CRISPR-Cas9 Gene Editing: A Promising Frontier in Revolutionizing Alzheimer’s Disease Management

Yuxin Jin

School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, China
Corresponding author: 1155174682@link.cuhk.edu.hk

Abstract:
Alzheimer’s disease (AD) is a globally devastating neurodegenerative disorder with a significant burden on public health. Currently, the pathophysiology of AD remains uncertain. Meanwhile, conventional pharmacological treatments for AD accomplished with limited efficacy and undesirable side effects necessitate the exploration of novel therapeutic approaches. This paper highlights the potential of clustered regularly interspaced short palindromic repeats-associated proteins nine systems (CRISPR-Cas9) as a promising approach to cure AD by examining its applications in genome editing and mechanism of action on specific AD-related genes. In addition to targeted therapeutic options for either familial or sporadic AD, the review also emphasizes the importance of utilizing in vivo and in vitro AD experimental models to evaluate the efficacy and safety of CRISPR-Cas9 interventions. The significance of this research lies in its potential to revolutionize AD management and serve as a valuable reference for future studies. However, unresolved issues, such as the complexity of on-target effects and the need for improved delivery efficiency, underscore areas for future ongoing investigation and development in the field of AD therapeutics.

Keywords: Alzheimer’s disease; CRISPR-Cas9; dCas9; genome editing; gene therapy.

1. Introduction

Alzheimer’s disease (AD) is one of the most common reasons for dementia, accounting for 60-80% of dementia cases and affecting over 55 million people worldwide [1]. This tremendous number is projected to reach 150 million by 2050 [1], undoubtedly being seen as a serious global public health challenge and adding a heavy burden on societies and economics. The impact of AD on individuals is also undeniably devastating. The major etiological profile of this progressive neurological disorder is characterized by impaired cognitive functions and gradual declines in memory formation as a result of irreversible neural degeneration and death [2]. Loss of judgment and recognition, depression, anxiety, and irritability are among the primary symptoms. Pathologically, the accumulation of β-amyloid (Aβ) plaques intracellularly and hyperphosphorylated tau protein extracellularly, along with overactive glial cells, are believed to be the significant disease hallmarks [2]. Yet, the pathophysiology of AD remains uncertain, requiring further extensive investigation.

The complexity of the human nervous system is a major obstacle worthy of attention in the onset and treatment of AD. In order to protect the central nervous system, the blood-brain barrier, involving pericytes, astrocytes, and capillary endothelial cells, forms a tight junction as a self-defense mechanism [3]. This selective permeable barrier not only stops toxins and pathogens from entering the CNS but also highly restricts the entry of many conventional drugs. Currently, the development and prospect of traditional pharmacological methods are confined. This explains the presence of new medication in treating symptoms of AD but seldom having available neurorestorative therapy to target the neurological alterations in the brain. Not to mention, the current symptomatic therapeutic options pose numerous side effects, including dizziness, confusion, constipation, and diarrhea [4].

The field of AD treatment is in need of new directions and novel strategies. A recently developed clustered, regularly interspaced short palindromic repeats-associated proteins nine system (CRISPR-Cas9) technology provides hope and attention to the scientific community worldwide due to its transformative potential to revolutionize the management of AD. This ground-breaking genome-editing tool has offered a promising approach to modifying AD-related genes directly, efficiently, precisely, and inexpensively. Meanwhile, this emerging tool can be used to construct humanized animal models for a better investigation. Several scientific studies have outlined the potential role of CRISPR-Cas9 in preventing the progression of...
AD, potentially saving millions of lives suffering from this horrible disease.

In this paper, the purpose of this review is to explore the potential of choosing the CRISPR-Cas9 gene editing tool as a treatment option for AD by examining its mechanism of action on several specific AD-related genes. The challenges of specificity and delivery systems in using this technology are worthy of discussion, contributing to a solid foundation and direction for future research in curing AD.

