Proceedings of the 4th International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/66/2024.18034

The analysis of whether CRISPR-Cas9 is the way forward for Alzheimer's disease treatment

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Abstract. Alzheimer's disease (AD) is a progressive and irreversible neurological condition which according to WHO affects more than 55 million individuals globally and is the leading cause of dementia and thereby poses a significant burden on healthcare systems worldwide. AD progresses through stages of mild cognitive impairment to severe dementia and is characterised by the accumulation of extracellular amyloid-beta plaques and neurofibrillary tangles concentrated with tau proteins in neocortical structures and the temporal lobe. Gene-editing technology such as the most prevalent CRISPR-Cas9 provides prospects for the treatment of AD by enabling precise modifications of the genetic mutations associated with the disease. This review explores the potential of CRISPR-Cas9 to revolutionise treatment by targeting and rectifying mutated genes but also examines the current state of Alzheimer's treatment. This paper examines recent advancements in preclinical studies and highlights the successes in reducing amyloid-beta plaques and tau neurofibrillary tangles, the pathological features of AD. By evaluating current CRISPR-Cas9 research and other treatments for AD, I aim to provide insight into its potential as a transformative gene therapy approach whilst evaluating its limitations.

Keywords: Alzheimer's disease, CRISPR-Cas 9, gene editing, amyloid-beta plaques, tau neurofibrillary tangles.

1. Introduction

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) with linked protein Cas-9 is a gene editing tool with the potential to transform the treatment of various genetic disorders such as AD. Precise modifications to targeted DNA sequences through sgRNA recognition, Cas-9 nuclease cleavage and repairing of the double strand through homology-directed repair (HDR) or non-homologous end-joining mechanisms enable researchers to directly target and correct genetic mutations implicated [1-2]. Most notably, mutations in genes such as APP (amyloid precursor protein), PSEN1 (presenilin 1), and the APOE4 allele have been linked to the development and progression of AD [3].

The employment of CRISPR-Cas9 in preclinical studies has shown promise in reducing the production of amyloid-beta and tau proteins. Targeting the APP gene, for example, to disrupt its cleavage, revealed significant decreases in amyloid-beta plaques using animal models [3]. Despite extensive research efforts, effective disease-modifying treatments have yet to be found because of a partial comprehension of synaptic deterioration and cognitive dysfunction mechanisms hence most current therapies mainly offer only symptomatic relief [4]. This highlights an urgent need for innovative therapeutic approaches that can target the root causes of AD. The rapid growth in CRISPR-Cas 9

DOI: 10.54254/2753-8818/66/2024.18034

research has potential in developing effective gene-editing therapies for AD and this review aims to provide an overview of the current state of treatments for the disease.

2. The physiopathology behind Alzheimer's disease

Regarding AD pathophysiology, the two hypotheses that are most widely accepted are the amyloid cascade hypothesis and the tau hypothesis. Therefore, understanding these mechanisms is essential in CRISPR-Cas9's potential in treating AD because it enables precise targeting of the genetic and molecular abnormalities underlying the disease.

The amyloid cascade hypothesis proposes that the deposition of amyloid-beta (Aβ) peptides in the brain is the primary event that causes the pathological cascade ultimately causing Alzheimer's disease pathology [5]. Amyloid precursor protein (APP) is fundamental to the pathogenesis of AD. Imbalanced toxic amyloid-beta, $A\beta_{(1-40)}$ and $A\beta_{(1-42)}$ segments, form when APP undergoes sequential proteolytic cleavages by beta-secretaries and gamma-secretases. Normally, these levels of peptides are controlled by degradation or clearance from the brain but in Alzheimer patients, this balance is altered because of mutations in the apolipoprotein E (APoE4) gene, APP gene, presenilin genes (PSEN1 and PSEN 2). Therefore, more Aβ proteins form which leads to toxic oligomers which then further transform into fibrillar plaques (Figure 1) [6].

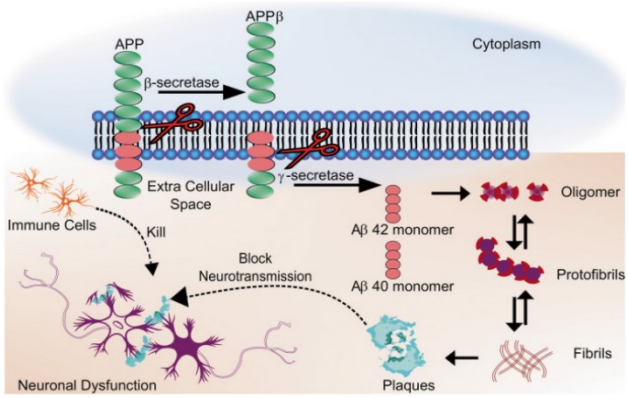

Figure 1. A schematic illustration of the amyloid cascade hypothesis based on the amyloidogenic pathway [6]

These plaques are believed to impair neuronal function, generate oxidative stress, and engage inflammatory mechanisms which collectively contribute to synaptic damage and neurodegeneration [7]. Furthermore, later studies showed that amyloid-beta oligomers have a wider effect than plaques on cell function since they lead to cognitive deficits by impairing synaptic plasticity and are particularly neurotoxic [8].

