The Molecular Efficacy of CRISPR-Cas9 in Treating Alzheimer's Disease

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Abstract: Alzheimer's disease (AD), a highly complicated type of neurodegenerative diseases is the brain ailment characterized by the progressive decline in mental function influencing millions of people globally. In spite the developments in molecular biology, it is not fully understood what causes this disease. Mutations in APP, PSEN1, and PSEN2 symptomize AD by building amyloid-beta, a plaque formed in the brains by which neurons communication is hindered triggering cognitive decline, neuronal impairment, and death. Current advances in gene editing have provided new insights into the significant genetic factors take part in the progress of AD. CRISPR-Cas9 is a cutting-edge genome-editing tool used to provide sincere efforts to target and silence infective proteins linked with AD. The main goal of this research is to assess the potential of CRISPR Cas9, as a treatment for Alzheimer's disease (AD) in addition to reviewing genes for both early and late onset AD.

Keywords: CRISPR-Cas9; Alzheimer's disease; amyloid-beta; neurons; gene editing

1. Introduction

During the course of brain development, the molecular mechanisms involved in normal homeostasis in the early ages are highly healthy. However, with age decline these molecular mechanisms experience gradual senescence thus, producing a wide variety of neurodegenerative disorders and mental issues leading to the dementia. Alzheimer's disease (AD) is one of the most predominant neurodegenerative disorders with a big socioeconomic problem. It affects over 50 million individuals worldwide [1],[2]. The social and economic implications linked to AD have had an impact, on health. It can lead to permanent neuronal death by deteriorating cognition over-activation glial cells, production of amyloid-beta 42 (Aβ-42) and phosphorylation of Tau in the brain [3],[4]. In spite of comprehensive research in this field, the pathophysiology, etiology, and mechanisms of cognitive impairment and synaptic dysfunction are poorly understood [5],[6].

Countless techniques and therapies have been developed to treat this devastating disease in recent years including pharmacotherapy and

non-pharmacological intrusions. For example, donepezil, cholinesterase inhibitors like galantamine, and rivastigmine are given to augment cholinergic neurotransmission and recover mental function [7]. Many factors such as cognitive stimulation remedies, physical exercise, and psychosocial interventions showed a positive influence on cognitive function and overall well-being in individuals with Alzheimer's. However, these available therapeutic options can only treat symptoms. Even though providing supportive care, they can have unpleasant side effects like depression, confusion, diarrhea, vertigo and constipation [8]. Despite the wealth of knowledge in terms of the molecular causes of AD, advancement toward active modifying therapies has proven problematic. For instance, numerous clinical trials failed to validate effectiveness in preventing the production, accumulation, and toxicity of Aβ [9]. Besides, gene editing technologies have made considerable advances over recent years, especially a technique known as Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated proteins 9 system (CRISPR-Cas9) gene editing. This review aims to assess the potential efficiency of CRISPR-Cas9 to treat AD.

2. Methodology

This review mainly intends to explore the significance of CRISPR-Cas9 as a molecular means to treat Alzheimer's disease. The most reliable and recent peer-reviewed articles were studied. We conducted searches on databases and websites such as the National Center for Biotechnology Information (NCBI), PubMed, Google Scholar, and the World Health Organization (WHO). The main focus of this paper was on CRISPR Cas9 and Alzheimers disease and related terminologies were used in this research like, APP, PSEN1, PSEN2, amyloid beta, Genome Wide Association Studies (GWASs) Homology Directed Repair (HDR) Non-Homologous End Joining, Delivery Methods of CRISPR Cas9, Adeno Associated Viruses (AAV), Tau protein. Additionally, we explored Early onset Alzheimer's disease and Late onset sporadic Alzheimer's disease.

3. Important Causes of Alzheimer's Disease

There are two types of AD, early-onset (also known as familial) and late-onset (also known as sporadic). Late onset AD is more common compared to the early onset, accounting for over 95% of cases. Early onset AD is relatively infrequent and is caused by mutations in three genes; APP, PSEN1 and PSEN2. Late-onset Alzheimer's on the other hand is more commonly detected in individuals aged 65 years and above and its heritability is high, estimated at 79%. However, a combination of genetic and environmental factors can influence the causation of late-onset Alzheimer's [10].