2. Overview of CRISPR-Cas9 Gene Editing

Initially discovered in 1987, the CRISPR-Cas system was later recognized as a vital component of the bacterial immune system, safeguarding against the integration and reinfection of undesirable viruses and conjugative plasmids [5]. This innovative and powerful tool for genome editing exhibits different categories based on its structure and components, involving two classes, six types, and 21 subtypes [6]. Class 1 encompasses types I, III, and IV, which comprise multiple Cas proteins working collaboratively. In contrast, Class 2, containing types II, V, and VI, utilizes a single Cas protein, offering a simpler and more favorable option for genome editing [7]. Among all, the type II CRISPR-Cas9 system has undergone extensive application in both laboratory settings and pharmaceutical development.

In the type II CRISPR-Cas system, a single-guide RNA (sgRNA) and a Cas9 enzyme are responsible for constituting the solid foundation. The pivotal role of sgRNA, comprising around 20 base pairs, leads the Cas enzyme to identify the target gene. The sgRNA is further constructed by CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA) [6]. crRNA carries sequences complementary to the targeted DNA, which ensures correct recognition and binding, while tracrRNA initiates the activation of host ribonuclease with concomitant pre-crRNA processing and crRNA maturation [6]. On the other hand, Cas9 is the sole protein functioning as an endonuclease in the type II CRISPR-Cas9 system [7]. These genetic scissors execute cleavage of the DNA double strands at the desired location, facilitating two subsequent intracellular repair processes: non-homologous end joining (NHEJ) and homology-directed repair (HDR). The NHEJ pathway aims to induce insertions and deletions (InDels) at DNA incision, attributing to DNA frameshifts, nonsense mutations by premature stop codons, and gene inactivation eventually. Meanwhile, the HDR pathway enables the amendment of mistaken or mutated sequences, taking the reference of correct sequences from a donor DNA template. The former allows higher efficiency, while the latter is a more reliable DNA repair mechanism yet restricted to the interphase of the cell cycle only [5].

Additionally, the Cas9 protein can be modified by employing specific mutations and converted into catalytically inactive Cas9, so-called dead Cas9 (dCas9). In this circumstance, dCas9 retains its DNA-binding ability but loses its cut function. Scientists have leveraged this property to develop CRISPR activators (CRISPRa) and inhibitors (CRISPRi) that can properly modulate the expression of the target gene [8]. Currently, this technology has also been employed for genome manipulation without relying on DNA double-strand breaks and DNA recombination, offering a faster and simpler method for precise gene regulation.

3. Overview of Alzheimer’s Disease

AD, as a prevalent neurodegenerative disorder, is primarily characterized by the accumulation of two prominent disease hallmarks: tau protein and Aβ. Tau constitutes intracellular neurofibrillary tangles; meanwhile, Aβ constructs extracellular amyloid plaques [2]. In fact, the majority of currently known disease mechanisms belong to familial AD, which constitutes only 1% of AD cases. Reports indicate that at least half of familial AD cases involve autosomal dominant mutations in one of three genes: amyloid precursor protein (APP), presenilin-1 (PSEN1), and presenilin-2 (PSEN2) in chromosomes 21, 14, and 1, respectively [9]. Typically, these mutations manifest before the age of 65, leading to early-onset AD [9]. On a pathogenetic basis, these mutations predominantly disrupt Aβ metabolism, serving as a crucial AD pathology. The ground-breaking CRISPR-Cas9 holds significant promise in effectively repairing or knocking out these autosomal dominant mutations.

On the contrary, sporadic AD accounts for more than 90% of AD cases, but this prevalent form of AD seriously lacks well-established underlying causes compared to familial AD. It involves a complex interplay of genetic predisposition and other environmental risk factors, including but not limited to aging, hormones, stress, and chronic diseases [2]. Although the exact triggers for sporadic AD remain largely unknown, ongoing research unravels the potential of CRISPR-Cas9 in explaining the intricate mechanisms underlying this late-onset AD and perhaps treating it.

