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Gene Editing and Clinical Applications of Gene Therapy

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Abstract. The swift evolution of genetic modification techniques has ushered in transformative prospects and challenges for the therapeutic utilization of gene therapy in medical practice. Over the past few years, the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system has swiftly ascended to prominence as the pivotal methodology in genetic engineering, attributed to its remarkable efficacy and accuracy. Innovations such as base editing and prime editing, which are offshoots of CRISPR technology, have significantly bolstered the refinement and productivity of genomic alterations. However, there are still many significant challenges in current research on gene editing, including the safety, off-target effects, and ethical issues. This paper introduces the basic principles of the CRISPR-Cas9 system and its applications, and the innovations and applications of base editing and prime editing by reviewing the latest research in related fields. Additionally, it discusses the current gene therapy applications of therapeutic strategies for hereditary diseases, as well as the challenges and prospects for their clinical application. This paper aims to provide an overview of the current state of gene editing technology and gene therapy development. The research in this paper will not only help to deepen the understanding of gene editing, but also provide novel insights and approaches for the clinical application of gene therapy.

Keywords: Gene editing, CRISPR-Cas9, Base editing, Prime editing, Gene therapy.

1. Introduction

What scientists have been seeking is the ability to modify and edit the genes of living organisms. As early as the 1990s, Woolf discovered a method for repairing gene mutations through oligonucleotide mediation, which revealed the potential for treating hereditary diseases and provided the technical basis and inspiration for later gene editing technologies. Subsequently, scientists used nucleases to modify genes, such as transcription activator-like effector nucleases (TALEN) and zinc finger nucleases (ZFN), which became important gene editing tools. Although both technologies have been proved to enable gene editing, there have many limitations due to their less efficient targeting and higher cost in terms of time and money[1]. It was not until scientists identified the existence of CRISPR within the genomes of archaea and bacteria that CRISPR technology began to replace ZFN and TALEN technology, becoming the core technology for gene editing due to its efficiency and accuracy.

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2. CRISPR-Cas overview

CRISPR is a distinctive adaptive "immune system" found in prokaryotic that works synergistically with Cas proteins to resist invasion by exogenous DNA.

The CRISPR system comprises CRISPR sequences and their associated Cas proteins. Based on the Cas genes and the proteins they encode, CRISPR can be divided into two broad categories, each group encompassing three distinct types [1]. The Cas9 protein is a pivotal component of the type II CRISPR system and plays a crucial role in the CRISPR system.

3. CRISPR-Cas principle

In 2012, the groundbreaking work of Jinek et al. showcased in laboratory settings that Cas9 proteins, guided by RNA, possess the capability to sever specific DNA sequences [2]. The CRISPR-Cas9 gene editing mechanism initiates with the fusion of a trans-activating CRISPR RNA (tracrRNA) and CRISPR RNA (crRNA) into a singular guide RNA (sgRNA), subsequently associating with Cas9 proteins to constitute the CRISPR-Cas9/sgRNA complex. This complex then orchestrates the editing process by triggering a double-strand breaks (DSBs) in the target DNA. The subsequent repair is executed either through Homology Directed Repair (HDR) or the Non-Homologous End Joining (NHEJ) pathway. Furthermore, the discovery of this system holds significant potential for the future development of genome editing. CRISPR editing technology was first used in human and mouse cells in 2013 [1]. CRISPR technology is developing very rapidly and researchers are constantly refining CRISPR to avoid off-targeting.

3.1. CRISPR-Cas applications

As researchers have delved deeper into the CRISPR system, the technology has been extensively utilized in a range of fields, encompassing gene therapy, functional genomics, agriculture, biotechnology, and diagnostics, among others [3]. At present, CRISPR technology is exerting a significant influence on gene therapy. Moreover, researchers are primarily employing CRISPR in the treatment of conditions like viral infection, cancer and genetic disorders.