Among the factors that support the amyloid cascade theory is the tau hypothesis, which concerns abnormal phosphorylation and aggregation of tau proteins. The microtubule-associated protein tau stabilises microtubules in neurons. Hyperphosphorylation of tau leads to its detachment from microtubules and aggregation into neurofibrillary tangles (NFTs). These tangles interfere with the microtubular network leading to axonal transport impairment, neuronal dysfunction and cell death (Figure 2)[9]. Therefore, the propagation of clinical symptoms through the brain corresponds with the progressive spread of tau pathology, indicating that misfolded tau can induce misfolding within adjacent neurons [10].

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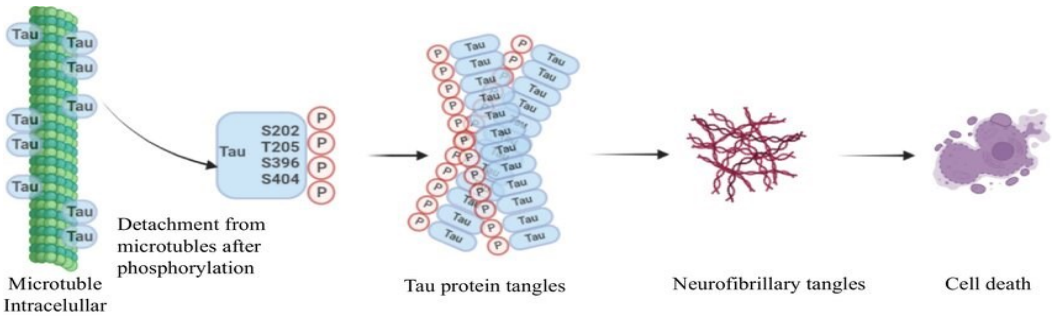

Figure 2. A schematic illustration of the tau hypothesis where hyperphosphorylation of tau leads to tau protein tangles, neurofibrillary tangles and eventually cell death. [9]

3. Applications of CRISPR-Cas 9 in Alzheimer's disease

In a study by György et al, Aβ production in targeted AD patient-derived fibroblasts was decreased by 60% by knocking out Swedish APP (APPswe), mutations located at the β-secretase site. To further test this gene disruption, the hippocampus of Tg2576 mice was injected with DNA-encoded Cas9 and SW1 guide RNAs. Indel formation in an average of 1.3% APPswe alleles occurred in all mice with the most common being one base pair insertions [3]. Due to a large number of target alleles, only 2% of transgenes were disrupted therefore behavioural and pathological changes were not assessed. Therefore further studies should assess the targeted cells in detail to reveal the efficiency of gene editing and its effects in vivo. In another study, the A673T mutation that could decrease β-secretase cleavage by 40% was inserted into APP genes in SH-SY5Y and HEK293T cells through a CRISPR-Cas 9 mediated system (Figure 3) [2].

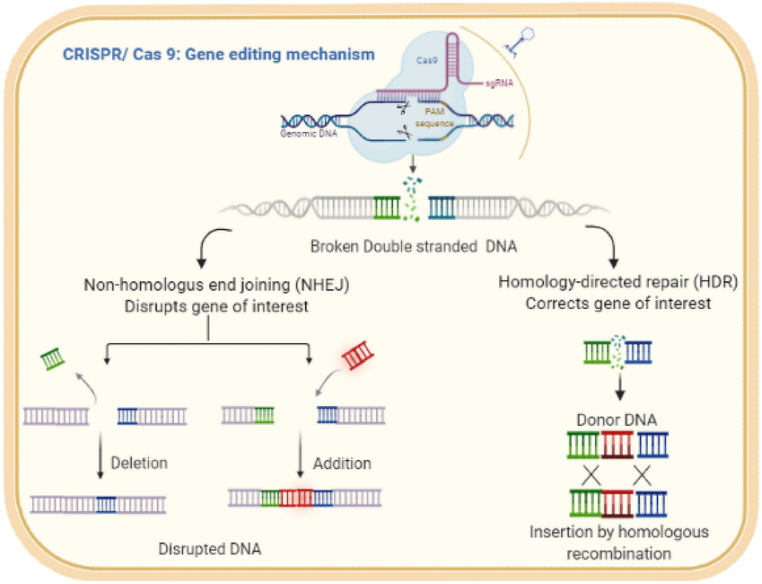

Figure 3. Double-stranded break on target DNA created through CRISPR-Cas 9 techniques. Reparation then occurs through non-homologous end joining (NHEJ) or homology-directed repair (HDR) [2]

