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The Application Prospects of Gene Editing in New Drug Development

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Abstract. Gene editing techniques have shown enormous application potential in the realm of novel drug development due to the rapid growth of biomedical technologies. With its accurate genome editing capabilities, the CRISPR-Cas9 system provides a revolutionary research tool for gene function analysis, disease mechanism exploration, and drug target discovery. Leveraging its high efficiency and operational flexibility, this system has been widely applied across various model organisms and cellular systems. This review summarizes the core mechanisms of CRISPR-Cas9, focusing on its key applications in drug discovery: high-throughput screening of disease-causing genes and drug targets, its value in constructing induced pluripotent stem cell disease models, and pathways for developing novel gene-editing-based therapies. Simultaneously, the paper delves into the technical bottlenecks and application limitations encountered during clinical translation, aiming to provide insights for advancing the deeper integration of CRISPR-Cas9 in future drug discovery efforts.

Keywords: CRISPR-Cas9, gene editing, new drug development, gene therapy

1. Introduction

To defend against viral threats in their environment, prokaryotes have evolved an adaptive immune system known as the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR). Scientists first discovered these spaced repeat sequences in Escherichia coli in 1987, subsequently finding them widely distributed across bacteria and archaea. It wasn't until 2013 that researchers successfully edited endogenous genes in mammalian cells using the CRISPR system for the first time [1]. With the rapid advancement of biomedical technology, gene editing thas emerged as a transformative force in drug discovery. In recent years, significant progress has been made in gene editing technologies like the CRISPR-Cas9 system, which enables precise editing of an organism's genome. This provides a powerful tool for studying gene function, disease mechanisms, and drug target discovery. As an efficient and flexible genome editing tool, the CRISPR-Cas9 system has been successfully applied across multiple model organisms and cellular systems, including human cell lines, bacteria, zebrafish, yeast, mice, fruit flies, nematodes, rats, common crops, pigs, and monkeys [2]. Furthermore, this technology demonstrates broad application potential in high-throughput screening, crop breeding and trait improvement, and gene therapy. In gene therapy, the CRISPR system enables precise genome editing to functionally modify cells, facilitating

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intervention and treatment for various diseases. Typical applications include mutation repair in single-gene hereditary disorders, optimization of chimeric antigen receptor (CAR) T-cell therapies, and applications in bioengineering and regenerative medicine [3]. However, several research gaps persist in this field. For instance, applying gene editing technologies to complex diseases remains challenging, including improving editing efficiency, reducing off-target effects, and achieving precise in vivo editing. Furthermore, ethical and safety concerns regarding gene editing in new drug development warrant further exploration. This review synthesizes existing literature to provide insights into the application of CRISPR-Cas9 technology in novel drug discovery.

2. Principles of the CRISPR system

CRISPR-Cas9 technology originated from the adaptive immune systems of archaea and bacteria against exogenous genetic elements, and has now become a revolutionary gene editing tool in the field of life sciences [4]. The operational mechanism of this system comprises the following stages.

2.1. Assembly of targeted complexes

The artificially engineered CRISPR-Cas9 system of type II primarily consists of two components: the Cas9 nuclease and the single-stranded guide RNA (sgRNA). The Cas9 protein possesses two distinct nuclease domains: HNH and RuvC [5]. The sgRNA is a chimeric RNA composed of two parts: its 3'-end contains the tracrRNA necessary for binding to the Cas9 protein, while its 5'-end consists of a crRNA segment of approximately 20 nucleotides responsible for target recognition [4]. The Cas9 protein binds to the sgRNA through electrostatic interactions and hydrogen bonds, forming a ribonucleoprotein complex. The conformation of this complex exposes the guide sequence, preparing it for subsequent target recognition.

2.2. Target site recognition and validation

The assembled Cas9-sgRNA complex begins scanning the vast genome to identify DNA target sites complementary to its guide sequence. This process relies on the principle of complementary base pairing between DNA and RNA. However, sequence complementarity alone is insufficient to trigger cleavage. A crucial recognition marker is the protospacer adjacent motif (PAM) located downstream of the 3' end of the target sequence. The PAM is a short sequence (typically 5'-NGG-3') specifically recognized by the Cas9 protein, not the sgRNA [4]. The presence of PAM serves as the key mechanism for Cas9 to distinguish self from non-self, effectively preventing erroneous attacks on the bacterium's own genome. Only when Cas9 confirms both sufficient complementarity between the sgRNA and target DNA and the presence of PAM is the complex fully activated.

