Exploring the Connection between CRISPR-Cas9 and Alzheimer's Disease Therapy

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Keywords: CRISPR-Cas9, Alzheimer's Disease, Gene Therapy.

Abstract: Alzheimer's disease (AD), which is a neurodegenerative disorder, has a pathophysiology that remains unclear, and there is a lack of effective treatments for this condition. The CRISPR/Cas9 gene - editing technique shows great promise in treating AD. It can target genes like APP, BACE1, and APOE to reduce Aβ production. However, its clinical application faces challenges. Delivery methods, including biological systems, local injections, and viral/non - viral vectors, have limitations such as poor blood - brain barrier penetration, invasiveness, and immunogenicity. Animal models created by CRISPR/Cas9 are beneficial for researching the pathogenesis of AD. However, issues such as off - target effects, long - term safety, and ethical concerns still need to be resolved. In upcoming research, it is essential to focus on improving delivery systems and gene - editing approaches. Only in this way can the potential of CRISPR/Cas9 in the treatment of AD be fully realized.

1 INTRODUCTION

Alzheimer's disease (AD), a condition of progressive neurodegeneration, has become a prominent global health matter. It places a heavy strain not only on patients and their families but also on healthcare systems around the world. Even with substantial research undertakings, the essential pathophysiology of AD remains only partially known, and there continues to be a lack of effective therapeutic tactics. The identification of amyloid-β (Aβ) metabolism abnormalities as a common denominator in AD patients—despite the relatively low prevalence of genetic mutations (about 1 in 100 patients)—has spurred the exploration of novel therapeutic approaches. Among them, the CRISPR/Cas9 gene - editing technology has demonstrated remarkable potential as a possible treatment method for curbing excessive Aβ production. CRISPR/Cas9, an adaptive immune system derived from bacteria and archaea, has revolutionized the field of gene editing. This system, which is composed of the Cas9 protein and a single-guide RNA (sgRNA), has the ability to precisely recognize and cleave target DNA sequences. The single-guide RNA (sgRNA) directs the Cas9 protein to the specific DNA site based on the base-pairing rules. The Cas9 protein, possessing various domains including REC I, REC II, the bridge helix, the PAM-interacting domain, HNH, and RuvC, induces a double-strand break in the DNA. The protospacer-adjacent motif (PAM) sequence is of crucial importance in allowing the Cas9 protein to identify the target DNA. When compared to other gene-editing technologies such as zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and meganucleases, CRISPR/Cas9 offers advantages such as its simplicity of operation, high efficiency, and low cost. These attributes have led to their rapid adoption in various research fields, including AD research.

In the context of AD treatment, several promising directions have been explored. Among the strategies being studied are making edits to the amyloid precursor protein (APP) gene, reducing the expression of the β-secretase 1 (BACE1) gene, and altering the apolipoprotein E ε4 (APOE ε4) gene. These approaches aim to directly target the molecular pathways involved in Aβ production and processing, potentially halting or reversing the progression of AD. However, translating CRISPR/Cas9 technology from bench to bedside for AD treatment is not without challenges. One of the major hurdles lies in the delivery of the

Lin, X.
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DOI: 10.5220/0014487000004933

In *Proceedings of the 1st International Conference on Biomedical Engineering and Food Science (BEFS 2025)*, pages 295-300
ISBN: 978-989-758-789-4

CRISPR/Cas9 components. While delivery via biological systems is feasible, it suffers from issues such as instability, low targeting efficiency, and poor blood-brain barrier (BBB) penetration. Local organ injection, such as direct injection into the brain, can enhance therapeutic efficacy but is highly invasive, limiting its widespread application. Delivery through the nasal cavity can bypass the BBB, yet its effectiveness and potential drawbacks require further clinical investigation. To better understand the pathophysiology of AD and evaluate the efficacy of CRISPR/Cas9-based therapies, animal models have been developed using this gene-editing technology. For example, specific mutations like F681Y, G676R, and R684H have been introduced into the APP gene of mice and rats, creating humanized animal models. These models have provided valuable tools for studying APP processing and uncovering new disease mechanisms of AD. This review article aims to comprehensively summarize the existing knowledge regarding the application of CRISPR/Cas9 in the treatment of Alzheimer's disease (AD). By critically evaluating the existing literature. The progress made in gene-editing strategies, the challenges associated with delivery methods, and the insights gained from animal models will be discussed. The objective is to offer an all - encompassing view of the field, recognize knowledge voids, and provide guidelines for future research, with the ultimate contribution being to the advancement of efficacious CRISPR/Cas9 - centered therapies for AD.