4. Role of CRISPR-Cas9 in the Management of Alzheimer’s Disease

4.1 Therapeutic Approaches for Familial AD

Several groups have explored the therapeutic potential of
CRISPR-Cas9 to knock out KM670/671NL mutation at the β-secretase cleavage site [10], hindering the generation of pathogenic Aβ. This variant, known as the Swedish APP (APPsw) mutation, is a specific alteration in the APP gene, which is one of the most related alleles in causing AD development. APPsw mutation inordinately activates the β-secretase enzyme in making excessive Aβ in brains. Cas9 enzyme and sgRNA designed to specifically target APPsw mutation can be delivered into AD patient-derived fibroblasts via recombinant adeno-associated virus, recording 60% less Aβ production [10]. Also, the same Cas9 and sgRNA were injected into the hippocampus of adult Tg2576 mice and primary cortical neurons of APPsw transgenic mice embryos, where both carrying manifold human APPsw mutation, 2% InDels in the APPsw gene were observed [10]. This reveals the ability of CRISPR-Cas9 to interrupt APPsw mutation. Besides tackling APP variations, the PSEN2 gene is also involved in considerable familial AD cases. For instance, the PSEN2N1411 point mutation is associated with an elevated Aβ42/40 ratio, promoting the formation of Aβ oligomers and fibrils in AD [11]. Recent studies have utilized the CRISPR-Cas9 system to correct this autosomal dominant mutation in induced pluripotent stem cells derived from AD patients carrying mutant PSEN2 gene, attributing to the generation of basal forebrain cholinergic neurons expressing normal levels of Aβ42/40 and reversing electrophysiological deficits [11].

Alternative strategies have been explored to control Aβ pathology in AD, such as employing CRISPRa or CRISPRi fused with dCas9 [8]. Currently, this system delivered via non-viral vehicles into AD animal models has demonstrated success in improving AD-like pathology. Taking BACE-1 as an example, researchers directly injected dCas9 nano-complexes targeting BACE-1 into the CA3 hippocampus of both APP knock-in and 5xFAD transgenic mice [12]. Notably, a 70% downregulated BACE-1 expression was captured in treated mice, along with improved cognitive impairment and reduced Aβ42 synthesis [12]. Additionally, the upregulation of the ADAM10 gene by utilizing dCas9 nano-complexes also results in significantly lower Aβ secretion [13]. This innovative approach holds great promise in potential therapeutic avenues for tackling AD.

Apart from accumulated Aβ, AD osteogenesis is also characterized by chronic neuroinflammation, where proinflammatory glia maturation factor (GMF) plays a significant role [14]. It is known microglia serve as the primary source of GMF. Recent CRISPR-Cas9 editing of the Gmf gene in BV2 microglial cell lines [14]. To this end, the inflammatory response in AD patients’ brains, mimicked by lipopolysaccharide, was alleviated with decreasing GMF expression. This discovery paves a new avenue for AD therapy by manipulating the Gmf gene.

4.2 Therapeutic Approaches for Sporadic AD

For late-onset AD, the APOE4 allele poses considerable associations with AD pathogenesis. APOE exists in different isofoms, with specifically APOE4 being linked to an increased risk of AD by promoting phosphorylation of tau protein [15]. Individuals carrying one copy of the APOE4 allele potentially have a three times higher risk, while those with two copies may even have a 15 times higher risk [5]. Notably, the majority of AD patients, approximately 80%, carry at least one APOE4 allele. Yet, a simple substitution of a single amino acid can convert this disease-linking allele into either APOE2, acting as a protective allele of AD with 40% less likely in developing AD, or a normal APOE3 gene. This molecular basis pushes the development of CRISPR-Cas9-mediated “base editing” specific to the APOE4 gene. In mouse astrocytic cells containing APOE4 and APOE3 genes, CRISPR-Cas9 delivered by lentivirus can selectively target APOE4 allele, suppressing up to 60% APOE4 protein production while leaving APOE3 unaffected [5]. In HKEK293T cell lines, Indels are induced in APOE4 by CRISPR-Cas9 systems fusing cytidine deaminase enzyme [15]. This selective base alteration strategy results in an irreversible conversion from APOE4 into APOE3 allele, correcting disease-related mutations and reducing hyperphosphorylation tau proteins.

