Review

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Role and application of CRISPR-Cas9 in the management of Alzheimer's disease

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Abstract

Alzheimer's disease (AD) is a serious health issue that has a significant social and economic impact worldwide. One of the key aetiological signs of the disease is a gradual reduction in cognitive function and irreversible neuronal death. According to a 2019 global report, more than 5.8 million people in the United States (USA) alone have received an AD diagnosis, with 45% of those people falling into the 75–84 years age range. According to the predictions, there will be 15 million affected people in the USA by 2050 due to the disease's steadily rising patient population. Cognitive function and memory formation steadily decline as a result of an irreversible neuron loss in AD, a chronic neurodegenerative illness. Amyloid-beta and phosphorylated Tau are produced and accumulate in large amounts, and glial cells are overactive. Additionally, weakened neurotrophin signalling and decreased synapse function are crucial aspects of AD. Memory loss, apathy, depression, and irritability are among the primary symptoms. The aetiology, pathophysiology, and causes of both cognitive decline and synaptic dysfunction are poorly understood despite extensive investigation. CRISPR/Cas9 is a promising gene-editing technique since it can fix certain gene sequences and has a lot of potential for treating AD and other human disorders. Regardless of hereditary considerations, an altered Aβ metabolism is frequently seen in familial and sporadic AD. Therefore, since mutations in the PSEN-1, PSEN-2 and APP genes are a contributing factor to familial AD, CRISPR/Cas9 technology could address excessive Aβ production or mutations in these genes. Overall, the potential of CRISPR-Cas9 technology outweighs it as currently the greatest gene-editing tool available for researching neurodegenerative diseases like AD.

Keywords: Alzheimer's disease, CRISPR-Cas9, gene editing

Introduction

Alzheimer's disease (AD) is a serious health issue that has a significant social and economic impact worldwide. The key aetiological signs of the disease are a gradual reduction in cognitive function and irreversible neuronal death[1,2]. Cognitive function and memory formation steadily decline as a result of an irreversible neuron loss in AD, a chronic neurodegenerative illness. Amyloid-beta 42 (A-42) and phosphorylated Tau are produced and accumulate in large amounts, and glial cells are overactive[3]. Additionally, weakened neurotrophins signalling and decreased synapse function are crucial aspects of AD. Memory loss, apathy, depression, and irritability are among the primary symptoms. The aetiology, pathophysiology, and causes of both cognitive decline and synaptic dysfunction are poorly

HIGHLIGHTS

- Alzheimer's disease (AD) is a serious health issue that has a significant social and economic impact worldwide.
- Cognitive function and memory formation steadily decline as a result of an irreversible neuron loss in AD.
- CRISPR/Cas9 is a promising gene-editing technique since it can fix certain gene sequences and has a lot of potential for treating AD.
- Mutations in the PSEN-1, PSEN-2 and APP genes are a contributing factor to familial AD, CRISPR/Cas9 technology could address excessive Aβ production or mutations in these genes.
- The potential of CRISPR-Cas9 technology outweighs it as currently the greatest gene-editing tool available for researching neurodegenerative diseases like AD.

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understood despite extensive investigation[4,5]. According to a 2019 global report, more than 5.8 million people in the United States (USA) alone have received an AD diagnosis, with 45% of those people falling into the 75–84 years age range. According to the predictions, there will be 15 million affected people in the USA by 2050 due to the disease's steadily rising patient population[6]. With the help of the recently developed and groundbreaking genome-editing tool—CRISPR/Cas9, diseases with few or no effective therapy choices can now be treated. Several studies have outlined a crucial role of CRISPR/Cas9 as a part of a bacterial immune mechanism, catering defense in opposition to the inadvertent integration of mobile genetic components like plasmids and viruses[7]. Recent in-depth research on CRISPR/Cas9 has greatly increased effectiveness of editing and reduced off-target effects despite being widely used for basic and translational research[8].