Genes responsible for both familial and sporadic AD have been studied by utilizing molecular genetics techniques. APP, PSEN1, PSEN2, and APOE were among several genes associated with AD. Additionally, genome-wide association studies (GWASs) have uncovered more than 20 loci that are related to AD risk. Most of these genes are involved in the lipid metabolism inflammatory response, and endocytosis pathways. Genes involved in lipid metabolism are represented by APOE, DSG2, ABCA7, SORL1, and CLU. Inflammatory response genes are denoted by CR1, CLU, CD33, ABCA7, EPHA1, MEF2C, HLA-DRB5, TREM2, INPP5D, HLA-DRB1, and MS4A. While genes linked with endocytosis pathways are signified by BIN1, CD2AP, SORL1, and PICALM. Sequencing technologies have further aided the identification of rare disease variants, such as PLD3, TREM2, UNC5C, AKAP9, and ADAM10. Although significant improvements were have been made in the past three decades, around half of the heritability for late-onset Alzheimer's remains anonymous. Ongoing research efforts are needed to reveal the missing genetic factors contributing to the disease [11].

4. Amyloidogenic and Non-amyloidogenic Processing Pathways of APP

In the amyloidogenic pathway, BACE1 first cleaves amyloid precursor protein (APP) at the Asp1 location to produce sAPPβ and a C-terminal fragment (CTF) C99 that is 99 amino acids long membrane-bound fragment (Fig.1). Then, γ -secretase breaks down C99 to release Aβ and CTFγ. Under physiological conditions (non-amyloidogenic pathways), APP is mainly processed by α -secretase at the Aβ Leu17 site, resulting in a secreted form of APP (sAPPα) and an 83-amino acid membrane-bound C-terminal fragment (CTF) C83, which stops the production of Aβ. BACE1 primarily processes APP at the Aβ Glu11 β-secretase site to yield C89, which is cut by γ-secretase to generate a truncated Aβ11-40. BACE2 slices APP at the Aβ-Phe20 θ-secretase site to produce C80 and stops Aβ formation. APP amyloid precursor protein, BACE1 β-site APP-cleaving enzyme 1, sAPP secreted APP, CTF C-terminal fragment, Aβ amyloid-β, tAβ truncated amyloid-β, BACE2 β-site APP-cleaving enzyme 2[12],[13].

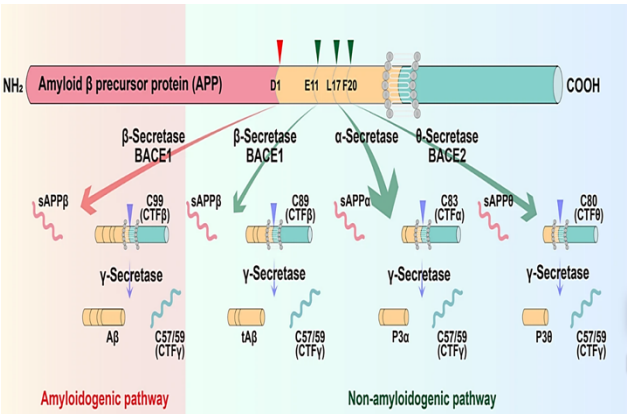

Figure 1: APP amyloidogenic and non-amyloidogenic processing pathways. Adapted from [12].

