DOI: 10.30651/jqm.v8i01.16054

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Literature Review

Tandem peptide lipid CRISPR-Cas9 complex combating APP and APOE4 gene abnormality in Alzheimer's disease

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ARTICLE INFO

Submitted : *29st November 2023*
Accepted : *16th May 2022*
Published : *25th January 2024*

Keywords:

Alzheimer, APOE4, APP, CRISPR, tandem peptide-lipid

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ABSTRACT

Alzheimer's attacks 24 million global population and dominates 60-80% of existing cases of dementia. It causes the accumulation of beta-amyloid (Aβ) plaques in the hippocampus and entorhinal cortex, resulting in decreased mass from the brain. Recent studies have shown that the manifestation of this disease is due to an overaccumulation of abnormal Aβ protein due to abnormalities in the APP and APOE4 genes. Point mutations in the APP gene will create the toxic form of Aβ protein, namely Aβ42, and the toxic APOE4 gene will accelerate the onset of Aβ42 deposition and pro-inflammatory activity that exacerbates the degenerative process of the brain. Gene editing as a potential definitive therapy was recently a concern by researchers. CRISPR-Cas9 repairs the APP gene and substitutes the APOE4 gene with APOE3 by modifying the gene's DNA sequence. Nano complex CRISPR-Cas9 tandem peptide lipid is a model for clinicians to target brain nerve cells. In vivo research on an Alzheimer's mouse model proved the potential of nano-complex-based peptides as carriers of CRISPR-Cas9 in brain nerve cells. This engineering technology offers satisfactory results with high precision, minimal side effects, and a relatively low price for long-term therapeutic effects and even a lifetime.

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INTRODUCTION

Aging is a physiological process that is inevitably experienced by all living organisms, especially humans. According to the 2019 World Population Prospects, the number of older people (age > 65 years) worldwide is expected to reach around 1.5 billion people (16%) by 2050 (United Nations, 2019). In other words, one in six people worldwide is expected to be 65 years or older. Increasing age is followed by an increased risk of geriatric-related diseases, including neurodegenerative diseases (Hou et al., 2019). The brain is one of the organs most vulnerable to aging, so it can manifest in various conditions, including Alzheimer's disease (Hou et al., 2019). Alzheimer's disease has become one of the most common neurodegenerative diseases in the world, surpassing Parkinson's, amyotrophic lateral sclerosis (ALS), and Huntington's disease (Erkkinen et al., 2018). This condition is estimated to have affected around 24 million global population and dominates 60-80% of existing cases of dementia (Sosa-Ortiz et al., 2012). This big problem needs to get more attention, considering that this neurodegenerative disease greatly affects the quality of life.

Alzheimer's is a chronic neurodegenerative disease that impairs short-term memory and causes behavioral changes (De-Paula et al., 2012; Dubois et al., 2016). Generally, this condition affects older people over 65 and is characterized by the main symptoms of dementia, often referred to as Alzheimer's dementia (Erkkinen et al., 2018). Studies say that the onset of Alzheimer's is influenced by external and internal factors (Breijyeh & Karaman, 2020). Early and repeated exposure to these factors can cause early-onset Alzheimer's disease (EOAD) at the age of ≤ 65 years (Giau, Senanarong, et al., 2019). Recent studies suggest that neuritic plaques and neurofibrillary tangles cause Alzheimer's disease, excess accumulation of amyloid beta plaques, and cytoskeletal changes due to hyperphosphorylation of microtubule-associated Tau protein (De-Paula et al., 2012). On the other hand, researchers believe that the chance for this disease to be inherited is quite large. Hence, hereditary factors and genetic mutations play a role in the pathogenesis of this disease (Breijyeh & Karaman, 2020).

Most Alzheimer's is caused by the accumulation of beta-amyloid (Aβ) plaques in the hippocampus and entorhinal cortex, resulting in a decrease in mass from the brain (D'Argenio & Sarnataro, 2020; Villegas-Llerena et al., 2016). Based on recent studies, increased Aβ accumulation is affected by mutations in the APP and APOE4 genes. The use of AChEI and NMDA drugs based on a Meta-Analysis study of 80 trial studies showed moderate efficacy with an average effect size of 1.08-1.10 points on cognitive examinations using the Mini-Mental State Examination (MMSE) at 3 to 12 months of treatment. These results indicate that giving AChEI for six months only provides cognitive improvement for the next three months and does not have a significant clinical effect (Knight et al., 2018). This approach causes patients to take long-term medication, even for life, and massive costs. The Indonesian government spends around USD 604 billion on this disease and more due to social care needs.