3.1.1. Viral diseases

Currently, the majority of viral infections including Human Immunodeficiency Virus (HIV) and Hepatitis B Virus (HBV) lack a definitive clinical cure. The primary reason for this is that the viruses remain undetected by the host immune system, as they exhibit the lowest activity during the latent phase. Consequently, the viral genome cannot be eradicated from the host, resulting in a chronic infection for the patient [1].

HIV infection continues to be a global public health crisis that resulting in tens of millions of people. Previous research has demonstrated that CCR5 is a crucial target for investigating the mechanisms of HIV-1 infection and for advancing drugs and gene therapy development. However, the CRISPR-Cas9 technology is capable of efficiently modifying the CCR5 gene [4]. Furthermore, numerous laboratories have successfully utilized CRISPR-Cas9 to knock down the CCR5 receptor in CD4+ T cells without significant side effects [3]. Indeed, multiple studies have shown confirmed that CRISPR-Cas9 is not only efficient but also highly mitigating the risk of off-target effects. The CRISPR technology exhibits conside potential to treat HIV, although there are still many problems with this gene editing therapy [4]. It needs to be continually improved and subjected to more in-vitro and in-vivo safety tests, but CRISPR has already pointed researchers in the direction of future research for HIV treatment.

In addition, hepatitis is a fatal disease that causes a significant global mortality rate. Based on the most recent World Health Organization (WHO) projections, by 2022, over 254 million individuals were afflicted with hepatitis B. However, by the end of 2022, merely 3% of the effected population (7 million people) had accessed antiviral treatment for hepatitis B [5]. In the past, people were only able to attenuate hepatitis infections. In contrast, the CRISPR-Cas9 has been demonstrated to be effective in inhibiting persistent cccDNA that induces chronic infection. It achieves this by direct targeting the

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HBV genome, thereby decreasing cccDNA levels, while simultaneously reducing the amount of HBV in infected cells. The antiviral effect of the CRISPR-Cas9 has already proved by in vivo hydrodynamic injection in mice [6]. Although it may need to be paired with an appropriate therapeutic agent for clinical use, the appearance of CRISPR offers a novel and effective therapeutic strategy for treating HBV infection.

Furthermore, the CRISPR-Cas system has demonstrated the capability to eliminate various viruses in vitro, including herpes virus infection, human papillomaviruses HPV-16 and HPV-18 [3].

3.1.2. Cancer

Cancer is a significant health problem affecting humans globally, and to date, there are no optimal treatments for such diseases. Conventional cancer treatments like surgery, radiotherapy, chemotherapy, and photothermal therapy can lead to radiation damage, drug toxicity, and other problems, even death in severe cases. Therefore, current cancer treatment methods urgently need innovation and expansion.

Chimeric antigen receptor T-cell (CAR-T) therapy is an innovative cancer immunotherapy approach that uses CRISPR-Cas9 to genetically modify the T-cells in patients so that the T-cells can recognize and attack cancer cells [3, 7]. Nowadays, this personalized treatment, combining CRISPR technology with CAR-T therapies, has great potential. In addition, CRISPR-Cas9 has been applied to high-throughput screening of genomes to discover new therapeutic targets, and it also has been utilized to design and develop individualized mRNA tumor vaccines and to control the tumor microenvironment to inhibit tumor progression[7]. These approaches have demonstrated their strengths and potential in preclinical and clinical trials, particularly CRISPR-enhanced CAR-T cell therapy and genomic screening. Both have already yielded positive results in several studies, showing great promise in cancer treatment.