Overview of CRISPR-Cas9

The technique known as "CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats, CRISPR associated nuclease, or CRISPR/Cas)" is advancing quickly and has some siginificant promise for use in both basic sciences and in the management of diseases[9]. "CRISPR/Cas9" system usually is a modifiable defense strategy for the bacteria and archaea, through which the organisms recognize and neutralize the conquering bacteriophages and other remote nucleic acids[10]. The "CRISPR gene-editing" technology mostly refers to as the "Type II CRISPR/Cas9", which is regularly used. Cas9 protein along with the sgRNA (single guide RNA—sgRNA) make up the CRISPR/Cas9 system, where the Cas9 may cleave the double strands of DNA (ds-DNA) while the sgRNA directs the system towards the target[11]. The sgRNA is necessary for accurate gene editing because of its 3′ duplex structure enabling an interaction with Cas9 proteins and its 20-nucleotide 5′-terminal sequence, which interacts with a target region in the DNA of host using "Watson and Crick" base pairing principles[12]. With the advancement of the gene-editing techniques, the CRISPR/Cas9 has been extensively used in gene engineering for things like gene therapy, knocking genes into mammalian cells and human disease modelling. The potential of the CRISPR/Cas9 system could be significantly raised using nanoparticles that are lipid-encapsulated as a multipurpose carrier, providing a much more successful strategy for tumours[13]. The CRISPR/Cas9 technology has advanced significantly in the past two decades. The CRISPR/Cas9 approach, which uses a manmade virus has numerous benefits over other lipofectamine and superinfect techniques such as, safety, higher efficiency, and minimal side effects. CRISPR/Cas9 technology is becoming more sophisticated and has the capacity to serve as a beneficial tool for clinical gene editing in various diseases[14].

Delivery method of CRISPR/Cas9 in the AD

The constituents of CRISPR must be supplied into the mammalian cells in order to permit the gene modification in the host cell for therapeutic application. Even though CRISPR/Cas9 treatment *in vivo* has advanced, issues with safety, tissue selectivity and biocompatibility still exist[15]. Various CRISPR/Cas9 components are provided depending on the genetic modification sought. The Cas9/sgRNA pair is enough for the most basic application, to disrupt genes (such as knockout), but for progressive bio-functions like insertion (knock-in) or gene repair, an additional piece of DNA must be sent[16]. There are several ways to instigate the CRISPR/Cas9 elements into cells, inclusive of mRNA, viral, protein-based and plasmid methods. The most popular means for dispersing CRISPR/Cas9 are viral ones. In recent times, the use of microvesicles for the therapeutic delivery of CRISPR/Cas9 has come to light. Microvesicles are the extracellular vesicular structures with a diameter of 100–1000 nm created and released into the media by means of the budding of cell membranes[17]. A 'producer' cell line is typically transfected with Cas9 protein, sgRNA, and a microvesicle-inducing protein. The cells secrete Cas9-sgRNA complex-containing microvesicles, which are then shed into the medium, purified, and reused to deliver their gene-editing payload to the desired cells[18]. Currently, CRISPR/Cas9 has been delivered using a variety of virus delivery methods, such as adenoviral vectors, adeno-associated viruses, and the lentiviral vectors. Given the benefits of each, studies involving the delivery of CRISPR/Cas9 therapies or siRNA, to the brain to treat AD used either local or intravascular modes of administration. Even though oral delivery is quick, noninvasive, and easy for patients it is exceedingly difficult because of the many obstacles the delivery system must overcome to get its gene-editing cargo into the blood[19]. On the other hand, the intranasal approach has drawn considerable attention since it is thought to make it possible to circumvent the blood-brain barrier swiftly and painlessly. This suggests nose-to-brain delivery as a potential means of advancing CRISPR/Cas9 therapies for AD into clinical investigation. Alternative methods, such as intraperitoneal and subcutaneous injections, may be used, but given their pharmacokinetics limitations, they would likely only be used as a last resort. Before creating the formula, it is crucial to contemplate the extensive size of CRISPR/Cas9. Because of its less size, the Cas9/sgRNA complex is picked over plasmid-assisted delivery strategies. Additionally, circulating nucleases and proteases can degrade each component of these formulations[8]. PEGylation (polyethylene glycol) simultaneously reduces cellular absorption and may produce specific PEG-antibodies, leading to immunogenic reactions, even though it is frequently utilized to decrease the recognition of these systems by the reticulo endothelial system (RES). The main barrier preventing real-world translatability is optimizing the various formulation elements since non-viral vectors are typically favored over viral ones for in-vivo applications[16].