5. CRISPR-Cas9

The CRISPR-Cas9 system is a crucial component of a bacterium's immune system, providing defense against the accidental integration of mobile genetic components like plasmids and viruses [14]. It was first recognized by Ishino in 1987[15]. The ground-breaking work of Doudna and Charpentier's allowed CRISPR-Cas9 to be established in laboratories, thus its potential could be explored. The Cas9 enzyme and the single-guide RNA (sgRNA) are the major parts of CRISPR-Cas9. The sgRNA distinguishes the target DNA sequence, with the design process taking into account numerous aspects to increase specificity [16]. The Cas9 protein functions as an endonuclease and cleaves the DNA double strands like a molecular scissor. The CRISPR/Cas system is branched into two classes: Class 1 (types I, III, and IV) and Class 2 (types II, V, and VI). Only one Cas protein is used in Class2, which makes it easier and more favorite for genome editing than Class 1, which uses a variety of Cas proteins that collaborate. The type II CRISPR-Cas9 system is one of the most studied and used systems in the manufacture of pharmaceuticals within Class 2 [17]. After a double standard break by the Cas9 protein, either homology-directed repair (HDR) or non-homologous end joining (NHEJ) might be started to repair this break (Fig.2). While the NHEJ route causes insertion and deletion, premature stop codons and/or DNA frameshifts, and ultimately gene inactivation, the HDR pathway subsidizes in replacing the erroneous or mutated sequence with the proper one. The correct DNA sequences are introduced into the target place to begin HDR with the assistance of a donor DNA template. Additionally, NHEJ may happen throughout every cell cycle phase, whereas HDR is only possible during the G or S phase. Although less efficient than the NHEJ process, the HDR pathway often offers a very reliable DNA repair method [18].

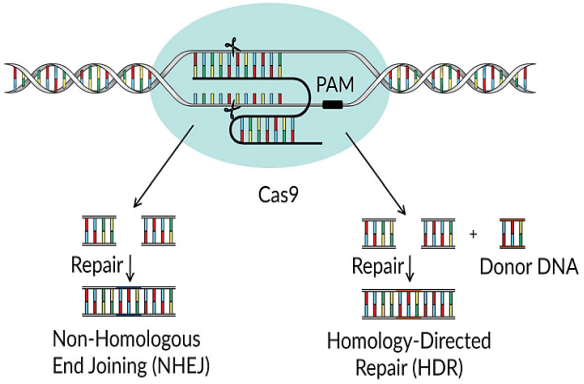

Figure 2: The Non-Homologous End Joining (NHEJ) and Homology-Directed Repair (HDR) pathways of CRISPR-Cas9. Adopted from [19].

6. CRISPR-Cas9 Delivery Methods and Alzheimer's Disease

The potential of CRISPR-Cas9 in genome editing makes it promising to fight AD. However, decoding this system into practical therapeutic applications is still a big responsibility. Many delivery methods have been tried to deliver CRISPR-Cas9 with AD. One of the most important methods of plasmid-

based CRISPR-Cas9 is a viral method. It is a typical technique in investigational models including cell lines and animals. Due to their high infectiousness, reasonable immunogenicity, and little integration into the human genome, adeno-associated viruses (AAV) are often exploited as vectors [20, 21]. (Fig.3). Two distinct AAV vectors were used to package gRNA specific to the APPsw and Cas9 that targets the KM670/671NL APP mutation that causes AD. *In vitro*, the viruses were studied in Tg2576 mouse embryonic primary neuronal cells and *in vivo* in Tg2576 animals after intrahippocampal injection. This potential therapy reduced Aβ production by around 60% in human fibroblasts. Long DNA inserts of 8–10 kb are fused by lentiviruses [22], although they do so with less success in spreading to the brain [23]. Opposing to AAV, lentiviruses may infect humans but are harder to be generate in large amounts [24].

