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Application of CRISPR/Cas9 gene-editing technology in Alzheimer's disease

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Abstract. Alzheimer's disease (AD) is a prevalent neurodegenerative disorder. It triggers metabolic disorders within the body due to genetic mutations, thus leading to irreversible damage to one's memory and brain. The primary pathogenic features of AD are intracellular neurofibrillary tangles caused by hyperphosphorylated Tau and extracellular amyloid plaques created by the buildup of β-amyloid β-protein (Aβ). However, up to now, there has been no effective treatment program for Alzheimer's disease. And after a long time of development, many gene editing technologies have been perfected and utilized in practice. Among them, CRISPR-Cas9 technology is the most efficient and practical. As a result, an increasing number of scientists have examined and debated the potential use of CRISPR-Cas9 technology in the management of Alzheimer's disease. This review addresses the use of the CRISPR/Cas9 system to treat Alzheimer's disease and presents the disease's pathophysiology, including the establishment of AD models, genetic screening for the cause of AD, and targeted therapy for AD.

Keywords: Gene-editing technology, Alzheimer's disease (AD), CRISPR/Cas9.

1. Introduction

In the whole world, Alzheimer's disease (AD) is the major neurodegenerative illness and the most prevalent trigger of dementia. As of March 2024, there were approximately six million and nine hundred thousand people with AD in the US, and this number is expected to continue to increase over the next few decades. When it reaches 12.7 million by 2050, there'll be an annual increase of 1.27 million people who get AD. In terms of mortality, 119,399 people have died from Alzheimer's disease in 2021, and the death rate usually rises sharply with age. The number of deaths from AD at ages over 85 is six times higher than that at ages ranging from 75 to 84 [1].

Despite the high morbidity and mortality rates, the pathogenic mechanisms of AD remain unclear. An intricate interaction of genetic, epigenetic, and environmental variables could be the cause. Early-onset FAD (familial AD) is predominantly driven by mutations in the APP, PS1 and PS2 genes, but merely accounts for around one percent of all factors. In contrast, prevalent late-onset Alzheimer's disease is closely related to the APOE4 gene, which significantly increases the risk of onset and lowers the age of onset. Other loci such as CLU, CR1 and PICALM, are also recognized as risk factors for AD. Extracellular amyloid plaques and intracellular neurofibrillary tangles, which lead to synaptic dysfunction and neurotransmitter imbalance, are the primary characteristics of Alzheimer's disease (AD). They would lead to a decline in cognitive function and neurodegeneration and ultimately result in severe impairment of memory and cognitive function.

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In addition, Alzheimer's disease itself brings so many problems. The proportion of people with this illness will rise as society gets older, forcing an increasing amount of healthcare resources to be allocated to it. It will undoubtedly put enormous economic and social pressure on the families of patients and the healthcare system. However, there is no means to completely cure AD, only to alleviate the symptoms. How to prevent and cure AD will become an urgent task for global medical research [1].

Compared to the first two generations of gene-editing systems, the CRISPR/Cas9 system offers several benefits, such as easy design, high efficacy, and low cost. As a result, it shows great potential in the treatment of AD. This review systematically introduces CRISPR/Cas 9 system and discusses the application of this system in AD.

2. CRISPR/Cas9 system

Without doubt, CRISPR/Cas9 system is an essential gene editing tool that is based on the bacterial immune system. Its core components include Cas9 protein and single guide RNA (sgRNA). Cas9 protein is an RNA-directed DNA endonuclease that recognizes the target DNA sequence by pairing with sgRNA. And then it could use its own nucleic acid cleavage activity to create double-stranded breaks. At the same time, Cas9 needs to recognize the PAM sequence on the target DNA strand to exert its cleavage activity.

2.1. Function mechanism of CRISPR/Cas9 system

Up to now, the key breakthrough of CRISPR-Cas9 is combining two key components-CRISPR RNA (crRNA) and tracrRNA-into a single sgRNA molecule. This sgRNA retains two key features: a base-pairing sequence that determines the sequence of the target DNA on the one hand, and a double-stranded RNA structure that binds to and stabilizes the Cas9 protein on the other hand. Through the modification of a small piece of the sgRNA, Cas9 may be engineered to target any DNA sequence using this straightforward two-component strategy. The simplification of sgRNA design makes CRISPR-Cas9 a cheap and easy-to-use genome editing tool.

