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A Review of Research on CRISPR/Cas9 Gene Editing Technology in Disease Treatment

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Abstract: The appearance of CRISPR/Cas9 gene editing technology marks a revolutionary advance in the field of gene editing. With its high degree of specificity and efficiency, this technology enables precise modification of genome-specific loci. This paper reviews the mechanism of CRISPR/Cas9 and its applications in various fields such as genetic disease treatment, cancer therapy, antiviral therapy, gene function research and virus detection. Also, the challenges of off-target effects are discussed and strategies to minimize them are proposed, The purpose of this study is to propose applications and innovations of CRISPR/Cas9 technology in medical therapeutics while addressing its current limitations.

Keywords: CRISPR/cas9, Disease treatment, Mechanism, Challenges.

1. Introduction

The appearance of CRISPR/Cas9 makes a progress in the field of gene editing. This technology is highly specific and efficient, enabling precise editing at particular locations within the genome. CRISPR/Cas9 technology originates from the immune defence mechanism of bacteria. Bacteria record and store gene fragments of invading viruses through CRISPR sequences and use these sequences to direct Cas9 proteins to cleave viral DNA, thereby fending off viral infections. Drawing on this mechanism, scientists have developed the CRISPR/Cas9 gene editing tool, in which the Cas9 protein, guided by gRNA, recognises and cuts a specific site of the target gene, and then the cell's own DNA repair mechanism attempts to repair the breaks created by the cut, which may trigger a gene mutation or the insertion of an exogenous gene in the process, thus enabling gene editing.

2. Introduction to the Mechanism of CRISPR/Cas9 Gene Editing Technology

The CRISPR/Cas9 system mainly consists of a guide RNA and the Cas9 protein, which functions as a nucleic acid endonuclease. During gene editing, researchers combine crRNA and tracer RNA to form sgRNA, which leads the Cas9 protein to cut the target gene. CRISPR is divided into two parts: the CRISPR sequence and the gene encoding the Cas protein[4]. The complete CRISPR array includes spacer sequences and repeat sequences. Repeat sequences (each 25–35 bp) are usually separated by spacer sequences (each 30–40 bp), and the Cas genes are usually situated close to the CRISPR array and are crucial for capturing and cleaving foreign genetic fragments.

The CRISPR/Cas9 system primarily involves three steps in gene editing: recognition, cutting, and repair. sgRNA recognizes the target sequence by pairing its 5'-crRNA with the target gene's bases, guiding the Cas9 nuclease to the target sequence. After the RNA-DNA hybrid forms, the Cas9 protein cuts the DNA at the 3 bp upstream of the PAM sequence, resulting in a blunt-ended double-strand break. The cell then repairs the double-strand break through two main steps: non-homologous end joining (NHEJ) and homologous recombination (HDR). NHEJ is the cell's primary repair mechanism for DNA breaks, which does not require a repair template and repairs DNA by directly joining the broken DNA ends. This process can lead to insertions or deletions (indels), potentially causing gene mutations or inactivation. In contrast, HDR relies on an external repair template that contains the same sequence as the target DNA region. The HDR process uses the repair template for precise gene repair or insertion, achieving accurate editing of the target gene. In addition, the CRISPR/Cas9 system also involves other extended technologies, such as CRISPR/Cas12 and CRISPR/Cas13, which have different characteristics and application potentials. CRISPR/Cas12 offers higher specificity and a simplified operation mode, while CRISPR/Cas13 is mainly used for RNA editing. Overall, CRISPR/Cas9 and its variants provide flexible and powerful tools for genome editing, widely used in basic research, therapeutic research, and agricultural improvement.[3]

3. Specific use of CRISPR/Cas Gene Editing Technology

3.1. Treatment of hereditary diseases

By correcting disease-causing genes, CRISPR/Cas9 is expected to cure some single-gene genetic diseases, such as sickle cell anemia and β-thalassemia [1]. Sickle cell anemia is caused by the replacement of the sixth amino acid, glutamic acid, on the β-peptide chain with valine, forming sickle-shaped hemoglobin. CRISPR/Cas9 can potentially correct the disease-causing gene to achieve therapeutic effects. β-thalassemia, alternatively, is caused by mutations in the β-globin gene, leading to ineffective erythropoiesis and subsequent anemia.