Non-viral methods on the other hand have been confirmed to be useful for delivering CRISPR-Cas9 to specific areas, which has been linked to their higher cost efficacy, realism, effortlessness of use, and versatility. They are, therefore, incredibly well fit for use in AD [25] (Fig.3). Another approach to delivering CRISPR-Cas9 is the nanocomplexes method. In this method, the positively charged CRISPR-Cas9 peptides are joined with negatively charged nucleic acid cargo [26] (Fig.3). This approach is less immunogenic compared to viral vectors, the nanocomplexes built of the R7L10 peptide is combined with Cas9-sgRNA ribonucleoprotein that targets BACE1 selectively, Park *et al*. [27]. This method yields an effective targeting, reducing BACE1 expression without significantly off-target mutation *in vivo*. Cas9-Bace1 nanocomplex was delivered directly into the hippocampal region of 6-month-old AD mice. Interestingly, after one month of injection, a significant reduction in BACE1 levels and β-cleavage products with APP was detected in the AD brain [13]. Intracerebroventricular and intrathecal administration were favorable over nanocomplex delivery due to the drawbacks accompanied by delivering nanocomplex in addition to the complications caused by the reticuloendothelial system (RES) (Figure3) [28].

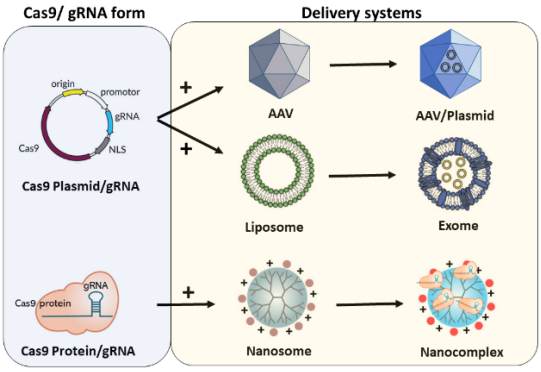

Figure 3: CRISPR-Cas9 Delivery Methods in Alzheimer's Disease. Some parts were Adopted and modified from [29] [19] [30]

7. Alzheimer's Genes and CRISPR-Cas9 Treatment

According to the recent studies, CRISPR-Cas9 can be employed to treat AD. For instance, György *et al*., 2020 showed that disrupting the APP mutant gene in an animal model of AD that expresses mutant human APP using CRISPR-Cas9 is enough to reduce AD-like pathology in Tg2576 mice [24]. Recently, it was established that this strategy is valid [31]. The disruption of the mutant, disease-causing allele, however, is only used as a therapeutic strategy in familial AD cases because these alleles are absent in sporadic cases. The endogenous APP gene's C-terminus has been targeted by the CRISPR-Cas9 system to cut down on Aβ production and get around this potential restriction. The authors (J. Sun *et al.*2019*)* specifically aimed to up-regulate the neuroprotective APP α-cleavage and shift APP processing away from the amyloidogenic β-cleavage. They demonstrated that their method was effective at reducing Aβ production with few off-site side effects both in vitro and in vivo [32].

In addition to β-amyloid, Tau is crucial for the pathogenesis of numerous other neurodegenerative diseases including AD [33],[34]. According to the "β-amyloid hypothesis," tau is associated with hyperphosphorylation and β-amyloid NFTs cause AD. [33],[34]. Clinical trial treatments aim to slow down the production of these proteins with the implicit presumption that these structural variations are the sole (or dominant) indicators of the causative event(s). Targeting tau and Aβ at the same time is essential for the effective treatment of AD. Numerous attempts have been made to get rid of the toxic tau forms, either using anti-tau antibodies or by utilizing gene silencing. The creation of a new tau knockout strain (tau1ex1) utilizing CRISPR-Cas9-mediated genome editing of the Mapt gene's intron1/exon1 in C57Bl/6J mice was described most recently by Tan et al. 2018[35]. A pair of guide RNAs that were 115 bp upstream and 18 bp downstream of the transcriptional start codon were utilized to introduce a modest deletion at the intron-1/exon1 junction of the MAPT gene using CRISPR-Cas9 genome editing [36]. Researchers in this study injected two guide RNAs of A673T and the CRISPR-Cas9 protein directly into the cytoplasm of the oocyte, which is previously fertilized [37], [38]. Targeting the ES cell gene yields the Tau-/- group, which requires considerable selective marker cassettes inserted into the MAPT coding region [39]. Using this strategy of selectable markers might have disadvantages by producing unwanted and unpredictable results.