Cas9 protein contains two independent nuclease domains, HNH domains and RuvC-like domains, which are responsible for cleaving the complementary and non-complementary strands of target DNA. The researchers conducted an analysis of the structures and sequences of tracrRNA and crRNA, which are key elements for Cas9 to localize and cleave the target DNA. Furthermore, it was discovered that the target DNA's protospacer adjacent motif (PAM) sequence serves as a crucial signal for Cas9 to attach to and cleave the target DNA. The changes in the PAM sequence could significantly affect the cleavage efficiency of Cas9 [2]. It’s a short sequence used in the CRISPR-Cas9 system to recognize the target sequence. By recognizing and binding to PAM sequences, Cas9 proteins can further examine the complementarity between the target sequence and the crRNA guide sequence to decide whether or not to cleave the target. Therefore, PAM is an important factor for self-/non-self-recognition and target recognition in CRISPR-Cas9 system. It has its own specific sequence characteristics and position in different types of CRISPR systems. Thus, it is also a key molecular switch for the immunization function of CRISPR systems.

NHEJ means non-homologous end-joining. It is one of the two main repair mechanisms after the CRISPR-Cas9 system induces genomic double-strand breaks. The cell starts the DNA repair process when the CRISPR-Cas9 system creates double-stranded DNA breaks (DSBs) at the target genomic location. At the DSB site, NHEJ is capable of creating insertion or deletion indels of different lengths. The inactivation of gene function can result from these indels, because they’re able to damage the reading frame of coding sequences or the binding sites of transcription factors in transcriptional regulatory sequences. In addition, HDR means homology-directed repair. It is the other main repair mechanism besides NHEJ, which has a key role in gene editing. HDR uses the provided exogenous DNA repair template to precisely repair the double-stranded DNA breaks introduced by CRISPR-Cas9. Compared with the HDR repair mechanism, NHEJ does not require additional screening markers, and the resulting mutations can be obtained by simple screening. This makes NHEJ one of the important mechanisms for efficient gene knockdown. In contrast, HDR enables more precise gene editing by

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introducing specific mutations as expected. Gene knock-out is a gene manipulation technique widely used in biomedical research. By purposefully deleting a specific gene, researchers can create cells or experimental animal models that lack the function of that gene. This approach helps researchers observe the effects of the absence of a target gene on an organism and understand the role of the gene in physiological processes. Gene knock-in is a technique that allows the precise insertion of exogenous DNA sequences into a cell's genome. Compared with gene knockout, knock-in not only introduces new genes, but also replaces or modifies endogenous genes.

Gene knock-out is a crucial component of the CRISPR-Cas9 gene editing system. By using this knock-out technology, researchers can create cell lines or experimental animal models that are missing a specific gene. At the same time, gene knock-in is also significant. By using gene knock-in technology, researchers can realize replacement of target genes, insertion of new genes, and modification of endogenous genes, thus opening up new possibilities for clinical applications such as gene therapy. In a word, CRISPR-Cas9 combines both knock-out and knock-in, greatly improving the precision and flexibility of gene editing.

2.2. Comparison of three generations of gene-editing system

TALEN is a novel customizable sequence-specific nuclease. It consists of two key components: a TALE-DNA binding domain and a FokI nuclease catalytic domain. TALE protein has a DNA binding module, each of which recognizes a single base in a DNA sequence. By assembling different TALEs, they can be created to recognize arbitrary DNA targets. When two TALENs bind to two sides of the target, the FokI catalytic domain forms a dimer and triggers a double-stranded break in the target DNA sequence. This DNA shearing is able to stimulate the cellular DNA repair mechanism, thus achieving precise gene editing. TALEN provides a new, efficient and customizable tool for genomic engineering. ZFNs mean zinc-finger nucleases. They are customizable DNA cleavage reagents that have been adopted as gene targeting tools. ZFNs consist of a DNA-binding domain that recognizes specific DNA sequences, and a DNA cleavage domain that cleaves DNA. This unique structure enables ZFNs to efficiently cleave the target genes, which can promote homologous recombination repair and non-homologous end joining. Finally, it could achieve targeted mutations and gene replacement [3]. Programmability and ease of use are CRISPR-Cas9's greatest benefits over TALENs and ZFNs. Without the need for intricate protein creation, the CRISPR-Cas9 method targets the Cas9 nuclease to a particular DNA sequence by designing a brief guide RNA. This makes the design and operation of CRISPR-Cas9 easier and faster. In addition, CRISPR-Cas9 could enable highly specific gene editing. Meanwhile, the cost of CRISPR-Cas9 is also relatively low, which further increases the feasibility and popularity of its application (Table 1).