In contrast, healthcare costs are relatively low due to the low diagnosis and limited treatment options. Besides that, Alzheimer's affects the social and mental conditions of sufferers and caregivers because of the burnout phenomenon experienced by caregivers. This fact indicates that dementia is a severe global problem that is difficult to identify at the outset, and there is a lack of renewable alternative therapies available (Korczyn, 2012).

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The potential for treatment through genetic engineering is becoming a concern, especially for treating degenerative diseases. One of them is the development of Clustered Regularly Interspaced Short Palindromic Repeats-Associate 9 (CRISPR-Cas9) as genetic engineering with high efficacy, affordable cost, and minimal side effects (D. Wang et al., 2020). CRISPR-Cas9 targets explicitly the mutant APP and APOE4 genes that produce abnormal Aβ plaque accumulation in the brain and cause progressive brain tissue damage. Clinically, the patient experienced a decline in cognitive, emotional, and psychosocial abilities. CRISPR-Cas9 repairs the APP gene and substitutes the APOE4 gene with APOE3 by modifying the gene's DNA sequence. This repairment and substitution suppresses the production of abnormal Aβ plaques and stops brain damage so that no clinical symptoms appear. This intervention is permanent because it targets the root of the problem, namely mutations of the APP and APOE4 genes, with precision and minimal side effects so that the duration of treatment can be shortened, minimize complications, and the costs required for the entire treatment (György et al., 2018; Lin et al., 2018). We are seeing the tremendous potential of CRISPR-Cas9 as an Alzheimer's therapy solution. Therefore, it is necessary to develop a specific CRISPR-Cas9 therapy targeting the APP and APOE4 mutant genes in Alzheimer's disease.

LITERATURE REVIEW

Alzheimer's disease

Alzheimer's dementia is a neurodegenerative disease caused by the abnormal accumulation of beta-amyloid (Aβ) plaques in the hippocampus and entorhinal cortex, which causes decreased memory, thinking, and emotional and behavioral control (Gale et al., 2018). The Aβ protein plays a vital role in the neuronal center and protects neurons from nerve damage (Giau et al., 2019). However, recent studies have shown that an overaccumulation of abnormal Aβ protein due to abnormalities in the APP and APOE4 genes is positively correlated with the manifestations of Alzheimer's disease (Park et al., 2019). Point mutations in the APP gene will cause errors in the arrangement of the Aβ protein to a more toxic form, namely Aβ42 (Tcw & Goate, 2017). On the other hand, the toxic APOE4 gene will accelerate the onset of Aβ42 deposition and pro-inflammatory activity that exacerbates the degenerative process of the brain (Zhao et al., 2018). In other words, the attenuation of abnormal activity of the APP and APOE4 mutant genes could be an alternative for advanced Alzheimer's dementia treatment. An illustration of the difference between a normal human brain and a person with Alzheimer's dementia can be seen in Figure 1.

Researchers have revealed that Alzheimer's disease is associated with multigene mutations and dysfunctions that control beta-amyloid formation. Two dominant genes have been identified to increase the risk of this disease, namely the amyloid-β precursor protein (APP) gene and apolipoprotein E4 (APOE4) (Swarup et al., 2019; Giau et al., 2019; Zhao et al., 2018). Under normal circumstances, the APP gene plays an essential role in producing the Aβ protein, which functions to protect neurons from nerve damage and is required for the continuity of the work of neuron centers (Zhang et al., 2011; Hu et al., 2017; Giau et al., 2019; Zhang et al., 2012). However, point mutations, both autosomal dominant and recessive in this gene, cause errors in the arrangement of the APP protein and cause an increase in the amount of Aβ protein and even a more toxic form, namely Aβ42 (Tcw & Goate, 2017; Gulisano et al., 2018). In addition, researchers also revealed that mutations in this gene are responsible for 10-15% of EOAD events (Giau et al., 2019).