2 THE APPLICATION OF CRISPR/CAS9 IN THE TREATMENT OF AD

Despite the fact that surveys suggest that, out of every hundred Alzheimer's disease (AD) patients, only one usually has a genetic mutation as the underlying cause of the disease, abnormalities in Aβ metabolism are truly a problem that all patients will face. CRISPR/Cas9 can indeed serve as a potential treatment method for correcting the overproduction of Aβ. The main treatment directions currently understood include the following.

Editing of APP gene: Mutations in the APP gene can lead to an increase in Aβ production, which is one of the important causes of AD onset. Studies have found that knocking out the APP allele using CRISPR/Cas9 technology can reduce Aβ protein expression. In a particular study, the hippocampus of an AD mouse model (Tg2576 mice) was injected with a viral vector that carried the sgRNA targeting the KM670/671NL mutation (APPsw) along with the Cas9 enzyme. Following the injection, after a period of one month, DNA sequencing results indicated that there was roughly a 2% insertion/deletion (InDels) in the APPsw allele. This discovery implies that the CRISPR/Cas9 system is capable of modifying the APP gene, thereby offering a possible method for the treatment of AD that is induced by APP mutations (Cao et al. 2021). Moreover, in numerous other studies, the CRISPR/Cas9 technology has been utilized to selectively modify the C-terminal part of the APP gene in both cellular and animal models. Through this editing, the interaction between APP and BACE1 has been inhibited, leading to a decrease in Aβ production. At the same time, it has promoted the neuroprotective APP-α cleavage process, thereby creating novel paths for the treatment of Alzheimer's disease (Zhang et al. 2022).

Inhibition of BACE1 Gene Expression: The Aβ protein is generated through the successive modification of APP by BACE1 and γ-secretase. This mechanism makes targeting BACE1 a feasible approach for treating AD. As illustrated in the research by Park et al., they formulated a nanocomplex composed of the R7L10 peptide and the Cas9-sgRNA ribonucleoprotein complex. This nanocomplex was then directly injected into the hippocampus of 5xFAD and App knock-in AD transgenic mice. As a result, the expression of BACE1 was successfully lowered, the production of Aβ was diminished, and the cognitive impairment in 5xFAD transgenic Alzheimer's disease (AD) mice was alleviated (Wang et al. 2020). This study indicates that using CRISPR/Cas9 technology to inhibit BACE1 gene expression holds promise as an effective method for treating AD.

Editing of the APOE ε4 gene: APOE4 represents the most potent genetic risk factor for sporadic Alzheimer's disease. Research findings have indicated that when CRISPR/Cas9 technology is employed to convert APOE4 into APOE3, it is capable of reducing tau phosphorylation levels, diminishing the accumulation of Aβ, and alleviating the pathological manifestations associated with Alzheimer's disease. For example, Wadhwani and his research team utilized the CRISPR/Cas9 system

to change the E4 allele existing in induced pluripotent stem cells (iPSCs) that were derived from two patients with AD into the E3/E3 genotype. They discovered that neurons with the E3 genotype demonstrated enhanced resistance to the cytotoxic effects induced by ionomycin and displayed decreased levels of tau phosphorylation (Li et al. 2021). This provides an important basis for gene therapy targeting APOE4, demonstrating the potential of CRISPR/Cas9 technology in improving genetic risk for AD.

3 THE CHALLENGES IN DELIVERY CHANNELS

The CRISPR/Cas9 technology also faces certain challenges in delivery methods, with the main delivery methods and the challenges they face including the following points.

Drug delivery through biological systems: Although this approach is workable, it is plagued by problems such as a lack of adequate stability, low targeting precision, and difficulties in penetrating the blood-brain barrier (BBB). For instance, nanocomplexes are formed by bringing together positively charged CRISPR/Cas9 peptides and negatively charged nucleic acid payloads. They possess a beneficial traits of low immunogenicity and the capacity to bind to ligands, which enables their application in a wide range of scenarios. Nevertheless, when these nanocomplexes are systemically administered, they encounter substantial hurdles in efficiently crossing the BBB. Moreover, the reticuloendothelial system (RES) will vigorously eliminate them from the bloodstream (Hu et al. 2020).