The APP gene is undoubtedly another good target for sporadic AD. The lentiviral CRISPR-Cas9 system was employed to selectively manipulate the C-terminus of the endogenous APP gene without disrupting the N-terminus [16]. Following this, the edited APP shifts away from a monogenetic gene that promotes the amyloidogenic pathway. Meanwhile, the crucial physiological roles of the N-terminus will not be interfered with. This approach has shown efficacy in reducing Aβ production and AD-like pathology in C57BL/6 mice, HEK293 cells, and neuro2a cells [16].

4.3 In Vivo Models for Familial AD

Over the years, AD animal models have primarily relied on overexpressed human genome mutations. Despite an advanced understanding of AD pathogenesis, these models are restricted from mimicking the complexity of the disease phenotype, especially in the absence of significant neurodegeneration [17]. To tackle this, CRISPR-Cas9 has been employed to tailor newer and more comprehensive AD in vivo models. APP, as a key disease-causative gene, is always on the hot spot for investigation. Some researchers incorporated
three independent point mutations (G676R, F681Y, and R684H) utilizing CRISPR-Cas9 into the endogenous mouse and rat APP gene, successfully humanizing the models for studying APP’s causal factor on AD progression [18]. On the other hand, some variations on APP confer protection against the disease. By employing CRISPR-Cas9 on APP-KI mice, specific deletions within the APP’s 3’-untranslated region demonstrated a dramatic decline in Aβ pathology [19]. In addition to Aβ, the pathology of another disease hallmark, tau protein, should be deeply investigated. In this regard, tau knockout C57Bl/6J mice were reconstructed by applying CRISPR-Cas9 to introduce a fine deletion in the transcriptional start codon in exon 1 of the tau-encoding Mapt gene [20]. The resulting animal model displays resistance to excitotoxicity and does not exhibit any memory deficits. This approach allows for a better understanding of tau’s role in AD pathology. Plcγ2 gene expressed in microglia has been implicated in AD, with different variants conferring varying risks of developing the disease. A team employed the CRISPR-Cas9 to develop a mouse model with a specific genetic alteration known as Plcγ2<sup>P522R</sup> knock-in, concluding this specific alteration offers a protective effect by enhancing microglial function and mitigating AD development [21]. These findings shed light on the molecular link between Plcγ2 and AD and pave the way for potential therapeutic interventions.

4.4 In Vitro, Model for Sporadic AD

SORL1, the gene responsible for encoding the SORLA protein, is a sorting receptor found in various cells within the central nervous system and plays a critical role in regulating the processing of APP. In patients with late-onset AD, a reduced expression of the SORL1 gene is reported [22]. CRISPR-Cas9 gene editing tool is employed to construct SORL1-deficient human induced pluripotent stem cell lines [22]. This creation of a sporadic AD-specific cell model provides valuable insights into the underlying mechanisms contributing to the accumulation of Aβ in AD, along with investigating the localization of APP within the endosomal network.

5. Challenges and Strategies of CRISPR-Cas9

5.1 Off-target Effects

CRISPR-Cas9 has revolutionized gene editing and manipulation by offering versatile prospects in clinical applications. Yet, the looming concern of off-target effects poses a significant challenge to its widespread adoption. Off-target effects occur when the Cas9 protein inadvertently cleaves DNA at unintended sites other than the target. This can end up with detrimental consequences, severely interrupting normal gene function and genomic stability. Indeed, this pioneering technology in AD heavily relies on the precise interaction between the sgRNA and the target DNA to achieve accurate therapeutic efficacy. However, some fluctuations in sgRNA sequence can influence the occurrence of off-target effects. Truncated sgRNAs, whose sequences are shorter than 17 nucleotides, usually exhibit insignificant or reduced activity compared to full-length sgRNAs with 20 base pairs [23]. In other words, it is crucial to keep the complete sequence length of each designed sgRNA to minimize potential mismatches. Similarly, the GC content of the sgRNA sequence should also be carefully considered. Both extremely rich and poor GC content can disrupt sgRNA performance, being associated with increased off-target possibility. For optimal targeting efficiency, a neutral GC content in sgRNA, approximately 40% to 60%, is the best to maintain [23].