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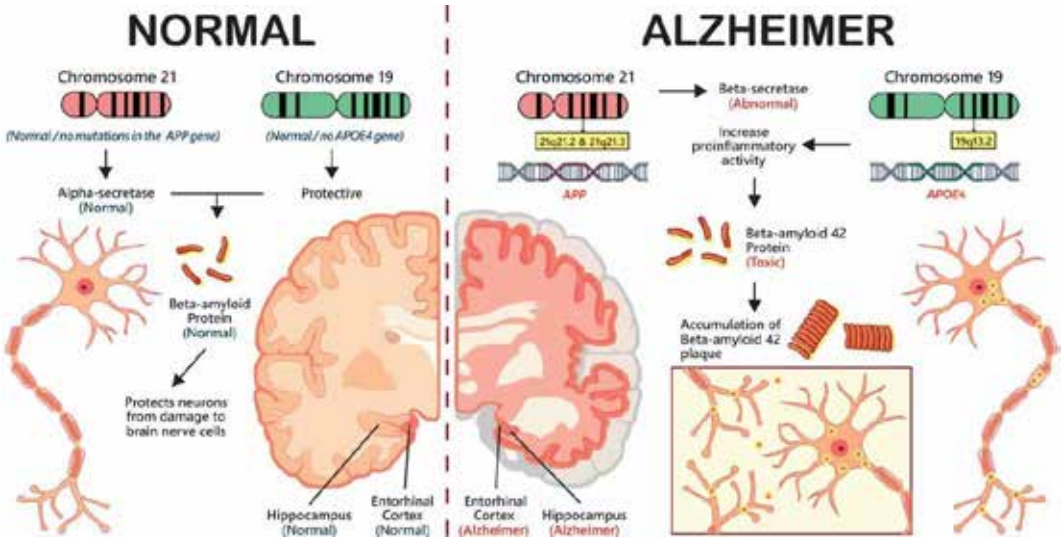

Figure 1. The difference between the normal human brain and Alzheimer's dementia (Source: Original picture).

In general, gene mutations can be caused by genetic inheritance or environmental factors (Wang et al., 2021). Alzheimer's disease is more dominantly caused by inherited genetic factors than mutations caused by environmental factors. However, these two factors are interconnected so that they can accelerate and aggravate the manifestations of Alzheimer's (Wang et al., 2021). Toxicants such as heavy metals, detergents, pesticides, and industrial waste products are known to cause damage to the neurological system. Exposure to heavy metals such as manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn) can induce oxidative stress in neurons and conformational changes of Aβ, causing neurodegenerative processes (Wang et al., 2021). Oxidative stress and nucleotide damage are known to increase in Alzheimer's disease but are always counterbalanced by the DNA repair process (Wang et al., 2021). If this condition persists, it will permanently impact genome structure or transcription (Wang et al., 2021). Mutation variants in the APP gene can be divided into two based on the inheritance characteristics: autosomal-recessive mutation and autosomal-dominant mutation (Tcw & Goate, 2017). In autosomal-recessive mutation, there is an amino acid substitution of alanine to valine at codon 673. In addition, four types of autosomal-dominant mutations are known to occur in the APP gene: APP duplication (entire sequence of APP gene), N-terminal APP missense mutation (1 point mutation), Aβ sequence APP missense mutation (11 point mutations), and C-terminal APP missense mutation (14 point mutations) (Tcw & Goate, 2017).

Typically, the APOE gene plays a role in manufacturing proteins that help transport cholesterol and fats in the bloodstream (lipid homeostasis) and synapse formation (Di Battista et al., 2016). Abnormalities in these processes increase the risk of Alzheimer's disease. The APOE gene consists of 299 amino acid proteins and has three isoforms, APOE2, APOE3, and APOE4 alleles, which differ in the amino acid sequence at positions 112 and 158 (Di Battista et al., 2016). Each person is known to inherit 1 APOE allele from each parent, resulting in 6 possible combinations 2/2, 2/3, 2/4, 3/3, 3/4, and 4/4 (Sienski et al., 2021). The APOE4

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gene is the only toxic APOE allele compared to the protective other alleles (APOE2 and APOE3) (Zalocusky et al., 2019). About 15% of people are estimated to inherit this allele, with 2% to 3% carrying two copies (Sienski et al., 2021). Studies state that individuals who inherit just one copy of the APOE4 gene are 2–3 times more likely to suffer from late-onset Alzheimer's disease (LOAD) (Zalocusky et al., 2019). Two copies of this allele increase the risk up to 12 times because it accelerates the onset of Aβ42 deposition and increases intense pro-inflammatory activity that exacerbates neurodegenerative disease (Figure 1) (Zhao et al., 2018; Yamazaki et al., 2019).