Table 1. Three generations of gene-editing technologies [3].

	CRISPR/Cas9 system	ZFN	TALEN
Nuclease	Cas9	FOKI	FOKI
Recognition	PAM sequences and the supplemental sgRNA site-DNA	Zinc finger proteins-DNA	RVDs-DNA
Mechanism	Simple	Complex	Complex
Adaptability	High	High	High
Expense	Low	High	High
Delivery	Easily	Limited	Limited
RNA edition	Yes	No	No
Specificity	High	High	High
Stability	High	High	High

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3. Application of CRISPR/Cas9 system in AD

Early-onset AD is mostly caused by more than 200 mutations in some genes. At the same time, CRISPR-Cas9 system could be used to accurately establish these mutations in cellular or animal models to mimic AD pathogenesis. These models can be used to observe the dynamic biological processes of AD pathogenesis. Meanwhile, CRISPR-Cas9 technology also supports the editing of multiple genes at the same time, which can be used to explore the effects of synergistic interactions between genes. Compared with the traditional cell line-based AD mutation study, CRISPR-Cas9 technology is faster, less costly and more reliable. It could help establish a large number of AD animal models in a shorter time. Therefore, CRISPR-Cas9 offers a revolutionary new instrument for creating animal models of AD. These models offer a novel experimental platform for medication screening and treatment advancement, as well as valuable insights into the pathophysiology of AD. This technology is expected to promote the progress of basic research on AD [4].

3.1. CRISPR/Cas9 is used in the construction of AD models

Through computer modeling analysis, the researchers found the differences in three key amino acids (G676R, F681Y, and R684H) between the human and rodent APP sequences. These variations had an impact on APP's affinities for the β-secretase BACE1, which in turn had an impact on the synthesis of Aβ. Subsequently, they constructed a humanized APP rodent model (Apphu/hu) by introducing these three amino acid substitutions in the mouse genomes by using CRISPR-Cas9 technology. Furthermore, they generated the double knock-in Apphu/hu; Psen1M139T mouse AD model by introducing the early-onset FAD (familial AD) mutation M139T in the mouse PSEN1 gene. It was found that both these humanized APP mouse and rat models produced approximately 3 times more Aβ peptide than the wild type, which validated the predictions of the computer model. This was due to the increased cleavage efficiency of BACE1 by the humanized APP sequence. And the double knock-in Apphu/hu; Psen1M139T rat model also produced higher levels of Aβ. These new humanized APP rodent models could better mimic the Aβ pathology of human AD and were more physiologically relevant than past transgenic overexpression models [5].

3.2. CRISPR/Cas9 is used in the screening of AD pathogenic genes

A study used the CRISPR-Cas9 technology to target the deletion of the 3' untranslated region (3'-UTR) of the App gene in an App knock-in mouse model. In particular, this model has three FAD mutations as well as a humanized Aβ sequence. By creating genetically chimeric mice, the researchers found that the higher the level of App 3'-UTR deletion, the less Aβ was deposited in the brain. It was caused by reducing the transcriptional and translational levels of APP. Further experiments further narrowed down the key regulation regions and revealed a 34 bp deletion near the 52 bp conserved sequence of the App 3'-UTR. This 34 bp deletion could significantly reduce Aβ pathology [6]. Meanwhile, CRISPR-Cas9 plays an important role in AD gene screening studies and provides a new powerful tool for exploring the pathogenesis of AD. Compared with traditional methods, CRISPR-Cas9 technology can edit the target genes more precisely, reduce the technical errors in the experimental process, and improve the reproducibility and reliability of the results [4]. In addition to editing gene sequences, CRISPR-Cas9 can catalyze dCas9 to regulate gene expression, thus providing a new tool for gene function research. It can be used to correct autosomal dominant mutated genes that cause early-onset familial AD. Mutations in these genes lead to overproduction of the Aβ protein, which triggers the onset of AD. CRISPR-Cas9 precisely corrects these mutated genes, thus preventing the overaccumulation of Aβ. For instance, it's used to edit the endogenous APP gene to inhibit β-cleavage and reduce Aβ production [7].