These mice show susceptibility limitation to excitonic convulsions and normal memory

formation despite lacking obvious phenotypic features.

G-protein coupled receptors (CysLT1R and CysLT2R) are the two main receptors by which inflammatory signaling cascades of cysteinyl leukotrienes start. According to the research [40], CysLT1R contributes to the emergence of AD. CysLT1R expression is brought on by elevated levels of Aβ1- 42. The levels of β-amyloid and neuroinflammation decrease when CysLT1R is deleted by using CRISPRCas9 [41, 42]. Further, the progression of AD can be limited by melatonin and melatonin receptors as well [43]. The toxic effects of β-amyloids that are known to contribute to the development of AD can be reduced by melatonin, through protecting neural cells.

Scientists investigated the potential therapeutic effects of activating the Melatonin or Mt1 gene gland using CRISPR-Cas9 technology to protect neurons from degeneration in AD [44]. These findings provide valuable insights into the potential therapeutic benefits of targeting melatonin receptors and the Mt1 gene in the treatment of AD [45]. Another study used CRISPR-Cas9 to downregulate the level of Thioredoxin-interacting protein (Txnip) in HT22 murine cells.

Since β-amyloid-induced protein cysteine oxidation modification is decreased, this downregulation could be a potential target for AD [46]. CRISPR-Cas9 was employed in the SH-SY5Y neuroblastoma cell line to prevent the expression of STIM1, the gene that is related to AD and other neurological diseases. Using this technique, scientists were able to further show that STIM1 deficiency causes cell death because it alters how calcium ions are transported via neurons' plasma membranes [33].

Mutations in the presenilin 2 (PSEN2) gene is connected with Early-onset familial Alzheimer's disease. In a study conducted by Ortiz-Virumbrales et. al, 2017, PSEN2 mutation in Induced pluripotent stem cells (iPSCs)-derived neurons was fixed using CRISPR. The basal forebrain cholinergic neurons (BFCNs) are one of the earliest cell types to be impacted by AD. The scientists used cell lines from PSEN2 mutation carriers/controls to create human BFCNs from iPSCs [47],[48]. The carriers also showed an electrophysiological impairment in addition to an increase in Aβ in BFCNs. The scientists were able to demonstrate normalization of the cells' electrophysiological activity and Aβ secretion after using CRISPR-Cas9 to fix the PSEN2 point mutation [49].

The other study used iPSCs-produced neurons from two distinct individuals with APOE4/E3 genotypes. When compared to neurons produced from control patients' iPSCs, APOE4/E3 neurons in culture showed enhanced APP processing, phosphorylation, and sensitivity to

calcium dysregulation. Isogenic neuronal cells expressing APOE3/E3 were created using CRISPR-Cas9 editing. Isogenic E4/E3 and E3/E3 neurons had equal differentiation and survival rates, as well as comparable soma density and average neurite length per cell, however, E3/E3 neurons had a greater number of neurite branch points per cell. E3/E3 neurons were more resistant to cytotoxins and had lower phosphorylation levels, but they did not process amyloid. These results designate the function of the APOE 4 allele in the development of AD in addition to a potential mechanism to protect neurons [50]. According to these findings CRISPR-Cas9 system holds great promise in developing innovative therapeutic strategies for neurological diseases, as well as promoting the general understanding of the intricate mechanisms underlying neuronal differentiation and function.

Conclusions

Alzheimer's disease is a complicated and devastating neurodegenerative disease that impacts millions of individuals' lives worldwide with no medication to prevent its progression yet. The advancement of molecular genetics has revealed several genes responsible for both familial and sporadic Alzheimer's disease. As a result, a greater knowledge of the genetic complexity of AD and the explanation of possible molecular pathways of neurodegeneration in AD were uncovered. The CRISPR-Cas9 gene editing system has been showing hopeful results that open new avenues for researchers to determine the potential contribution of certain genes that are associated to the disease and provide insight into the disease mechanisms. That could lead to the development of effective prevention and therapeutic strategies for AD.

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