Attenuating abnormalities in the APP and APOE4 genes could become a new approach to Alzheimer's treatment. Utilizing Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) genetic engineering as gene therapy can potentially reduce abnormalities and correct mutations in the APP and APOE4 genes that cause Alzheimer's disease. Gene therapy is a technology that allows researchers to edit the genome as needed and desired so it can potentially treat diseases associated with genetic mutations, such as Alzheimer's disease (Meiliana et al., 2017). This literature review aims to determine the potential and challenges of CRISPR as a gene therapy for Alzheimer's disease.

Genetic Engineering CRISPR-Cas9 as a Gene Therapy for Alzheimer's Disease

CRISPR gene therapy is a defense mechanism possessed by prokaryotic bacteria against harmful genetic material, such as viruses and plasmids, by creating single-guide RNA (sgRNA) that matches the genetic material, then deactivating it (Meiliana et al., 2017; Hanafy et al., 2020). Utilization of CRISPR isolated from bacteria is used to indicate the location of mutations in the APP (21q21.2 & 21q21.3) and APOE4 (19q13.2) genes (Ortiz et al., 2015; Irfannuddin, 2018). Meanwhile, CRISPR-associated 9 (Cas9) plays an active role in cutting DNA bases and the genes that CRISPR wants (Hanafy et al., 2020; Meiliana et al., 2017). This system activates the CRISPR-Cas9 nuclease to induce a double-stranded break (DSB) (Giau et al., 2018). The base pairs that have been cut will be repaired through a nonhomologous end-joining (NHEJ) mechanism, which includes insertions, non-specific deletions, and other mutations (InDels). In addition, DSB can also be repaired through a homology-directed repair (HDR) mechanism that uses a DNA template that has been repaired or knocked from a specific mutation (Giau et al., 2018). The CRISPR-Cas9 complex repairs the point mutation from the APP gene in chromosome 21. Meanwhile, the APOE4 gene can be inactivated by this complex by converting toxic APOE4 to nontoxic APOE3 by replacing one nucleotide position 112 (arginine in APOE4 to cysteine in APOE3) (Ortiz et al., 2015; Irfannuddin, 2018; Safieh et al., 2019; Mamun et al., 2020). The mechanism of action of CRISPR-Cas9 as a genetic therapy for Alzheimer's dementia can be seen in Figure 2.

In vitro studies of fibroblast cells isolated from Alzheimer's patients with the APP mutant gene demonstrated the extraordinary potential of the CRISPR-Cas9 complex. The fibroblast cells were injected with gRNAs and Cas9-2A-GFP complex isolated from *Streptococcus pyogenes* and then cultured. This study examined extracellular levels of Aβ40 and Aβ42 using an enzyme-linked immunosorbent assay (ELISA). As a result, knocking out the APP gene via CRISPR-Cas9 reduced Aβ40 levels by 60% and Aβ42 by 50% (György et al., 2018). This evidence is consistent with a study conducted in Japan, which found that a 70% deletion of the APP 3'-UTR segment indicated a significant reduction in Aβ accumulation (Nagata et al., 2018). On the other hand,

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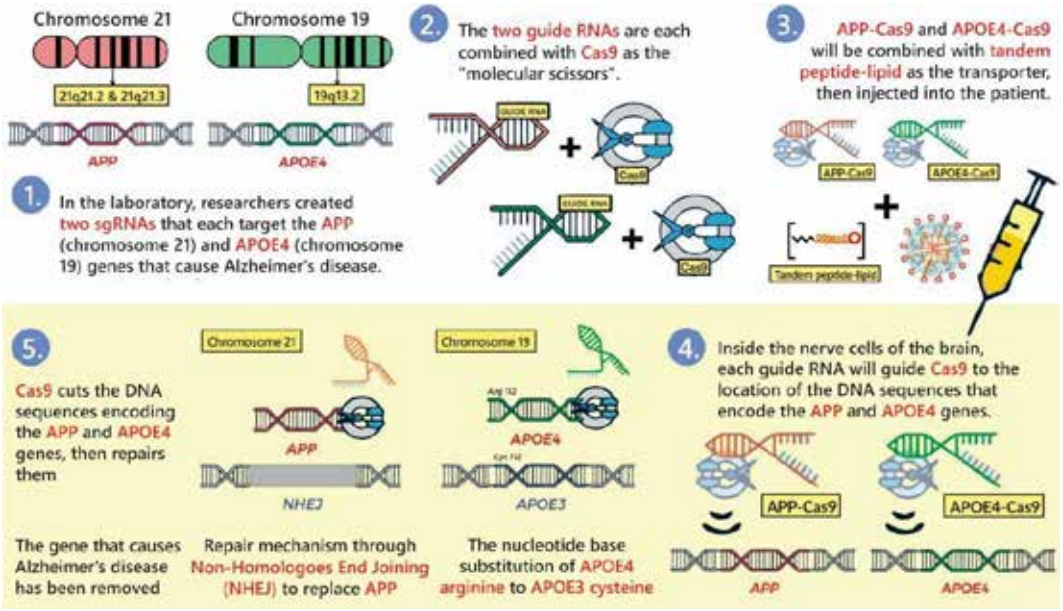