3.1.3. Others

In addition to its extensive applications in medicine, CRISPR also plays a critical role in agriculture, environmental science, and other fields. The primary focus of CRISPR-Cas9 technology in agricultural applications is to edit plant genomes to improve crop traits. For example, CRISPR-Cas9 has been used to edit rice susceptibility genes, enhancing resistance to white leaf blight. Additionally, genes related to grain size regulation have been precisely edited in rice to increase yield. Furthermore, rice genes have been modified to boost vitamin A content, thereby enhancing the nutritional value of rice [8]. Traditional breeding methods are time-intensive and costly to achieve desired traits, while CRISPR-Cas9 can significantly reduce breeding time by directly editing target genes. In the realm of environmental science, CRISPR also has substantial potential. It is employed to modify microbial genes to enhance their ability to degrade environmental pollutants, such as improving bacterial capacity to break down oil pollution. Moreover, researchers have explored using CRISPR to edit the genomes of endangered species to enhance their resilience, thus aiding their survival in changing environments[8].

3.2. Base editing technique principle

Base editing technology is a new genome editing method derived from CRISPR technology. It enables single base mutations directly in cellular DNA or RNA without causing DSBs. In contrast, CRISPR technology requires the induction of cellular HDR via DSBs for gene editing, integrating sequences from exogenous DNA templates into DSB sites. Although traditional CRISPR technology has become a powerful tool in various fields due to its convenience and numerous advantages, it still has drawbacks such as being error-prone, limited by the state of cell division, and having high competition for NHEJ[1, 9]. Therefore, a more precise technology is needed to edit the genome more efficiently and accurately. Base editing can directly and irreversibly convert one base pair to another at the target site without causing DSBs or requiring HDR, making it more efficient and less error-prone[9].

In 2016, David Liu et al. developed a single-base editor, named Cytosine Base Editor (CBE), by fusing the rat cytosine deaminase APOBEC1 with dCas9[1, 9]. They combined the dCas9 protein,

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APOBEC1, and uracil glycosylase inhibitors into a complex that binds to specific sgRNA. The dCas9 protein directs the complex to its target via sgRNA, APOBEC1 catalyzes the deamination of cytosine to uracil, and a uracil glycosylase inhibitor prevents the removal of uracil from DNA [10]. The Adenine Base Editor (ABE) was reported again by David Liu et al. in 2017. Like CBE, tRNA adenine deaminase (TadA) binds to modified Cas9 proteins to form ABE, which uses TadA to catalyze the deamination of adenine to inosine (I). When the DNA is replicated, the inosine is copied to guanine, thus completing the substitution from A-T to G-C [3, 10]. Hence, base editing can complete four conversion mutations [1].

3.3. Base editing applications

Currently, base editors are utilized in gene therapy, the establishment of relevant animal models, and functional gene screening [10]. They are primarily applied to the study or treatment of point mutations that cause hereditary diseases [9]. For instance, Liu and his colleagues demonstrated that base editing could correct mutant cytosine to thymine to treat diseases such as sickle cell anemia and thalassemia [10]. Furthermore, a clinical study that is currently in progress aims to assess the viability and effectiveness of administering a solitary dosage of autologous hematopoietic stem cells, specifically modified through CD34+ base editing (designated as BEAM-101), for the treatment of individuals suffering from profound sickle cell anemia [11]. In addition, for rare genetic diseases, base editing offers a more efficient method of gene repair, enabling precise gene correction without causing DSBs. There is also substantial evidence showing that base editing is effective in mutant mice, rats, and rabbits in animal studies [9]. Despite the current limitations and immaturity of base editing technology, its potential remains significant. Researchers are continuously addressing new challenges, and base editing technology is now widely adopted by academic labs and therapeutic companies. It is progressively being applied in therapeutics, agriculture, and scientific research [3].