3.3. CRISPR/Cas9 is used in targeted therapy for AD

The core and known mechanisms of AD include β-amyloid deposition and tau protein hyperphosphorylation, but there may be other age-related, protective or predisposing factors involved. CRISPR-Cas9 technology could also be used to repair or knock out some AD-related genes, such as APOE, TREM2, etc., to explore how these genes work in the pathogenesis of AD. By using CRISPR-

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Cas9 technology, the researchers knocked out the TREM2 gene in iPSCs, and verified that these iPSCs were indeed deficient in TREM2 protein expression. Through transcriptome analysis, the researchers found that TREM2 deletion led to changes in the expression of 390 genes in human microglia, involving calcium signaling, cell migration and other important biological processes. Further experiments revealed that knockdown of TREM2 increased the dependence of microglial cells on the survival factor M-CSF and showed higher apoptosis sensitivity. Meanwhile, TREM2 deficiency also inhibited the phagocytosis of microglia toward apolipoprotein E (APOE). In order to undertake single-cell sequencing analysis, researchers transplanted people microglia with TREM2 deletion into a rat model of Alzheimer's disease. The results showed that TREM2 deletion resulted in human-derived microglial cells being unable to fulfill their normal damage response to β-amyloid plaques. It could also be considered that disease-associated microglial cell (DAM) function was suppressed. This finding provides additional evidence for the important part that TREM2 plays in controlling human microglia function, particularly in response to pathogenic processes associated with Alzheimer's disease [8].

Screening and repairing AD-related key pathogenic genes with CRISPR-Cas9 technology can directly target the root mechanism of AD. These disease-causing genes include APP, PSEN1, PSEN2 and APOE, which play key roles in the pathogenesis of AD. By precisely editing these genes with CRISPR-Cas9, the relevant gene defects can be repaired, thus preventing the occurrence and progression of AD [9]. Early-onset FAD is commonly associated with mutations in the APP, PSEN1, and PSEN2 genes, which lead to accelerated production of Aβ42. However, a study utilized CRISPR-Cas9 technology to correct an autonomous dominant mutation in basal forebrain cholinergic neurons that were derived from iPSCs from individuals with the PSEN2N141I mutation. As a result, it restored the stability of the Aβ42/40 ratio and reversed the electrophysiological defects. In addition, other studies have reported that CRISPR-Cas9 technology can be employed to correct mutations in the PSEN gene in the iPSCs of FAD patients. One study also found that Swedish mutations accelerated Aβ production. CRISPR-Cas9 technology had been used to knock out Swedish APP mutations in patient-derived fibroblasts, resulting in a 60% reduction in Aβ [7]. A study team presented a CRISPR/Cas9-based method to modify the amyloid pathway in Alzheimer's disease. The researchers designed sgRNAs targeting the APP C-terminus, which were able to effectively edit the APP gene in cell lines, human embryonic stem cells, and mouse brains. APP editing resulted in a decrease in β-cleavage but a rise in α-cleavage of APP, which inhibited Aβ production and enhanced the neuroprotective pathway. Meanwhile, APP C-terminal editing did not affect the physiological functions of APP, such as axon growth, synaptic structure and function. Mechanistic analysis revealed that APP C-terminal editing attenuated the interaction of APP with BACE-1. This was mainly achieved by inhibiting the endocytosis of APP, rather than affecting the intracellular transport of APP. This approach is not limited to a single gene mutation, but targets key proteins involved in disease development, and has broad therapeutic potential [10]. Gene therapy for Early Onset AD (EOAD): EOAD is usually caused by mutations in the APP, PSEN1 and PSEN2 genes. However, the CRISPR-Cas9 technology is able to be used to repair the mutations in these genes, such as the PSEN2 N141I mutation. Targeting APP^{Swe} mutations to inhibit the production of pathogenic Aβ peptides is also a potential therapeutic strategy. Gene therapy for Sporadic Alzheimer's Disease (SAD): SAD is multifactorial, and the main risk factors include gene variants such as APOE4. CRISPR-Cas9 could also be used to modify the APOE4 gene to APOE2 or APOE3, reducing the risk of AD in APOE4 carriers. CRISPR-Cas9 can also target other newly discovered AD-related genes, such as ABCA7 and BIN1 [11].

4. Conclusions

With the rapid development of genomics and genetics technologies, as well as the growing knowledge of the brain's molecular and electrical processes, it is possible to construct models of human brain diseases to elucidate their pathogenesis and then carry out effective intervention and treatment. The CRISPR-Cas9 technology offers a novel framework for scrutinizing the intricate molecular mechanisms underlying various complex neurodegenerative disorders. By precisely editing the relevant genes, it can help to better understand the pathological mechanisms of AD. CRISPR-Cas9 can be used to discover

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key signaling pathways that regulate neuroinflammatory and neurodegenerative processes, thus providing many chances and opportunities for the future of novel therapeutic targets.

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