Figure 2. CRISPR-Cas9 treats Alzheimer's dementia through APP and APOE4 gene repair (Source: Original picture).

although the alleles of the APOE4 and APOE3 genes differ only by one nucleotide base, the APOE4 gene can be easily converted into APOE3 using CRISPR-Cas9 selectively. This experiment was conducted on HEK293T cells and APOE4-containing mouse astrocyte cells. The results show that this base editing system produces permanent corrections of 15–75% of DNA with <1% InDels when tested on target cells (Komor et al., 2016). This potential is supported by other studies that compare Aβ42 levels, which are 20% higher in APOE4 cultures than APOE3 so that modification of APOE4 to APOE3 in brain cells can attenuate several pathologies related to Alzheimer's dementia (Lin et al., 2018).

Tandem peptide-lipid as CRISPR-Cas9 Transporters

The selection of a "systemic vehicle" for CRISPR-Cas9 needs special attention in implementing this idea. The use of non-viral transporters such as nano complex-based peptides was chosen because the positive charge of this peptide easily forms a complex with a negative charge on Cas9-sgRNA (Meiliana et al., 2017; Hanafy et al., 2020). This transporter has more potential than viral vectors such as adeno-associated virus (AAV) and lentivirus because of its safe use, better cost-adequacy, and size flexibility for packaging the CRISPR-Cas9 complex itself (Barman et al., 2020). An illustration of the construction and mechanism of action of the Cas9-sgRNA nano complex can be seen in Figure 3 (Jain et al., 2019).

In vivo research on an Alzheimer's mouse model proved the potential of nano-complex-based peptides as carriers of CRISPR-Cas9 in brain nerve cells (Park et al., 2019). In this study, the R7L10 amphiphilic peptide was combined with the Cas9-sgRNA complex to form a nano complex system, which was then aimed at suppressing the activity of the BACE1 gene, the cause of excess Aβ protein accumulation and definitive cognitive function in experimental animals. As a result, the nano complex system

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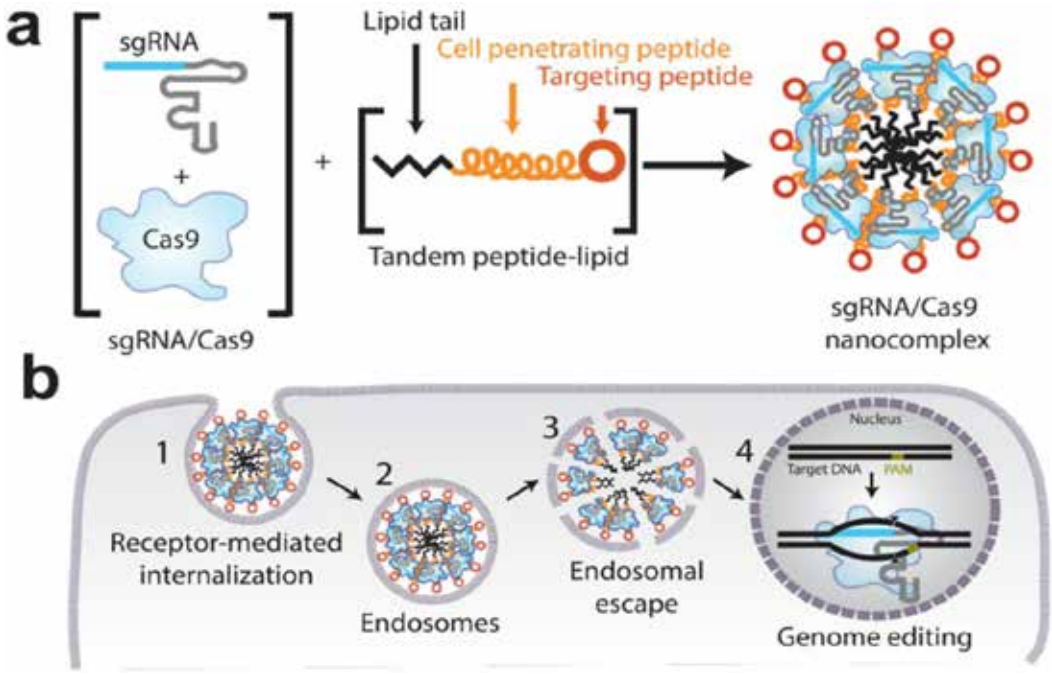

