CRISPR-Cas9: A Promising Tool in Developing Alzheimer's Disease Treatment

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Abstract. Alzheimer's disease (AD) impacts millions of people worldwide, causing cognitive impairment that can get more and more severe as the patients age. Such a disease can also severely impact patients' lives in the late stage. AD is a sporadic and familial disease. Beta-amyloid (Aβ) and hyperphosphorylated tau protein aggregates directly link to AD pathogenesis, leading to synaptic dysfunction and cognitive decline. When amyloid beta precursor protein (APP) has mutations on the genetic level, patients are likely to have familial AD. In addition, the presenilin (PSEN) gene is another target gene for AD research. Unfortunately, the existing therapies are still ineffective as they can only delay AD progression but cannot stop nor reverse it. Recently, CRISPR-Cas9 has emerged as a promising gene-editing tool in AD research. CRISPR-Cas9 is an outstanding tool compared with the first two generations of gene editing techniques, as CRISPR-Cas9 is cheaper and more efficient. It can create desired mutations in precise positions to target gene sequences, thus potentially manipulating the genes related to familial AD. By mitigating the overproduction of Aβ and tau protein aggregates, researchers aim to slow down or halt the disease progression. CRISPR-Cas9 has significant potential in constructing AD models, screening AD pathogenic genes, and developing targeted therapies for AD. Such a technique offers scientists a better understanding of AD pathogenesis and enlightens more efficient genetic therapies. This review systematically illustrates the CRISPR-Cas9 system and further explains its implementation in AD model constructions, pathogenic genes searching, and potential treatment for AD.

Keywords: Alzheimer's disease, beta-amyloid, CRISPR-Cas9, gene therapy

1. Introduction

Alzheimer's disease (AD) is a disease that occurs progressively and mainly after 65 years old. AD that happens earlier than 65 is early-onset, and such a population comprises less than 5% of the patient cohort [1]. Early-onset cases generally account for familial AD, which has inherited genetic mutations. The late-onset ones do not have mutated genes that can contribute to AD pathogenesis, and they are usually sporadic AD. The amyloid precursor protein (APP) gene, a gene responsible for producing the precursor of the amyloid protein found in the brain, and the presenilin gene, a gene encoding an essential component of the γ-secretase enzyme complex, can lead to the development of familial Alzheimer's disease (AD) when they are mutated. The presenilin gene can then divide into two subcategories: the PSEN1 gene and the PSEN2 gene [2]. In addition, AD's essential characteristics are the beta-amyloid (Aβ) extracellular plaques and aggregated hyperphosphorylated tau protein [3]. APP employs beta-secretase and gamma-secretase to generate soluble Aβ deposition [2]. AD patients usually have a cognitive impairment, postural instability, and failure to perform daily tasks. The cognitive impairment progression has different stages, and it can be more and more severe as the patient ages. The earliest expression can be a mental ability reduction without cognitive impairment performance. The patients then progress to mild cognitive impairment, but the essential functions are still preserved. The late stage involves severe impairment that can negatively affect the patient's daily life. Such symptoms include hallucinations, anxiety, depression, and sleep disorders.

However, AD pathogenesis can be more complicated than what is mentioned above. Other elements contribute to AD pathogenesis, such as oxidative stress and neuroinflammation. Therefore, it is difficult to develop sufficient clinical treatments for AD. Compounding the complexity, patients may exhibit diverse reactions toward the same manifestations of AD, further confounding treatment approaches. The heterogeneity among individuals demands personalized and tailored therapies,

adding another layer of difficulty to the quest for effective interventions. Clinical treatments for AD mainly focus on delaying the progression of symptoms and cognitive decline. While these treatments can provide temporary relief and improved quality of life, they do not offer a complete cure. Ultimately, despite these interventions, many patients will inevitably progress to the late stages of the disease's pathogenesis. Consequently, there is a pressing need for more comprehensive and innovative research to identify novel targets and therapeutic strategies. The development of disease-modifying therapies that can halt or reverse the neurodegenerative process remains an elusive goal in the field of AD research.