The alanine codon in cells was then converted into a threonine codon with deaminated cytosine 1 and 2. The introduction of the A673T mutation along with new mutation E674K (A673T/E674K-APP) into 53% of HEK293T cells revealed a decrease of 44% decrease in Aβ40 and 53% decrease in Aβ42 peptides. Furthermore, investigations into possible off-target events to evaluate the specificity of this base editing approach led to 1 possible site in a non-coding DNA sequence with no other off-target's found which demonstrates precision and safety [11]. With changes in DNA methylation being associated

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with the progression of AD and more specifically the hypomethylation of APP, Park et al used DNA methyltransferase enzyme (Dnmt3a) fused to catalytically dead Cas 9 (dcas9) on the locus of the APP gene in a mice model to cause DNA methylation. Using the dCas9-Dnmt3a system, -189 single guide RNA significantly reduced protein levels and APP messenger RNA in mouse embryonic fibroblast (MEF) and NIH/3T3 cells. This effect was also observed in the targeted site of APP knock-in (APP-KI) mouse primary neurons through increased CpG methylation. This led to decreased amyloidogenic processing of APP due to the decreases in Aβ42 and Aβ40 peptides. No significant off-target effects occurred from the analysis of 6 predicted off target sites[12]. Providing proof of concept strategy for selective APP silencing, sun et al edited endogenous APP at the extreme C-terminus in mouse brains and human iPSC-derived neurons to decrease β-secretase cleavage and therefore Aβ production. APP and beta-secretase 1 (BACE1) approximation is reduced as well as abrogating their convergence [13]. Since this method does not rely on gene editing on APP mutations, it could be used on sporadic AD rather than the rarer familial AD that accounts for 5-10% of cases.

Another significant application is the editing of the APOE gene, particularly the APOE4 allele, which is a major genetic risk factor for sporadic and late-onset AD. Whilst isoform APOE3 does not contribute to the possibility of developing AD and having 1 copy of APOE2 can reduce the risk by up to 40%, APOE4 is associated with increased Aβ deposition and impaired clearance and around 65-80% of diagnosed individuals have 1 or more copies of this allele. Using CRISPR-Cas9, researchers have been able to convert the APOE4 allele to the more benign APOE3 variant, resulting in reduced Aβ accumulation and neuroinflammation in cell and animal models [14]. This genetic correction holds promise for reducing the risk or delaying the onset of AD in individuals carrying the APOE4 allele. This is further supported by another study in which a CRISPR-Cas 9 variant was used to selectively knock out the APOE4 allele while preserving the APOE3 allele in neuron cells. Overall, this led to decreased amyloid-beta production [15].

Autosomal dominant mutations in presenilin 1 genes (PSEN1) can also be corrected with this technology. Being a key component of gamma-secretase, Aβ42 that are prone to aggregation is generated due to the M146L mutation in PSEN1. In human fibroblasts, selective disruption of the PSEN1 M146L allele through the CRISPR-Cas9 system leads to more than 50% of mutated alleles being disrupted which partially restores the Aβ42/40 ratio[16].

CRISPR-Cas9 is also being explored for its potential to target and mitigate tau pathology. By editing genes involved in tau production and phosphorylation, CRISPR-Cas9 can reduce tau aggregation and its associated neurodegenerative effects. For instance, modifying the MAPT gene which encodes tau with adenine base editor NG-ABE8e to correct the MAPT mutation in the hippocampus of mice decreases the number of insoluble tau proteins and improves neuronal health[17].

4. Transport Methods of CRISPR-Cas9 for Alzheimer's Treatment

For any therapeutic application in Alzheimer’s disease, efficient delivery of CRISPR-Cas9 into the brain is key and both viral and non-viral approaches to this have been used to achieve this.

4.1. Viral Delivery

The high transduction efficiency and wide range of infected cell types make viral vectors, especially adeno-associated viruses (AAVs), a common choice for delivering CRISPR-Cas9 components. Since AAVs are capable of sustained gene expression, they can cross the blood-brain barrier which makes them useful tools for targeting AD-related genes such as APP, PSEN1 and PSEN2. Furthermore, studies have shown that AAV-mediated delivery of CRISPR-Cas9 can reduce amyloid-beta levels in the brain. This leads to improved AD pathology in animal models, an example being György et al's study using AAV vectors to deliver CRISPR-Cas9 targeting the APP gene in a mouse model of AD. This resulted in decreased amyloid plaque formation and improved cognitive function [3].