Figure 3. a) Construction of sgRNA/Cas9 and tandem peptide-lipid into sgRNA/Cas9 nano-complex, b) mechanism of action and fusion of Cas9-sgRNA nano-complex in target cells (Source: Jain et al., 2019)

successfully targets the BACE1 gene without significant off-target mutation rates (Park et al., 2019). However, due to its size, this transporter is challenging to penetrate the blood barrier. However, this can be anticipated through intrathecal or intracerebroventricular injections (Barman et al., 2020).

Opportunity and Challenges in CRISPR-Cas9 as Gene Therapy

CRISPR-Cas9 genetic engineering technology has potential that is no less promising than using drugs that only temporarily relieve symptoms of Alzheimer's dementia. If the APP and APOE4 gene abnormalities can be corrected, the accumulation of beta-amyloid plaques can be suppressed (Meiliana et al., 2017; Hanafy et al., 2020). Other genetic therapies, such as zinc finger nucleases (ZFNs) and transcription activation like-effector nucleases (TALENs), have weaknesses that lie in the level of difficulty and the length of time required for the construction of DNA-binding proteins. The use of sgRNAs is also considered technically and cost-effectively more efficient than these ZFNs and TALENs (Stepanichev, 2020). In addition, CRISPR-Cas9 tends to be more easily packaged in transporter vectors and offers high precision with lower cytotoxicity (Ortiz et al., 2015). This technology is also much better than RNA interference (RNAi), which only reduces gene expression at the messenger RNA (mRNA) level (knockdown). At the same time, CRISPR-Cas9 completely and permanently silences genes at the DNA level (knockout) (Boettcher & McManus, 2015).

Unfortunately, clinical research on CRISPR in Alzheimer's is still limited to animal studies, with the experimental animals that are often used are mostly rodents (Lu et al., 2021). Rodent models cannot fully describe or resemble conditions in the human brain, considering that Alzheimer's is strongly

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influenced by age, while rodents have a short life span (Lu et al., 2021). Further research using primate subjects can be considered to adjust the age-dependent characteristics of Alzheimer's disease. However, the possibility of off-target cleavages by CRISPR-Cas9 can still occur when CRISPR induces cutting and repair at unintended genomic locations (Liu et al., 2021). This process is due to the characteristics of gRNA that can still tolerate mismatches, thus reducing the precision of CRISPR. However, it can still be anticipated by the specialized algorithm of gRNA (Liu et al., 2021). In addition, CRISPR-Cas9 still has some challenges in terms of delivering method and immunogenicity, so it requires more clinical research so that the application of CRISPR-Cas9 in Alzheimer's disease can be realized in humans (Liu et al., 2021).

CONCLUSION

The use of CRISPR-Cas9 genetic engineering in suppressing abnormalities in the APP and APOE4 genes has the potential to be a renewable step in the treatment of Alzheimer's dementia in the future. This engineering technology offers satisfactory results with high precision, minimal side effects, and a relatively low price for long-term therapeutic effects and even a lifetime. The CRISPR-Cas9 complex can one day replace AChEI and NMDA drugs, which only relieve symptoms of dementia but do not treat the direct source of the disease, namely the abnormalities of the APP and APOE4 mutant genes. In addition, nano complex-based peptide transporters are considered better and safer than viral vectors, making them the best choice for CRISPR-Cas9 mobilization.

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