Local drug injection into the organ: For instance, intracerebral injection, while enhancing the effect of drug action, also possesses a strong invasiveness, making widespread application quite challenging. Direct intracerebral injection requires invasive procedures, which may increase the risk of infection and has strict limitations on the volume and frequency of injections, making it unsuitable for repeated dosing. Administration of drugs through nasal ducts: This method can effectively avoid the issue of BBB permeability, but the specific effects and whether there are other disadvantages still lack further clinical research. Current research suggests that nasal drug delivery could be a promising non - invasive method of administration. However, additional clinical investigations are required to establish its safety and effectiveness. Moreover, it is necessary to identify any potential problems that might be associated with this delivery route.

In addition to the aforementioned common delivery methods, there are other delivery options available. Regarding viral vector delivery, which encompasses adeno - associated virus (AAV), adenovirus (AdV), lentivirus (LV), and others. Among these, adeno-associated virus (AAV) is extremely popular and extensively applied. This is because of its characteristics including high infectivity, low immunogenicity, and a minimal probability of integrating into the human genome. In the course of the research, two different AAV vectors have been used to encapsulate the amyloid precursor protein with the Swedish mutation (APPsw)-specific gRNA and the Cas9 protein. These are targeted at the KM670/671NL mutation of the APP, a mutation that is of great significance in the pathological process of Alzheimer's disease. In both in vitro primary neuronal cell cultures and in vivo Tg2576 mouse experiments, the production of Aβ was reduced by approximately 60%. However, the packaging capacity of AAV is limited to only about 4.7kb, making it difficult to package some larger genes. LV can accommodate longer DNA insertion fragments (8 - 10kb), but its propagation efficiency in the brain is low, and there are risks of immunogenicity and gene integration. AdV can efficiently express genes, but it has a strong immunogenicity, which may trigger severe immune responses (Gao et al. 2021, Wang et al. 2022, Sun et al. 2020). Non - viral carriers like DNA nanocages can be fabricated via rolling circle amplification. They are capable of carrying sgRNA/Cas9 complexes and exhibit favorable stability along with efficient cellular uptake properties. In one study, DNA nanocages loaded with the sgRNA/Cas9 complex targeting enhanced green fluorescent protein (EGFP) were locally injected into the tumors of EGFP tumor - bearing mice. After 10 days, the expression of EGFP decreased by about 25%, providing new insights for the delivery in AD treatment. However, DNA nanocages may also induce immunogenic reactions, requiring further study (Chen et al. 2021).

4 CRISPR/CAS9 GENE EDITING TECHNOLOGY

The CRISPR/Cas9 system, which has its roots in the adaptive immune mechanism of bacteria and archaea, is engineered to precisely identify and effectively neutralize invading bacteriophages or other foreign nucleic acid entities. The CRISPR/Cas9 system primarily consists of the Cas9 protein and single-guide RNA (sgRNA). The sgRNA, in accordance with the base-pairing principles, guides the Cas9 protein to the specific target DNA sequence and performs cleavage on the double strands of the DNA. The Cas9 protein consists of multiple distinct structural domains. Among them are REC I, REC II, the bridge helix, the PAM-interacting domain, HNH, and RuvC, among others. The PAM sequence is of vital importance in the process of Cas9 recognizing the target DNA (Jinek et al. 2020). From the perspective of its development, the CRISPR/Cas9 technology has gone through a process from its initial discovery to its gradual application in the genome editing of mammals. In 1987, Ishino et al. first discovered CRISPR sequences in Escherichia coli, but their function was not clear at the time. Subsequently, following several years of intensive research, in 2012, two separate laboratories independently verified that the CRISPR/Cas system reconstituted in vitro indeed has the biological capability to cleave a specific single DNA sequence. In 2013, three independent American teams successfully utilized Cas9 to edit bacterial and mammalian genomes. Subsequently, the CRISPR/Cas9 technology experienced a swift progression and has been widely applied in the field of genetic engineering (Ishino et al. 2020, Ran et al. 2021).