Recently, gene-editing techniques have been rapidly developed, and Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR-Associated 9 (CRISPR-Cas9) has shown great potential in therapeutic candidate investigation. Studies have generated feasible treatments for genetic-related diseases utilizing CRISPR-Cas9 on mouse models. Therefore, such success indicates the possibility of generating AD treatments using CRISPR-Cas9. Initially, this review will provide an introduction to the evolution of the CRISPR-Cas9, followed by a comparison between CRISPR-Cas9 and other gene-editing techniques currently accessible. After that, this article will summarize the current applications of CRISPR-Cas9 in AD.

2. CRISPR-Cas9 System

2.1. Mechanisms of CRISPR-Cas9 Technique

CRISPR-Cas9, derived from the defense system of bacteriophage to combat phage invasion, is an efficient and precise gene-editing tool. The first CRISPR locus was observed in Escherichia coli (E. Coli) as E. Coli has developed RNA-guided adaptive immune systems to achieve anti-virus immunity [4]. In the bacterial immune system, the CRISPR loci have short pieces of repeated DNA sequences with spacers inserted among them [5]. The spacers are unique DNA sequences derived from viruses' nucleic acids that have previously invaded the bacteria. Such genetic record stimulates bacterial immune response if they reencounter the same virus [4]. Therefore, the spacers function as recognition signals to destroy invading genomes. In addition, the CRISPR activity requires the CRISPR-associated (Cas) genes, which can be translated into a protein critical to the immune response.

The CRISPR-Cas immune system has three stages: adaptation, maturation, and interference. In the first stage, the new recognition gene is inserted into the spacer of the CRISPR locus, leading the CRISPR system to recognize a new invading sequence [5]. Secondly, in the maturation stage, the precursor CRISPR RNA is first generated by CRISPR gene, and the Cas gene is translated into Cas protein. Cas protein facilitates the precursor CRISPR RNA into mature CRISPR RNA [5]. In the interference stage, the CRISPR RNA and Cas protein target the invading nucleic acid and destroy it together [5].

The CRISPR-Cas9 is one category of the CRISPR-Cas family that cleaves the target DNA sequence and repairs the cleavage with a designed template. CRISPR-Cas9 technology has two key elements: one guide RNA and one endonuclease.

Single-guide RNA (sgRNA) is a 20-bp scaffold DNA sequence attached to Cas9 and can complementarily pair with the initial sequence of the target organism [6]. The complementary gRNA locates close to the desired mutation site, and the PAM site is followed behind the gRNA. The PAM site is an NGG sequence, and N can be any nucleotide. The cleavage site is three bp upstream of PAM. Cas9 is an endonuclease and contains a Rec I domain, a bridge helix, and a PAM-interacting domain. Rec I domain is responsible for gRNA binding, and the PAM-interacting domain is specific to the PAM site. Therefore, Cas9 cleaves where the guide RNA (gRNA) and PAM site target since the [6]. Once Cas9 mediates the double-stranded break (DSB), generally there are two repair mechanisms. The first one is Non-homologous-end-join (NHEJ), which can typically create insertions or deletions at random places. The second one is homology-directed repair (HDR), which uses the homologous section of DNA as a repair template. The prior one is more common and error-

prone, and it can lead to random mutations, while HDR template is around 200 bp and has similar DNA sequences to the wild type (WT) around the cleavage site except for the mutated nucleotides [6].

2.2. Comparisons of three generations of gene-editing tools

In addition to CRISPR-Cas9, there are two genome editing tools, and they have their advantages and disadvantages.

Like CRISPR-Cas9 system, a typical Transcription Activator-Like Effector Nuclease (TALEN) compound consists of several sections and domains: a DNA-binding domain (DBD), an acidic domain, and Fok I nuclease [7]. In the TALEN system that edits the genome, two units of DBD bind to FOK I [7]. A linker sequence connects the DNA-binding sequence and Fok I nuclease, and a spacer sequence separates them [6]. The TALE DBD can be manipulated to recognize and bind to particular DNA sequences by customizing its amino acid sequence [7]. Like Cas9 can recognize PAM sites, TALENs have functions to recognize specific sequence in the genome. TALENs have repeated residues in DBD to bind to single nucleotides in the target sequence, such as NN and NG [7]. This specificity allows TALENs to target and bind to specific genes or gene regions. Once TALENs are adhered to the genome sequence, Fok I dimerizes and form DSB. This break stimulates the cell's natural DNA repair machinery, which can introduce genetic changes during the repair process [8, 9]. By providing a DNA template with the desired genetic modification, such as introducing specific mutations or inserting new DNA sequences, scientists can harness the repair process to precisely edit the target gene [7].