3.4. Prime editing principle

CBE and ABE in base editing are available for base pair substitutions between four pyrimidines or purines (i.e., Interconversion between cytosine and thymine, and between guanine and adenine), whereas effective tools for the other eight base pair substitutions between pyrimidines and purines(e.g., between cytosine and adenine, etc.) are lacking. In 2019, Liu and his colleagues developed a novel gene editing technique called prime editing(PE), which uses a catalytically inactivated nCas9 and reverse transcriptase (RT) fusion. Their team also developed guide RNA (pegRNA) for PE. The pegRNA not only directs the fusion protein to the target gene site, but also encodes the required genetic information through the reverse transcription template sequence contained within it. When the prime editor is functional, the complex of nCas9 and pegRNA first binds to the target DNA and forms single-strand breaks at specific locations. Then, the target DNA homologous binding site (PBS) on the pegRNA binds to the target DNA fragment, directing the reverse transcriptase to transcribe the RNA template into DNA. Finally, the newly synthesized DNA repairs the target DNA while the original DNA fragment is removed. To improve editing efficiency, David Liu's research group has successively improved the PE system in several versions [10]. The PE technique enables efficient insertion, deletion, and substitution of all 12 types of point mutations without causing DSBs and produces few byproducts.

3.5. Prime editing applications

Currently, PE has been successfully applied in animal cells, plant cells, and microorganisms. Traditional CRISPR technology in plant applications faces problems such as low efficiency of HDR and difficulty in providing sufficient donor repair templates (DRT). In contrast, PE can be better applied to crop breeding improvement because it enables precise editing of target genes without HDR and DRT. Numerous experiments have demonstrated the use of PE in the genetic breeding of rice, wheat, maize, and dicotyledonous plants, showing success in improving crop yield and enhancing disease resistance. In addition to human cells, PE has also played a significant role in non-mammalian

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and mammalian models. The PE methodology has been effectively employed in various model organisms, including Drosophila, zebrafish, and mice [12]. These experiments have highlighted the prospective utility of the PE system across a broader spectrum of animal models, thereby serving as an invaluable asset to medical investigations. Primarily, the PE system has been utilized to rectify genetic anomalies that underlie human illnesses. A significant proportion, roughly 90%, of disease-inducing genetic variations are solitary nucleotide alterations, and the PE technology is capable of rectifying these, along with inserting, deleting, or substituting all 12 possible base pair configurations. Initially, PE was deployed to rectify genetic faults accountable for disorders like sickle cell anemia and Tay-Sachs disease. The technique was later used to introduce protective mutations in the PRNP gene to reduce the risk of developing prion diseases, demonstrating its potential in preventing genetic diseases. Additionally, PE can insert fluorescent proteins into genes to aid in the observation and study of proteins [10].

4. Gene therapy

Gene therapy treats disease by introducing healthy genes (DNA or RNA) into the cells of the patient to promote or inhibit the expression of target genes, or modify target genes. There are different strategies for gene therapy, including in vitro and in vivo gene delivery, gene addition, as well as gene editing. Its main applications are in the treatment of inherited and acquired diseases, as well as somatic and germ cell gene therapy. In vitro gene delivery, where cells taken from the patient are modified outside the body and then re-introduced, is commonly used for hematological disorders and certain types of cancer. In contrast, in vivo gene delivery directly modifies genes in the body and is suitable for diseases where the target cells are difficult to extract or widely distributed, such as certain hereditary eye diseases, muscular diseases, and neurological disorders. Gene addition involves the direct addition of normal genes to the patient's cells, often used to treat diseases caused by a lack of specific gene function. Gene editing, on the other hand, is the precise genetic modification of the genome using tools such as CRISPR, base editors, and prime editors [13]. Currently, gene therapy relies on three main types of vectors: adeno-associated virus (AAV), adenovirus (Ads), and lentiviral vectors (LV). Among them, lentiviral vectors (LV) are single-stranded RNA retroviruses with large carrying capacity, which can integrate genes into host genes and achieve long-term stable expression [14]. This makes them suitable for treating hereditary blood disorders such as sickle cell disease.