When compared to other gene - editing technologies such as Zinc Finger Nucleases (ZFN), Transcription Activator - Like Effector Nucleases (TALEN), and meganucleases, the CRISPR/Cas9 technology has significant advantages. These include a relatively simple operation process, high efficiency, and lower cost. During the gene editing process, the CRISPR/Cas9 system recognizes the target DNA sequence through the sgRNA, and the design process of the sgRNA is quite straightforward. In contrast, both Zinc Finger Nucleases (ZFN) and Transcription Activator-Like Effector Nucleases (TALEN) necessitate intricate protein design and construction procedures. Moreover, CRISPR/Cas9 exhibits a greater editing efficiency and is capable of attaining effective gene editing across a diverse range of cells and organisms. Regarding the cost aspect, the reagents and equipment needed for CRISPR/Cas9 technology are relatively widely available and common. This availability leads to a reduction in the overall expenses associated with research and practical application.

During the process of gene editing, upon the Cas9 protein's recognition of the target gene sequence, the CRISPR/Cas9 system induces double-strand breaks within that specific sequence. Following that, the cell proceeds with the repair process through either the non-homologous end joining (NHEJ) or the homology-directed repair (HDR) mechanism. NHEJ serves as the primary mechanism for mending double-strand breaks in the cellular DNA. It directly links the ends of the fragmented chromosomal DNA. However, this process is prone to mistakes. There is a likelihood of random insertions or deletions (indels) of nucleotides, which in turn can lead to gene disruption. In contrast, HDR repairs DNA through the process of homologous recombination. It precisely rectifies DNA breaks by substituting mutated or incorrect sequences with the accurate ones. Nevertheless, the efficiency of HDR is relatively low and it predominantly occurs during the S or G2 phases of the cell cycle. In practical applications, depending on various research goals and needs, the appropriate repair pathway can be selected to achieve gene knockout, insertion, or correction. CRISPR/Cas9 causes double - strand breaks in the target DNA, and these breaks are repaired through either the NHEJ or HDR pathways. NHEJ directly ligates broken DNA ends, prone to errors causing indels. HDR repairs breaks accurately during S or G2 phases, but with lower efficiency. Choice of pathway depends on research goals for gene knockout, insertion, or correction.

5 ANIMAL MODEL CONSTRUCTION

In order to have a deeper comprehension of the pathological mechanisms that underlie AD, the CRISPR/Cas9 technology has been utilized to create AD animal models. For instance, through the utilization of this technology, researchers have inserted specific mutations including F681Y, G676R, and R684H into the amyloid precursor protein (APP) gene of mice and rats. As a result, they have successfully created humanized animal models. These carefully crafted animal models offer potent

tools for the investigation of amyloid precursor protein (APP) processing. They are of vital importance in uncovering and revealing novel pathological mechanisms of AD. In the process of establishing cell models, the CRISPR/Cas9 technology has also had a significant impact. For example, Wang and his co-researchers made use of the CRISPR/Cas9 system to lower the concentration of thioredoxin-interacting protein (Txnip) within HT22 cells. This method successfully alleviated the protein cysteine oxidation modification induced by amyloid β, indicating that Txnip might have the potential to be an effective target for the therapy of AD. CRISPR/Cas9 technology has been used in constructing both AD animal and cell models. In animal models, specific mutations were introduced into the APP gene of mice and rats to create humanized models for studying APP processing and AD pathology. In cell models, Wang et al. downregulated Txnip in HT22 cells with this technology, suggesting Txnip as a potential AD treatment target. Song et al. determined that reducing the expression of KIBRA in HT22 cells impacts cell growth and apoptosis and elicits a response to Aβ1 - 42 oligomers. Sun et al., on the other hand, utilized CRISPR/Cas9 to knockout the PSEN1 gene in N2a cells. Their findings indicated that the introduction of exogenous PSEN1 mutant protein led to a reduction in the production of Aβ42 and Aβ40. However, due to differences between these cell lines and real neurons, the constructed AD models can't accurately clarify the molecular mechanisms of AD onset. So, the induced pluripotent stem cells (iPSCs) that are derived from patients have been attracting more and more attention. In 2016, Paquet and his research team introduced mutations into the APP and PSEN1 genes—genes linked to the development of AD—within human induced pluripotent stem cells (iPSCs). Subsequently, they detected higher levels of Aβ in neurons derived from both homozygous and heterozygous human iPSCs carrying the mutations. This indicated that CRISPR/Cas9 technology could simulate AD-related mutations in human neuronal cells.