Gene editing technologies, especially CRISPR/Cas9, offer a promising therapeutic approach. The first step in gene editing involves identifying and localizing the mutated β-globin gene locus. Specific sgRNAs are designed to direct the protein to the mutation site, where the Cas9 protein induces double-stranded breaks. In the in vitro approach, hematopoietic stem cells are extracted from the patient, edited genetically outside the body, and then reinfused, with the goal

that these cells will produce normal red blood cells in the bone marrow. Alternatively, in vivo editing involves directly injecting the CRISPR/Cas9 system into the patient to target the hematopoietic stem cells within the body.

Duchenne muscular dystrophy (DMD) causes from mutations in the DMD gene. CRISPR can be employed to correct or bypass these mutations, leading to the production of partially functional dystrophin protein. Eliminating the mutated transcripts can yield a truncated but functionally adequate dystrophin protein. For instance, Tabebordbar [13] used adeno-associated viral vectors to deliver CRISPR/Cas9 into an mdx mouse model, deleting the mutated exon 23. This enhanced muscle function, restoring the Dmd reading frame and enabling potential treatment for Duchenne muscular dystrophy.

The team led by Dennis M. K. found that the preprotein convertase subtilisin/kexin type 9 (PCSK9) is a gene participated in cholesterol metabolism. Humans carrying loss-of-function mutations in PCSK9 experience no significant adverse effects and have lower LDL cholesterol levels, lowering the risk of various cardiovascular diseases. This suggests that targeting and disrupting PCSK9 with CRISPR/Cas9 technology could offer a treatment for familial hypercholesterolemia [14].

3.2. Cancer treatment

CRISPR/Cas9 can change the cancer-related genes and improve the ability of immune cells to recognize and get rid of cancer cells, opening up new avenues for cancer immunotherapy.[6] CRISPR/Cas9 technology allows for screening cancer cells to determine their gene specificity. By targeting and deleting these genes, CRISPR/Cas9 can decrease the viability of cancer cells. Additionally, it can identify genes related to drug resistance in cancer cells and facilitate research on overcoming this resistance [8].

For example, immunosuppressive genes such as PD-1 can be knocked out by CRISPR to enhance the anti-tumor ability of the immune system[5]. The Rupp, L. J. team demonstrated this approach by utilizing CRISPR/Cas9 to delete the PD-1 gene in T cells., rendering them unsuppressed and thus improving their anti-tumor activity. The team found that PD-1 knockout T cells exhibited stronger anti-tumor effects in a mouse model, significantly prolonging the survival time of the mice [19].

Genome-wide screening can also be performed using CRISPR gene editing technology to identify and validate new cancer therapeutic targets. Researchers used CRISPR/Cas9 to conduct a genome-wide knockout screen in cancer cell lines, revealing several new tumor suppressor genes. Inactivating these genes can significantly promote tumor growth and provide new targets for cancer therapy [20].

It is also possible to examine the genomes of cancer patients for partial gene inactivation and use CRISPR gene editing to transplant or modify these genes to inhibit tumor growth, thereby potentially prolonging patients' lifespans.

3.3. Antiviral therapy

For viral infections, CRISPR/Cas9 can be used to cut viral genomes and inhibit viral replication, such as in the case of HIV and Hepatitis B[7]. In 2013, Ebina's research group explored the feasibility of using CRISPR/Cas9 to modify the HIV-1 genome and inhibit its expression. They focused on CRISPR/Cas9 assemblies targeting the HIV-1 long terminal repeat (LTR) sequence for their experiments.

The CRISPR/Cas9 components were delivered into T cells expressing the HIV-1 LTR, both in silenced and inducible states, to examine their effect on expression driven by the LTR.[11]. Ebina's group found that the CRISPR/Cas9 system significantly reduced LTR-driven expression in response to stimulation. Sequence analysis confirmed that the CRISPR/Cas9 system effectively cleaved and mutated LTR targets. In addition, the system was able to remove internal viral genes from host cell chromosomes. CRISPR/Cas9 technology can block the transcriptional expression of the HIV-1 virus or excise latent HIV-1 proviruses directly from the host genome. The results demonstrated that CRISPR/Cas9 can indeed block HIV-1 transcription and directly remove latent HIV-1 proviruses from the host genome [12].