Zinc Finger Nuclease (ZFN) is another gene-editing method that is similar to TALEN, which also induces DSB with the facilitation of Fok I. ZFN has two domains: DNA-binding and DNA cleavage, which are originated from Fok I. Fok I has been demonstrated to have both binding and separating functions [8]. The DBD contains zinc finger sets, and there are approximately 30 amino acids in each set, linking to a zinc atom and binding to 3 bp of DNA [6, 8]. Cleavage domain functions similarly to TALEN, where it forms dimers and cleavage the opposite strand [6]. To achieve the cleavage with the highest efficiency, scientists can join a monomeric cleavage domain to one set of zinc fingers that target the neighboring DNA sequence then repeat this process. Therefore, relatively high concentration of dimer interface can facilitate the dimer formation and DNA cleavage because there are two sets of zinc fingers and their corresponding cleavage domain [8].

Although ZFN and TALEN have shown their outstanding capabilities in genome editing, CRISPR-Cas9 gradually become the more popular tool. CRISPR-Cas9 has demonstrated to be less labor-intensive and more economically practical. In addition, ZFN and TALEN have less specificity in DNA sequence recognition and are less successful in homozygous mutants' creation. Furthermore, because CRISPR-Cas9 has the HDR template, it possesses the ability to introduce multiple mutations at different positions simultaneously.

3. Application of CRISPR in AD

3.1. CRISPR-Cas9 is utilized in the AD models developement

Approximately 1% of the entire AD patient population is familial AD. Although only a small portion of AD cases has genetic mutations, those mutations can lead to severe impacts like increased Aβ deposition. Therefore, cell models generated by CRISPR-Cas9 plays a significant role in exploring the mechanism of AD. Since it is efficient to mimic the disease progression and less time-consuming to develop cell models in AD research compared with animal models, they have been widely applied into research of AD.

Txnip is an inhibitor of Trx and can inhibit its activity in maintaining thiol redox homeostasis. Scientists have reduced the level of a protein interacting with Thioredoxin (Txnip) utilizing CRISPR-Cas9 in HT22 cells and thus created a model to inhibit the protein cysteine oxidative modification caused by Aβ accumulation [9]. AD patients have fluctuated thiol levels in different redox states

compared with healthy people [9]. To maintain a stable thiol redox, thioredoxin (Trx) can reverse cysteine oxidative reformations and facilitate the capture of H2O2 and other peroxides [9]. Inhibiting the activity of Txinp can release Trx from the restraint and thus keep stable thiol level, which indicates that Txinp can be a potential target for AD therapies.

Induced pluripotent stem cells (iPSCs) are popular tools in cell-line research. CRISPR-Cas9 corrected autosomal dominant mutations in basal forebrain cholinergic iPSCs with PSEN2N141 point mutation. The repaired cells have decreased Aβ42/40 ratio and successfully recovered electrophysiological deficits [10]. Basal forebrain cholinergic neurons have early dysfunction in all forms of AD, and mutations in PSEN2 is related to early onset of familial AD [10]. PSEN2N141 point mutation can elevate the Aβ42/40 ratio and further lead to Aβ oligomers and fibrils [10].

3.2. CRISPR-Cas9 is employed in screening of AD pathogenic genes

Genetic screening is used to search in the genome of a whole population and identify certain risk factors in the genome. Such screening can facilitate the diagnosis, prevention, and potential treatment of certain disease. AD's pathogenesis involves the genetic factors, but it can be more complicated because the environmental factors and other disease can also contribute to AD formation. Therefore, it is important to examine the genetic portion of AD pathogenesis to better understand the interaction among genetic factors and other ones. CRISPR-Cas9 can facilitate the screening of AD pathogenesis genes because scientists can either knockout (KO) or overexpress some genes and examine the outcomes.