CRISPR-Cas9 in the management of AD

The great majority of cases of AD are sporadic, and only a tiny proportion are caused by autosomal mutations that are dominant in one of three genes, the presenilin (PS) 1 or 2 or the "amyloid precursor protein (APP)"[20]. From the past few years, many animal models for AD were created through overexpression of human genes that were altered to produce tau and Aβ proteins. All these models have shown to be quite effective for comprehending various elements of pathogenesis of AD, but their applicability is constrained as most AD mice models do not genuinely experience neurodegeneration[21]. Newer models of AD have been developed recently by utilizing CRISPR-Cas9 technology to understand pathophysiology, better representation of the disease phenotype, exploring the harmful genes, and eventually identifying a satisfactory treatment for the pernicious condition like AD[22,23]. CRISPR-Cas9 was employed by Serenels

and colleagues and his team to create fresh animal models of AD, where they have specifically introduced three distinct point mutations namely "F681Y, G676R and R684H", into the endogenous rat and mouse APP gene to change it into the human version. They produced humanized animal models as a result, which will be helpful for researching APP processing and revealing novel disease pathogenesis processes[24]. To learn more about how this protein is involved in AD, tau knockout mice are helpful. Tan and colleagues employed CRISPR-Cas9 to create a brief deletion in the tau-encoding Mapt gene's exon 1's transcriptional start codon. This novel animal model, developed from a pure background of C57Bl/6J, is immune to excitotoxicity and exhibits no memory impairment. Although most of the existing APP gene mutations are the causal factor for the disease, few of these variants or mutations are protective against Alzheimer Disease[20,21]. In line with this, Nagata K and colleagues discovered protective deletions in the APP 3′-UTR. The pathology associated with Aβ was dramatically reduced when such deletions were incorporated utilizing the CRISPR-Cas9 within the APP of an AD animal model—APP-KI mice, that is caused by three human mutations in the APP gene of mouse. Furthermore, CRISPR-Cas9 has been utilized to determine the significance of recognized risk factors of AD in the pathophysiology of the disease[25]. Plc2 is an expressed gene in the microglia that has been linked to AD and there are various variants of the Plc2 gene, some of which are connected to a higher risk of advancing AD and others of which are connected to a lesser risk. The Plc2-P522R mutation is one among these that reduces the risk of AD. Using CRISPR-Cas9, Christian Haass *et al*. created a Plc2-P522R knock-in mouse model for determining the molecular relationship in between Plc2 and AD. They found that through improving microglial function, this variation may reduce the incidence of AD[26,27].

Application of Cas9 inhibitors or activators is a different strategy to lower Aβ production, wherein a Cas9 which is inactive catalytically is paired with the gene inhibitors or activators. Particularly, the guide RNA directs dCas9 to the target gene[28,29]. The expression of target gene will instead be affected by a generic transcriptional activator or inhibitor that it will carry. Various groups have developed nanocomplexes of non-viral delivery vehicles and Cas9 activator with outstanding probable therapeutic uses, including for neural illnesses, to aid in the administration of Cas9 activators[30,31]. In-vivo experiments using these methods have been successful in reducing BACE-1 expression or elevating ADAM10 expression in animal models of AD[32,33]. Gyorgy and colleagues demonstrated the CRISPR-Cas9 attributed deletion of the APP mutant gene has been enough to lessen pathology of Alzheimer's like disease utilizing the Tg2576 mice, a mutant human APP-expressing AD animal model. (Table 1) Targeting the endogenous APP gene's C-terminus with the CRISPR-Cas9 method has been found to be instrumental to decrease the Aβ synthesis[34].

