recent advancements have significantly expanded the capabilities of conventional CRISPR - Cas nucleases. Technologies such as base editing, prime editing, epigenome editing and RNA editing offer greater precision and in some cases, reduce dependence on double strand DNA breaks.^[28,34,35] In parallel, improvements in viral and non-viral delivery systems, including lipid nanoparticles and other targeted delivery approaches, are helping overcome major barriers to therapeutic application.^[28,29] These innovations are accelerating the transition of CRISPR-based technologies from experimental research toward clinical medicine.^[28,34,35] Despite remarkable progress, several challenges remain before CRISPR -Cas -mediated therapies can achieve widespread clinical applications. Major concerns include off - target editing, unintended genomic alteration, delivery efficiency, tissue specificity, immune responses, editing heterogeneity, and uncertainties regarding long-term safety.^[30,36,38] Ethical, regulatory, and accessibility issues also require careful considerations. Therefore, this review aims to discuss the emerging applications, recent advancements, and future perspectives of CRISPR - Cas - mediated genome editing for human disease therapy^[28,34-36] with particular emphasis on its therapeutic applications technological developments, current limitations, and the strategies required to establish safer, more effective, and personalized genome-editing treatments.

History of gene engineering

CRISPR - Cas9 technology emerged in 2012.^[5] Since then, the techniques for targeted and precise manipulation of DNA sequences in living cells have played a crucial and dominant role in biology. Although CRISPR has become almost a synonym with gene editing. The history of genome editing goes back to the 1970's when research successfully employed transgenic mice.^[1] It was however, impossible to carry out targeted insertion into the genome of a cell by this technique. The limitations have led scientists over the world to a united effort to develop different gene targeting technologies. The first gene - targeting system introduced in 2005, was zinc finger nucleases (ZFNs), the recognition of the specific sequences of DNA and targeted insertion into the genome could be executed.

Discovery of CRISPR - Cas system

CRISPR - Clustered regularly interspaced short palindromic repeats - were first discovered in the sequences of DNA from *Escherichia coli* bacteria and described in 1987 in Ishino^[11] from Osaka university (Japan) at that time sequencing of these difficult to study DNA fragments took several months but neither their origin nor their significance in the bacterial cell were understood by their discoveries. The biological functions of the CRISPR System had not yet

been elucidated, scientists had already proposed a way to use the information encoded in CRISPR in medical research, for genotyping various strains of bacteria initially on mycobacterium tuberculosis and later on streptococcus pyrogenes, As it turned out, CRISPR loci had a high degree of polymorphism in different strains of the same, species of pathogenic bacteria,^[12,13] which enabled the identification of bacterial strains in clinical conditions. A significant breakthrough in understanding the biological function of CRISPR loci occurred with discovery of Francisco Mojica of the university of Alicante (Spain), who came across similar structure in the archaeal genome of *Haloferax mediterranei* in 1995.^[14]

Structure and functional components of CRISPR Cas systems

CRISPR - Cas systems are adaptive immune systems found mainly in bacteria and archaea^[15,16,18] that protect against invading genetic elements such as bacteriophages and plasmids. Their function occurs through three major stages. Adaptation, crRNA expression/processing and interference.^[16,18] During adaptation, Cas and Cas participate in acquiring foreign DNA fragments and incorporating them as spacers into the CRISPR array. The CRISPR array is transcribed into precursor crRNA which is processed into mature crRNA. In type I systems, tracrRNA participates in crRNA processing and associates with Cas9; crRNA and tracrRNA can be combined into a single guide RNA (sgRNA) for genome editing applications. The Cas9 - guide RNA complex recognizes target DNA and an appropriate PAM (Protospacer and Adjacent Motif)^[20,23] target DNA which together enable programmable DNA recognition and cleavage.

Mechanism of CRISPR/Cas 9 Genome Editing

The mechanism of CRISPR - Cas - 9 genome editing can be generally divided into three steps: Recognition, cleavage and repair.^[20,23]

The designed sgRNA directs Cas 9 and recognizes the target sequence in the gene of interest through its 5' crRNA complementary base pair component. The Cas 9 - protein remains inactive in the absence of sgRNA. The Cas-9 nuclease makes double-stranded breaks (DSBs)^[5,20,23] at a site of 3 base pairs upstream to PAM. PAM sequence is a short (2-5 base pair length) conserved DNA sequence downstream to the cut site and its size varies depending on the bacterial species. The most commonly used nucleases in the genome editing tool, Cas 9 protein recognizes the PAM sequences at 5' NGG-3' (N can be any nucleotide base). Once Cas-9 has found a target site with the appropriate PAM, it triggers local DNA melting followed by the formation of RNA-DNA hybrid, but the mechanism of how Cas-9 enzyme melts the target DNA sequence was not early understood yet. Then the Cas-9 protein is activated for DNA to produce predominantly blunt-ended DSB. Finally the DSB is repaired by the host cellular machinery.