In terms of animal model construction, in addition to the models introduced above with APP mutations in mice and rats, other studies have utilized CRISPR/Cas9 technology to construct different models. As Komor and his colleagues utilized the CRISPR/Cas9 system to convert APOE3r into APOE4, they carried out point mutation modifications within mouse astrocytes (Kim et al. 2020). Park and his associates administered Cas9 nanocomplexes that were targeted at the tyrosine hydroxylase (Th) and BACE1 genes to the primary neurons of mice. They then assessed the efficiency of these Cas9 nanocomplexes and discovered that there were almost no off-target effects. Furthermore, they administered the Cas9-BACE1 nanocomplex into the hippocampus of 6-month-old mice with AD. Afterward, they observed that, four weeks following the injection, the expression levels of BACE1 as well as the β-cleavage products of the amyloid precursor protein (APP) in the hippocampus had significantly dropped. Takalo and his colleagues devised a Plcγ2 - P522R knock - in mouse model. Subsequently, they conducted an evaluation of the protective effect that this specific variant had. They found that the Plcγ2 - P522R knock-in mice exhibited enhanced microglial function, providing a new potential avenue for the treatment of AD. Takalo and his collaborators created a Plcγ2 - P522R knock - in mouse model and then evaluated the protective effect this variant exerted. They discovered that the Plcγ2 - P522R knock - in mice demonstrated augmented microglial function, thus presenting a novel potential approach for the treatment of AD. Although there is currently no AD primate model designed through CRISPR/Cas9 technology, studies have successfully achieved genetic modification in the crab-eating macaque. In the future, constructing an AD primate model based on CRISPR/Cas9 technology will help to delve deeper into the pathogenesis of AD and promote the progress of AD treatment research. CRISPR/Cas9 technology has not yet been used to create an AD primate model, but it has been used to genetically modify crab-eating macaques. Future models based on this technology could advance understanding of AD pathogenesis and treatment research.

6 CONCLUSION

CRISPR/Cas9 technology has achieved substantial headway in the research regarding Alzheimer's disease (AD) treatment. In terms of gene editing, editing strategies targeting key genes such as APP, BACE1, and APOE have shown potential in reducing Aβ levels and improving AD pathology. By constructing AD animal models, a powerful tool has been provided for in-depth research into the pathogenesis of AD, and certain achievements have been made in screening pathogenic genes and exploring the role of inflammatory molecules in AD. However, CRISPR/Cas9 technology still faces numerous challenges in the clinical application for

AD treatment. In terms of delivery systems, both viral vectors and non-viral vectors have their own limitations. For instance, viral vectors face issues with immunogenicity and packaging capacity, while non-viral vectors encounter challenges with blood-brain barrier (BBB) penetration and cellular uptake efficiency. Moreover, the off - target effects inherent in CRISPR/Cas9 technology have the potential to trigger unforeseen genetic alterations, thereby impinging on the safety and effectiveness of treatment. Furthermore, the long-term safety and ethical issues of this technology also need to be further explored and studied.

In the future, it will be necessary to further optimize the delivery system of CRISPR/Cas9 technology, enhance its targeting and delivery efficiency, and reduce its immunogenicity. The pursuit of more accurate gene editing approaches is crucial for minimizing the incidence of off - target effects. Moreover, surmounting the hurdles related to blood - brain barrier penetration and enhancing the cellular uptake efficiency of non - viral vectors are essential steps for the progress of gene therapy applications within the central nervous system. Moreover, continuous research is required to assess the long-term safety and address the ethical concerns surrounding the use of CRISPR/Cas9 technology, ensuring its safe and effective translation into clinical practice. Despite these obstacles, the potential of CRISPR/Cas9 technology in revolutionizing AD treatment remains promising. Efforts are underway to refine the technology, address its limitations, and harness its full potential. With ongoing advancements and a deeper understanding of AD pathogenesis, the hope is that CRISPR/Cas9-based therapies will eventually offer new avenues for treating this devastating disease, bringing relief to patients and their families.

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