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4.2. Non-Viral Delivery

Being less immunogenic and carrying a lesser risk for insertional mutagenesis, they are an alternative to viral vectors. Lipid nanoparticles (LNPs) are a key non-viral delivery system that can package CRISPR-Cas9 components for transportation to the brain. LNPs can be designed to target specific cell types, and have shown efficacy in transporting CRISPR-Cas9 into neurons. Gold nanoparticles are another non-viral approach that can be engineered to transport CRISPR-Cas9 components to brain cells efficiently. In their work, Rosenblum et al showed that lipid nanoparticles could target and deliver CRISPR-Cas9 [18].

While both viral and non-viral delivery methods are being optimized to enhance the precision, safety, and effectiveness of CRISPR-Cas9-based therapies for Alzheimer's disease, there is still room for further improvement in these techniques. This could potentially lead to more effective gene-editing treatments for AD patients in the future.

5. The current state of Alzheimer's treatment

Only 2% of clinical trials for AD treatment have succeeded from 2003 to 2023 with the rest of phase II and III compounds considered unsuccessful due to adverse effects that prevented continuation of trials, trial efficacy endpoint not being met, or discontinuation [19]. Presently, the treatment options available for Alzheimer's disease (AD) mainly concentrate on relieving symptoms rather than curing or slowing down the progression of the disease. To date, there are various FDA-approved drugs designed for managing AD symptomatology.

Donepezil (Aricept), rivastigmine (Exelon), and galantamine (Razadyne) are examples of cholinesterase inhibitors that raise acetylcholine levels in the brain – a neurotransmitter essential for memory and cognition. Generally, these drugs are prescribed at mild to moderate stages of AD and improve cognitive symptoms while not halting the underlying progression [20].

Memantine (Namenda), which is an N-methyl-d-aspartate receptor antagonist, is given during moderate to severe stages of the condition by blocking current flows in NMDA receptor channels. This regulates glutamate activity, an excitatory neurotransmitter whose excess production leads to excitotoxicity and destruction of neurons.

Amyloid-targeting therapies, including aducanumab (Aduhelm), lecanemab (Leqembi) and Donanemab (Kisunula), have been recently approved by FDA. This monoclonal antibody targets aggregated amyloid-beta protein and its intended usage is to reduce amyloid plaques in the brain. Though it has been approved, aducanumab has stirred up controversy because clinical trials yielded mixed results in terms of its effectiveness at slowing down cognitive decline [21]. Lecanemab is another monoclonal antibody developed by Eisai and Biogen that displayed significant promise in reducing amyloid plaques as well as slowing cognitive decline in patients with early-stage AD, where clinical trials showed modest reductions in amyloid burden and a slower rate of clinical deterioration although not improving[22].

These interventions are significantly beneficial to address the amyloid pathology associated with AD, though their continuous assessment for long-term safety and efficacy remains essential. Moreover, the safe use of these treatments necessitates the management of adverse effects with the most common being amyloid-related imaging abnormalities (ARIA) [21].

6. Conclusion

In understanding AD, the ability to make targeted genetic modifications is of great significance to understanding its mechanisms and opens up possibilities for therapeutic remedies that are effective. Nonetheless, the move from experimental and conceptual research to clinical trials is challenging and raises controversies regarding approval. It is essential to establish efficient delivery of CRISPR-Cas9 components into the brain whilst avoiding and minimising off-target effects as well as considering ethics related to gene editing. Therefore, more research will need to be carried out in these aspects considering that genome editing is irreversible. In the future, research should focus on optimising delivery approaches such as viral and non-viral vectors that will enhance the specificity and efficacy of gene

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editing. The Shortcomings of this paper acknowledge the current lack of clinical trials in this area of research and current limitations in delivery efficiency. Therefore, future directions of this paper will focus on rigorous preclinical and clinical trials to confirm the safety and efficiency of CRISPR-Cas9 therapeutics. Also, there must be developed ethical frameworks and regulatory guidelines for responsible navigation through the complex process of human gene editing. This gene-editing approach with ongoing improvements in CRISPR-Cas9 technology and joint efforts from scientific and medical communities could change how Alzheimer's disease is treated. Breakthrough therapies based on the accuracy and adaptability of CRISPR-Cas9 might address not only symptoms but also underlying genetic reasons for AD.

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