2.3. Cleavage of double-stranded DNA

Successful recognition and binding induce a significant conformational change in the Cas9 protein, transitioning it from an inactive search state to an active cleavage state. Subsequently, its two nuclease domains are activated sequentially: the HNH domain cleaves the DNA strand complementary to the sgRNA guide sequence, while the RuvC domain cleaves the DNA strand lacking base complementarity with the sgRNA [4]. Both cleavages occur approximately 3 base pairs upstream of the PAM, ultimately generating a blunt-ended double-strand break (DSB) in the DNA double helix.

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2.4. Cellular repair mechanisms and editing outcomes

DSBs trigger two primary intrinsic cellular repair pathways, which researchers can leverage to achieve different editing objectives [1]. One is non-homologous end joining (NHEJ), where cells directly join broken DNA ends in the absence of repair templates. This error-prone repair process frequently introduces base insertions or deletions (Indels), leading to gene knockouts. If Indels occur within a gene's coding region, they readily cause frameshifts, resulting in loss of target gene function. The other is homologous directed repair (HDR). When an exogenous DNA donor template with homologous arms matching sequences flanking the breakpoint is available, cells can utilize this template for repair [6]. By designing specific donor templates, researchers can achieve gene knock-in, site-directed mutation correction, or insertion of specific sequences. HDR provides the theoretical foundation for precise gene therapy.

3. Application scenarios of CRISPR-Cas9 technology in new drug development

The traditional model of new drug development is costly and plagued by high failure rates. The maturation of gene editing technology has injected revolutionary momentum into this field.

3.1. Discovery and validation

CRISPR-Cas9 technology can be employed for genome-wide library screening, enabling high-throughput gene knockout or regulation to study and identify disease-associated genes [7-10]. The genome-wide CRISPR screening workflow begins with the in-silico design and synthesis of tens of thousands of single-guide RNAs (sgRNAs), allocating two to four distinct sgRNAs per gene to maximize both genomic coverage and phenotypic reliability. These oligonucleotides are then cloned to generate a comprehensive CRISPR-Cas9 knockout library [11]. Next, select suitable cell lines for experimentation. Then, an appropriate cell line, often a transformed or primary human line that faithfully models the biology under investigation, is selected and rigorously validated. Finally, the pooled library is introduced into these cells at low multiplicity of infection through physical, viral, or non-viral delivery systems, thereby establishing a genetically heterogeneous population ready for selective enrichment or depletion analysis. Among these, adeno-associated virus is frequently chosen due to its high safety profile and low genotoxicity [12-16]. Genetically specific knockout cells are then subjected to selective screening conditions. Genomic DNA is extracted, and integrated sgRNA cassettes are amplified and sequenced to determine the abundance of cells with specific gene knockouts, thereby analyzing the phenotypic effects of different gene deletions [17]. Finally, high-throughput sequencing analysis identifies and confirms key genes associated with target biological processes or disease phenotypes.

3.2. Drug design and development

The emergence of induced pluripotent stem cells (iPSCs) provides a groundbreaking tool for research in cell biology and regenerative medicine. CRISPR technology enables precise gene editing, allowing effective introduction or correction of pathogenic mutations to construct isogenic disease models. Furthermore, applying this technology to genetically engineer iPSCs helps enhance therapeutic safety and efficacy, driving their widespread application in disease research, drug screening, and cell therapy [18-20]. Traditional pathological research and drug development primarily rely on animal models and animal-derived cells. However, interspecies differences prevent these models from fully mimicking human physiology and disease characteristics, leading to poor

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prediction of drug performance in clinical trials [21]. iPSC-derived in vitro models offer powerful tools for simulating disease complexity. Three-dimensional organoid technology can reproduce spatial structures and cell-cell interactions that are difficult to replicate in conventional models [22]. Bissoli et al. developed a novel heart failure organoid model that, upon endothelin-1 (ET-1) stimulation, successfully reproduced pathological features of heart failure. This included molecular-level alterations in marker gene expression and microRNA profiles, alongside functional reductions in contractility, multidimensionally simulating key manifestations of human heart failure [23]. Pérez et al. generated pituitary protease metallopeptidase 1 (PITRM1)-knockout iPSCs using CRISPR-Cas9 technology and further developed brain organoid models. It demonstrated that this model developed typical pathological features of Alzheimer's disease, including protein aggregate accumulation, tau pathology, and neuronal death [24]. These results validate the feasibility of using CRISPR-iPSCs to establish organoid models that more closely resemble human physiological states for studying complex etiological diseases and conducting drug design and development.