Types of CRISPR -Cas system

1. CRISPR - Cas9

Introduction

CRISPR-Cas9 is an RNA - guide genome - editing system^[5,20] derived from the adaptive immune system of bacteria. Cas9 is a CRISPR - associated endonuclease that can recognize a specific DNA sequence using a programmed guide RNA (gRNA) and introduce a targeted double - strand break (DSB). Its simplicity, efficiency and programmable sequence specificity have made it one of the most widely used genome - engineering tools.^[20,23]

Mechanism

Cas9 contains several functional domains, including RECI, RECII, the arginine - rich bridge helix, PAM - interacting domain, HNH nuclease domain and RuvC nuclease domain. The guide RNA binds to cas9 and activates the protein. Cas9 then search for a target sequence adjacent to a PAM, commonly 5' - NGG - 3' for *S. pyogenes* cas9. after target recognition and RNA - DNA hybridization, cas9 generates a double - strand DNA break approximately 3 BP upstream of the PAM.^[5,20] The HNH domain cleaves the target strand, where as the RuvC domain cleaves the non - target strand.

2. CRISPR - Cas12

Introduction

Cas12 is an RNA - guide DNA endonuclease belonging to the type v CRISPR system and serves as an alternative to cas9 for genome editing. Important cas12 variants include AAS cas12a, isolated from *Acidaminococcus*, and LbCas12a, from *Lachnospiraceae* bacterium. Unlike cas9, cas12 can process precursor crRNA itself and does not require tracrRNA or Rnase,^[23,24] which makes the system useful for multiplex genome editing.

Mechanism

Cas12 requires crRNA for target recognition and contains RuvC and nuclease lobe (BUC) domains involved in cleavage. The cas12 - crRNA complex recognizes a target DNA sequence adjacent to a suitable PAM. Target recognition leads to formation of an R - loop, involving base - pairing between crRNA and target DNA strand. Cas12 subsequently uses its RuvC domain to cleave DNA and produce a staggered DNA break.^[23,24]

3. CRISPR - Cas 13

Introduction

CRISPR - Cas 13 is an RNA - targeting CRISPR system belonging to type VI. Unlike cas9 and cas12, which primarily target DNA., cas13 specifically recognizes and cleaves single - stranded RNA (ssRNA). the cas13 family includes cas1a and cas13b, with cas13a originally identified from *Leptotrichia shahii*.^[24]

Mechanism

Cas13 is activated by a single crRNA and contains NUC lobes and two HEPN (higher eukaryotes and prokaryotes nucleotide - binding) RNase domains involved in RNA targeting. The crRNA recognizes a complementary RNA sequence, typically around 22- 28 nucleotides, and cas13 cleaves the target ssRNA. Cas13 can perform RNA cleavage without requiring tracrRNA.^[24]

Clinical application of CRISPR - Cas system:

1. Cancer treatment:

Cancer is a complex disorder caused by genetic and epigenetic mutations in oncogeneses and tumor suppressor genes. CRISPR -Cas9 can be used to manipulate the genome in tumor research and to investigate the causes of tumor initiation progression and metastasis. This system has been widely used in cancer treatment investigations in recent years by correctly mutations (or) knocking out certain genes.

The CRISPR -Cas9 applications are now mostly focused on single gene disorders, viral infections and cancers. CRISPR based therapies are currently used to treat blood cancers such as leukemia and lymphoma. In addition, a lung cancer trail in china was recently completed. Another clinical trail in the united states using CRISPR - based cancer

immunotherapies has also been completed. Numerous other clinical trails using CRISPR -based immunotherapies are on going for treatment of cancers.^[29,32,36,37]

2. Infectious diseases:

To date, infectious disease are one of the most common global causes of serious and lethal human disorders. These diseases are the cause of frequent care and are sometimes difficult to treat. In addition, many of them are resistant to antibiotics (or) have vaccine - escape mutations viral and bacterial pathogenesis are major global health care challenges. In the last two decades, the WHO introduced a number of infectious diseases as “public health emergency of international concern. Infectious disease mainly include bacterial and viral infections. Bacterial infections are commonly treated with antibiotics, but the over use and improper use of antibiotics can cause bacteria to become resistant to many medicines. These are called multi drug resistant (MDR) bacteria and they are a major health problem, CRISPR - Cas9 may help to overcome this problem because it can identify and target specific DNA sequences in resistant bacteria. It can potentially remove antibiotic resistance genes and help to control resistant bacteria. CRISPR Cas9 is a promising new approach for the treatment and control of infectious disease.^[28,29,34]

3. Human immunodeficiency virus (HIV)

HIV/AIDS is one of the fatal diseases that has infected 39 million people worldwide. even though life long antiviral therapy can take HIV replication under control in a “shock - and - kill manner, the persistence of HIV pro - viral known as latent infection, is a barrier to HIV cure. There are two strategies for HIV treatment, both of which are based on gene therapy strategies; targeting the pro-virus and targeting host genes that are crucial for the entrances of virus into cells (CCR5 and CXCR4). now a days, these approaches are achieved by CRISPR Cas technique HIV was the first infectious disease targeted by gene therapy in clinical trial. It has been shown that CCR5 - null blood cells are resistant to HIV - 1 entry and this raises the possibility that CCR5 knocking out via CRISPR - Cas may be alternative approach to clear HIV pro-virus from the cells.