5. Gene therapy applied to sickle cell disease

Sickle cell disease (SCD) is an inherited hemoglobinopathy caused by a point mutation (A → T) in the sixth codon of the hemoglobin β-globin gene on chromosome 11. It affects approximately 20 million people globally and 300,000 to 400,000 newborns each year [15]. Currently, the only drug available to alleviate the symptoms of SCD is hydroxyurea, which works by increasing the level of fetal hemoglobin (HbF). However, it does not cure the condition or prevent exacerbations. Although newly developed drugs such as Voxelotor and Crizanlizumab have emerged in recent years, they have not been widely adopted by patients due to their immature development and lack of longitudinal evaluation. With advancements in gene editing technology and the rise of gene therapy, more reliable and effective therapeutic methods for treating SCD are now available. One such method involves autologous hematopoietic stem cell transplantation through gene addition. This strategy modifies hematopoietic stem cells (HSCs) with lentiviral vectors to introduce normal β -globin genes, compensating for the defective HBB genes. Another therapeutic approach is to induce HbF production by activating γ-globin gene expression or to inhibit adult hemoglobin (HbS) expression through site-specific mutagenesis, resulting in amino acid substitution. In addition to these gene therapies, CRISPR and base editing technologies also show great potential. These gene editing techniques can directly repair or replace mutations in the HBB gene, offering a promising therapeutic approach for SCD [15].

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6. Gene therapy applied to cystic fibrosis

Cystic fibrosis, a genetic ailment triggered by alterations in the CFTR gene's structure, manifests predominantly through complications within the pulmonary and gastrointestinal tracts, resulting in the excessive accumulation of mucus that blocks the respiratory and pancreatic ducts. For many years, due to the inability to correct the CFTR gene defect, treatment of cystic fibrosis has been limited to symptom management and has failed to eradicate the disease. Previous therapies have used adeno-associated virus (AAV) and adenovirus (Ad) vectors to introduce the normal CFTR gene into the epithelial cells of patients. However, these therapies have exhibited low persistence of gene expression and are prone to gene loss. In recent years, with the emergence and improvement of genome manipulation technologies, gene therapy has brought about new therapeutic breakthroughs. Mutations in the CFTR gene can now be directly repaired using CRISPR-Cas9, base editing, and prime editing. The HDR pathway of cells can be utilized to replace the mutated sequence with the correct one, although this replacement method is highly inefficient and not suitable for widespread clinical use [16]. To improve the therapy, the Clevers team applied base editing to correct mutated CFTR genes. Due to the low conversion efficiency of this method (less than 8%), Jiang et al. proposed a new method of delivering base editors in the form of chemically modified mRNA [16]. Additionally, PE technology allows for precise and efficient treatment of cystic fibrosis by inserting three nucleotides into the mutated ΔF508 motif [10].

7. Conclusion

Currently, the development of gene editing technology has made remarkable progress. CRISPR-Cas9 has gradually replaced earlier gene editing tools as the core technology due to its high efficiency and precision. In recent years, CRISPR-based base editing and PE have further improved the efficiency and accuracy of gene editing, revolutionizing medicine and agriculture, as well as opening up new pathways for gene therapy and other applications. However, the technology has also prompted consideration of ethical, legal, and social implications. Genome editing applied to somatic cells has generated the least amount of controversy, while heritable genome editing is the focus of discussion. Unlike somatic genome editing, gene editing in the germ line can pass on edited genes to offspring, and the safety of this remains unclear. In addition to safety concerns, the larger issue with heritable gene editing is ethical, such as the possibility of "designer babies," genetic discrimination, and genetic enhancement. The current variability and inconsistency of national regulations complicate global cooperation, but most countries permanently prohibit any application of heritable gene editing [17].

The future development and application of genome editing technologies will require global cooperation. Therefore, it is essential to develop a unified global regulatory framework and standards, and each country should implement a strict ethical review mechanism to ensure that the application of these technologies adheres to ethical standards [17]. At the same time, the safety and effectiveness of gene editing technology will also be central to future governance. Researchers need to continue to optimize the technology to minimize off-target effects and other safety risks and enhance editing efficiency. Moreover, a combination of in vivo and in vitro experiments is needed to assess and validate the effects and potential risks of gene editing.

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