One example of utilizing CRISPR-Cas9 in AD gene screening is that TREM2 KO can causes severe impairment in different functions of iPSCs [11]. The TREM2 KO microglia has low viability and degrade less synaptosomes and Aβ [11]. Additionally, microglia show decreased mobility towards Aβ and neurons that produce Aβ [11]. By utilizing CRISPR-Cas9 to knock out TREM2, researchers demonstrated that TREM2 is critical in the clearance of Aβ, making it crucial for assessing the risk of AD.

Another genetic factor that was discovered by using CRISPR-Cas9 is L-Moses, a Lysine Acetyltransferase 2B inhibitor that can reduce neuronal cell death and endoplasmic reticulum (ER) stress [12]. AD patients tend to have stressed ER and dead neuronal cells. Tunicamycin (Tun) is a glycoprotein biosynthesis inhibitor in ER that can induce ER stress. Researchers performed KAT2B KO that protects iPSC cortical neurons from Tun-mediated neuronal cell death [12]. This KO cell model highlights the importance of CRISPR-Cas9 in screening genes involved in AD etiology.

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

Based on the research in model construction and pathogenesis screening, therapies targeting the causal genes of AD utilizing CRISPR-Cas9 have been gradually developed. To form genetic therapies, potential target genes normally need to pass the pathogenesis screening. Then those genes must demonstrate a statistically significant difference in pathology expression in before and after getting KO in the AD models. Scientists injected adeno-associated viral (AAV)-vector containing a gRNA with designed APPSW (Swedish)-deletion mutants and a Cas9 in the of transgenic mice's hippocampus region [13]. Such allele disruption causes increase in β-secretase activity, and thus lead to significantly high level of enzymatic cleavage and reduction in the expression of Aβ [13], [14]. Therefore, CRISPR-Cas9 can function as target therapy for APP mutations in AD.

In addition, there is another way to generate genetic therapy besides insertion and deletion. Inactive Cas9 (dCas9) can fuse with other domain to generate CRISPR activation and thus form Cas9 activators. Those activators can manipulate the expression of target genes without causing DSBs [15]. It can be a technical challenge to utilize Cas9 activators to develop therapeutic candidates. Researchers activate Adam10 using Cas9 activator nanocomplex to overcome such challenge and alleviate Aβ deposition and cognitive deficits in AD mice [15]. Furthermore, Adam10 overexpression can also increase α-Secretase activity. α-Secretase can then cleave and inhibit the Aβ peptide

expression [15]. Therefore, Aβ production can be diminished via modifying Cas9 activators, thereby forming a genetic therapy without taking the DSB risks.

4. Conclusion

AD is a neurodegenerative disorder with extensive Aβ plaques and hyperphosphorylated tau protein, leading to cognitive impairment and functional decline to millions of people. CRISPR-Cas9 system have shown promising potential in the investigation and treatment of AD as it allows for precise and efficient modification of genes, offering valuable tools for creating AD models, screening for pathogenic genes, and developing targeted therapies. Through the construction of AD models, scientists have gained insights into the disease's mechanisms and identified potential therapeutic targets. Genetic screening using CRISPR-Cas9 has also revealed key genes involved in AD pathogenesis, further expanding our understanding of the disease.

There are still challenges in implementing CRISPR-Cas9 in clinical trials. One of the significant challenges is the efficiency of non-viral vector delivery. The vectors should be capable of carrying the CRISPR-Cas9 gRNA to the target region and pass the cell membrane to enter the cell without being degraded. One of the successful techniques is utilizing lipid nanoparticles as a transportation tool for CRISPR-Cas ribonucleoprotein [14]. Previous studies have also shown that the Cas-9-gRNA complex is more preferable than the delivery via plasmid because the former one has smaller size [16]. Another challenge for CRISPR-Cas9 utilization in clinical AD therapies is that much research has been done on animal models, which cannot ideally mimic human AD cases. In addition, humans have more uncontrolled variables like lifestyle and living environments than animal models, making genetic therapy less effective than the tested results.

In conclusion, CRISPR-Cas9 has involved into a powerful tool in the fight against AD, offering new avenues for understanding the disease's complexities, identifying therapeutic targets, and potentially developing effective treatments. Continued research and collaboration among scientists, clinicians, and regulatory authorities will be essential in harnessing the full potential of CRISPR-Cas9 and eventually making meaningful strides in the battle against Alzheimer's disease.

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