Surprisingly, the world’s first genome edited babies (twin girls) were born in 2018. The study was done by the southern university of science and technology of china a woman with genome edited embryos in which the pathway that HIV uses to infect cells were genetically disabled. Research used the CRISPR - Cas 9 system to destroy the CCR5 gene the human germline.^[28,29,34]

Advantages of CRISPR - Cas system technology:

The CRISPR Cas system offer several advantages over earlier genome - editing technologies its major strength include high versatility, simplicity of programming, ease of use low cost, rapid application, and efficient DNA targeting, in like ZFNs and TALENs, which require modification of DNA - binding protein I domains, CRISPR - Cas technology primarily requires changes to the guide RNA sequence, making target design relatively simple and flexible. The system also support multiplex genome editing, allowing multiplex genomic sites to be targeted simultaneously. In addition, CRISPR - Cas can generate genome modifications without necessarily leaving foreign DNA in the final organism, which is particularly advantageous for application in the plant genome editing these features have contributed to the wide spread adoption of CRISPR Cas technology in research biotechnology and therapeutic development.^[25,26,34,35]

Limitations of CRISPR /Cas 9 Genome editing

Despite its potential as a powerful therapeutic technology, CRISPR / Cas9 genome editing has several limitations that need to be addressed for safe and effective clinical applications. Off - target editing is one of the major concerns, particularly in complex mammalian genomes, where the guide RNA may recognize sequence similar to the intended target and produce unintended genetic alterations. Although bio informatics prediction tools, high - fidelity Sp Cas9 variants, optimized guide RNA's, and transient expression of Cas9 can help minimize these effects, complete prevention of Off - target activity remains difficult[5,30,38]. Immunogenicity is another important limitation, as Cas9 proteins, guide RNA's, and delivery vectors may be recognized by the immune system. Pre - existing immunity against cas proteins or viral vectors can trigger innate and adaptive immune responses, which may reduce editing efficiency^[39] and cause adverse reactions. Efficient delivery of CRISPR components to the appropriate target cells and tissues is also a major challenge, especially for in vivo therapy. The editing components must research the target tissue, enter the appropriate cells, avoid degradation, and reach the nucleus while maintaining sufficient editing activity. Viral vectors are effective delivery vehicles but may have limitations such as immune responses, restricted cargo capacity, and concerns associated with prolonged exposure. In contrast, non - viral approaches, including lipid - based nanoparticles and other nano-materials - based systems, may reduce some viral - associated concerns but can have limitations related to toxicity, tissue targeting, stability, and large scale manufacturing.^[34,39] Precise gene correction or knock - in is also more difficult than gene knockout because efficient and accurate repair of disease - causing mutations remains challenging. This problem is particularly important in cancer, where multiple mutations in different gene and tumor heterogeneity may require targeting several genetic abnormalities simultaneously, increasing the complexity of treatment and the possibility of off - target effects. Furthermore, achieving appropriate localization and distribution of the editing machinery within tissues is difficult because inadequate delivery may result in uneven editing among target cells. Therefore, improving editing specificity, delivery efficiency, immune compatibility, transient control of cas9 activity, and long - term safety remains essential for the successful translation of CRISPR / Cas9 - based therapies in to routine clinical practice.^[36,38,39]

Future perspectives

Future development of programmable genome insertion technologies should focus on improving **efficiency, safety, precision, and delivery** in different cell types, particularly mammalian cells. An ideal genome-insertion system should be compact, capable of efficiently delivering large DNA cargo, highly accurate with minimal unwanted editing, and adaptable to different applications. Future research should therefore aim to better understand the mechanisms responsible for **on-target and off-target insertion**, improve existing knock-in approaches, and develop novel DNA genome-insertion technologies. These advances could facilitate safer and more effective applications of genome editing in gene therapy and cell-based therapeutics.

CONCLUSION

Genome editing technologies have advanced rapidly over the past decade and have become valuable tools in both fundamental biological research and therapeutic applications. Programmable genome insertion enables targeted insertion of genes, exons and therapeutic sequences at a specific genomic loci. This approach has potential for correcting genetic disorders, developing engineered cells and improving therapeutic gene expression. Targeted insertion of therapeutic genes can provide greater control and may offer advantages over random integration. However efficient and precise insertion of large DNA fragments remain a major challenge.

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