3.4. Gene Function Studies

By knocking out or activating specific genes, we can study their functions and mechanisms of action in diseases, thereby advancing basic medical research. Donehower, L.A. and his team found that mice with the P53 gene knocked out exhibit a high frequency of spontaneous tumorigenesis, including lymphomas, osteosarcomas, and other types of tumors. This observation suggests that p53 plays a crucial role in tumor suppression. Mouse cells lacking p53 are unable to effectively arrest cell cycle progression after DNA damage, leading to genomic instability [16]. This highlights the progress of this gene editing technology in studying gene function studies.

3.5. Virus detection

Cas9 proteins are single effector complexes synthesized from TracrRNA to gRNAs that localize the cleavage of target sequences by recognizing PAM sites. The specificity of the PAM site can be utilized for virus detection. Joung, J.'s team employed the SHERLOCK technique to detect SARS-CoV-2 viral RNA using the CRISPR-Cas13a system. This technology first reverse transcribes the viral RNA into cDNA and then recognizes the viral RNA sequence with CRISPR-Cas13a in combination with a specific gRNA. Cas13a activity cleaves the reporter RNA, producing a fluorescent signal that signals the presence of the virus. This assay is highly sensitive and specific, capable of detecting low concentrations of SARS-CoV-2 RNA in a short period of time [17]. Gootenberg, J. S. also utilized SHERLOCK technology to detect dengue viral RNA using the CRISPR-Cas13a system. They first amplified the viral RNA through reverse transcription and isothermal amplification. Subsequently, the CRISPR-Cas13a system was used to recognise and cleave specific viral RNA sequences, generating a fluorescent signal indicating the presence of the virus. SHERLOCK technology can rapidly detect the dengue virus and differentiate between different serotypes [18]. The application of CRISPR technology in virus detection demonstrates its strong potential and advantages, such as high sensitivity, specificity, rapid detection, and portability. These effective cases highlight the utility of CRISPR technology in detecting SARS-CoV-2, HPV, and dengue viruses.

4. Challenges and Perspectives of CRISPR/Cas9 Technology

CRISPR/Cas9 technology faces a number of challenges and areas for further research in its practical application, despite its wide range of applications. The main challenges include off-target effects, delivery issues, immune response,

genetic stability and ethical issues. Off-target effects refer to the possibility that the CRISPR/Cas9 system may cut DNA at a non-specific location other than the target DNA, resulting in unwanted gene mutations or interference with normal gene function[10]. Despite improvements through optimization of sgRNA design and Cas9 protein variants, off-target effects remain an important concern. Delivery issues relate to the efficiently introducing CRISPR/Cas9 components into cells, which may require different delivery strategies for different cell types and tissues, such as viral vectors, nanoparticles or electroporation. Immunoreactivity refers to the possibility that Cas9 proteins may be recognized by the body's immune system as exogenous substances, generating an immune response that could affect therapeutic efficacy and bring about side effects. Genetic stability issues, on the other hand, relate to the possibility that genetic modifications introduced by CRISPR/Cas9 may be destabilized during cell division. Ethical issues relate to the fact that gene editing techniques, especially in embryos and germ cells, may give rise to ethical controversies, requiring a balance between technological advances and ethical norms. Looking ahead, research will aim to enhance the specificity of gene editing technology and reduce its negative effects, develop Cas protein variants with higher specificity and optimize sgRNA design, and explore more efficient and safer delivery systems including novel nanoparticles, smart viral vectors, and so on, in order to improve editing efficiency and reduce side effects. Long-term safety assessments will ensure the stability and effectiveness of the technology, and personalized treatment plans will tailor editing to the genomic characteristics of specific patients, potentially greatly improving treatment efficacy. At the same time, ethical norms and regulatory frameworks for gene editing technology need to be developed and improved to safeguard the rights and safety of patients. Finally, the use of CRISPR/Cas9 technology will also be expanded to agriculture, biomanufacturing and environmental protection, exploring its potential in crop improvement, disease prevention and ecological restoration.

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