Addressing the hurdle of off-target effects requires innovative strategies to explore advancements in Cas9 protein engineering. In recent years, scientists have actively developed a range of Cas9 variants to enhance the specificity of DNA cleavage. Meanwhile, the on-target activity of Cas9 should be well preserved. These hyper-accurate variants, such as SpCas9-HF1, Sniper-Cas9, eSpCas9 1.1, HypeCas9, and xCas9 [23], have shown remarkable success in minimizing non-specific DNA contact, offering hope for improved precision in gene editing. Algorithms designed for picking sgRNA with higher specificity are another strategy [24]. Researchers are then allowed to choose optimal sgRNA sequences, reducing the potential for unintended DNA cleavage. In parallel, active investigation toward the precision in CRISPR-Cas9 targeting is currently underway. These efforts aim to mitigate the off-target effects when applying this technology, ultimately paving the way for its successful clinical application in AD.

5.2 CRISPR-Cas9 Delivery System

Currently, there are three possible CRISPR-Cas9 delivery strategies commonly used: plasmid-borne CRISPR-Cas9 system [5], Cas9 protein/sgRNA complex [12], and finally Cas9 mRNA/sgRNA mixture [5].

Thanks to the assistance of plasmid, the first approach allows both Cas9 protein and sgRNA to be incorporated into the same vector. This means their simultaneous co-expression within the same cell can be ensured, offering stability. Meanwhile, the inclusion of multiple sgRNAs within the same plasmid is acceptable, granting researchers greater versatility in their experimental design. Containing genes encoded fluorescent protein is another key feature of this
plasmid-based approach, enabling the visualization and tracking of fluorescent-labeled cells expressing Cas9. The good reproducibility and cost-effectiveness always make the plasmid-borne CRISPR-Cas9 system a better option. One concern of this delivery strategy, however, is its low transfection efficiency, particularly when working with primary cells. Random insertion of plasmid fragments into the targeted gene is considered a danger to have unintended genetic modifications, not to mention potential cytotoxicity.

Moving on to the complex of Cas9 protein and sgRNA, this approach stands out for its remarkable simplicity because the complex formed effortlessly, requiring neither transcription nor translation of Cas9 protein. In short, this streamlined method is rapid and efficient with minimal off-target effects. Nevertheless, it is a tough task to deliver the large-sized Cas9 protein into target cells. Innovative techniques are essential to overcome this obstacle and achieve successful intracellular transportation of Cas9. Besides, the cost of the purification process in eliminating endotoxin contamination can incur considerable costs.

The Cas9 mRNA/sgRNA approach presents notable advantages over the plasmid-mediated CRISPR-Cas9 delivering strategy in terms of reduced off-target effects and cell toxicity. However, it is essential to acknowledge a hurdle in RNA instability. RNA molecules are susceptible to degradation, which can compromise the efficiency and longevity of the Cas9 mRNA within the cellular environment.

6. Conclusion

As a gene editing tool, CRISPR-Cas9 undoubtedly demonstrates its promising potential throughout many scientific fields. Not restricted to its application in laboratory settings, it further extends its purpose as a treatment option for diseases with limited or ineffective therapeutic options, including AD as one of those. This revolutionized system can properly manage both familial and sporadic AD, focusing on its construction of AD models or therapeutics for Alzheimer’s disease and other amyloidosis. CRISPR-Cas9 gene editing: New hope for Alzheimer’s disease therapeutics. Journal of Advanced Research, 2022, 40, 207-221.

References