3.3. Clinical trials and therapeutics

Gene editing technology is undergoing a transformation from revolutionary tools to revolutionary drugs. Precision gene editing components, exemplified by the CRISPR-Cas9 system, are evolving into “drug” entities with defined active ingredients, administration methods, and a suite of quality control standards when combined with delivery vectors. These entities are now directly entering clinical development pipelines. Regulatory bodies including the U.S. FDA, EU EMA, and China NMPA have explicitly incorporated in vivo and in vitro gene editing products into the regulatory frameworks for Advanced Therapy Medicinal Products (ATMPs) or gene therapy products. In 2016, Lu You's team at West China Hospital of Sichuan University pioneered the application of the CRISPR/Cas system for human tumor therapy. In this trial, researchers first isolated autologous T cells from patients, then targeted knockout of the PD-1 immunosuppressor gene within these T cells. The edited T cells were subsequently infused back into the patients, with edited T cells detectable in peripheral blood post-infusion. Results demonstrated that CRISPR/Cas9-edited T cell therapy is clinically safe and feasible with low off-target risk [25]. Frangoul et al. pioneered the use of CRISPR-Cas9 for treating sickle cell disease (SCD) and transfusion-dependent beta-thalassemia (TDT). Two patients with TDT and SCD underwent bone marrow ablation followed by infusion of autologous $CD34^+$ cells edited with CRISPR-Cas9 targeting the BCL11A enhancer. One-year follow-up revealed high levels of allele editing in both bone marrow and peripheral blood, with comprehensive increases in fetal hemoglobin, achieving transfusion independence. The SCD patient experienced no recurrence of vascular occlusion [26]. In 2023, this therapy became the world's first approved gene therapy for market release.

4. Challenges faced

With the rapid advancement and expanding applications of gene editing therapies, their associated safety concerns remain a focal point of attention across all sectors.

Despite the revolutionary precision offered by the CRISPR-Cas9 system, off-target effects remain the primary safety challenge hindering its transition to clinical therapy. Off-target cleavage may induce mutations at unintended genetic loci, potentially disrupting tumor suppressor genes or activating oncogenes. Despite the development of high-fidelity Cas9 variants, optimized sgRNA design algorithms, and enhanced off-target detection through whole-genome sequencing (e.g., CIRCLE-seq), eliminating off-target risks remains impractical within the complex human

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environment [27-30]. This necessitates stringent preclinical off-target assessment requirements from regulatory bodies and demands extremely rigorous long-term safety monitoring plans for the design of first-in-human trials, significantly increasing both development time and costs.

Insufficient delivery efficiency and targeting remain core translational challenges for in vivo gene editing therapies. Inefficient delivery may prevent adequate therapeutic editing components from reaching target tissue cells, leading to suboptimal efficacy or treatment failure. Currently, LNP technology has become the mainstream non-viral platform for gene drug delivery, demonstrating significant clinical potential in liver-targeted siRNA therapy [31]. However, achieving efficient, specific, and safe delivery remains challenging in the face of the human body's complex physiological barriers [32]. Drug developers must carefully consider vector selection, dosing regimens, and indication suitability. Regulatory bodies require stricter assessments of in vivo biodistribution, editing activity, and potential toxicity for delivery systems.

However, the application of gene editing technology in the medical field also raises profound and complex social and ecological ethical challenges. At the societal level, its high costs and patent monopolies may exacerbate inequalities in the distribution of medical resources, turning cutting-edge therapies into privileges for the few. This could deepen global and internal social inequalities, creating a new “genetic divide.” At the ecological level, this technology disrupts natural evolutionary processes. The release of edited products, unforeseen gene mutations, and new genetic defects could threaten the long-term security of the human gene pool and destabilize ecosystem balance. Therefore, advancing this technology requires not only scientific breakthroughs but also establishing a social governance framework ensuring equitable access and a global regulatory system safeguarding against ecological risks. This approach guarantees that technological progress benefits all humanity without sacrificing social equity or planetary ecology.

5. Conclusion

As a gene editing tool characterized by high efficiency, simplicity, and low cost, the CRISPR-Cas system has revolutionized the life sciences, providing powerful new tools for human disease research and clinical treatment. It is not merely an efficient tool for basic research but has become a disruptive force spanning drug screening, clinical studies, and even direct therapeutic development. By enabling precise manipulation at the genomic level and constructing disease models that more closely mimic real human pathological states, it accelerates the discovery and functional validation of drug targets while creating an entirely new class of gene editing therapies. From high-throughput CRISPR libraries screening potential drug targets in laboratories to in vitro cell editing therapies that have successfully cured β-thalassemia in clinical settings, gene editing technology has demonstrated its application value in advancing from “understanding disease” to “curing disease.” It offers hope for fundamental cures for numerous genetic disorders and intractable cancers that currently lack effective treatments. This review covers only selected literature and lacks experimental validation, indicating that challenges persist on the path toward this promising future. Technologically, potential off-target effects and bottlenecks in in vivo delivery efficiency remain critical hurdles to overcome. Ethically and socially, concerns about fairness stemming from exorbitant treatment costs and potential harm to ecosystems demand the establishment of broad societal consensus and comprehensive regulatory frameworks.

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