Challenges and future directions of utilizing CRISPR-Cas9 for AD

The effective management of AD resulting from CRISPR/Cas9 brain delivery utilizing non-viral vectors meets several challenges. The vectors should ideally be steady and effective at transporting the weight to the target location[31]. The vectors should be internalized when they come in contact with the targeted cells in order to prevent lysosomal breakdown and target the nucleus. Non-viral vectors are favored only for in-vivo applications, but real-world applications can only be expanded by exploring the varied formulation properties[32]. Despite the problems with the stability and targetability of the delivery vector, the systemic route is extensively researched because of its in-vivo viability in the case of AD patients. Stereotaxic microinjection surgery to administer medication intracerebroventricularly and intrathecally can be difficult for AD due to the broad nature of A-β pathology of AD[33]. The intranasal route is another interesting strategy that can be explored because it can bypass the blood-brain barrier. More clinical investigations on the nasal administration of CRISPR/Cas9-based therapies are nevertheless required to establish the therapeutic safety of the procedure[34,35]. The CRISPR/Cas9 technology impacts the somatic cells significantly in comparison to germline cells. As a result, gene editing would only be applicable in those individuals who receive the treatment and cannot be transferred to the future generations[38,39]. Because genome editing is irreversible, more study is required to guarantee the security of CRISPR/Cas9 technology and its application. Additionally, there are still not enough studies examining potential off-targets and the long-term effects, thus ethical considerations and future studies are required before the full-fledged application of CRISPR/Cas9 in humans[38,39].

Limitations of the CRISPR-Cas0 technology

CRISPR-Cas9 technology elevates the advancement of molecular engineering through genome engineering in comparison to existing editing methods since it offers improved feasibility, efficiency, and multi-role application clinically. Nevertheless, the use of this

Table 1

Showing therapeutic application of CRISPR/Cas9 in managing Alzheimer's disease

Genetic target	Mutation	Cell line/organism	Impact	References
APP gene	Deletion of F681Y, R684H and G676R	Mice	Humanized animal models	Serneels *et al.*[19]
	3′-UTR of APP	APP-KI mice	Reduced Aβ pathology	Nagata *et al.*[20]
	C-terminus	Tg2576 mice	Decreased Aβ production	Sun *et al.*[22]
Plcγ2-P522R variant	P522R	Mice	Knock-in mouse model of Plcγ2-P522R	Gyorgy *et al.*[29]
Microtubule-associated protein Tau (MAPT) gene	Transcriptional start codon deletion	Tau knockout mice	Background resistant to excitotoxicity of C57Bl/6J	Tan *et al.*[23]
APOE-E3/E4	Endogenous expression of E4	Human-induced pluripotent stem cells	Reduction in APOE-E3/E4 and Tau protein hyperphosphorylation	Wadhwani *et al.*[24]

APOE, apolipoprotein E; APP, amyloid-β precursor protein; KI, knock-in; Plcγ2, phospholipase C gamma 2; Tg, transgenic; UTR, untranslated region.

approach may be complicated by the potential for off-target cleavages by Cas9[40]. In fact, the therapeutic effectiveness of CRISPR-Cas gene therapy in AD largely depends on the precision of the genetic alterations and the interactions in between the target DNA and gRNA[41]. Nonetheless, when DNA breakage and repair caused by CRISPR occur at an undesirable site of genome, erroneous (off-target) genome editing may ensue. For instance, when the cell does homology-directed repair and/or non-homologous end joining, various alleles of an altered gene are produced. These occurrences, which are caused in part by the fact that gRNAs may tolerate many mismatches, are detrimental in a therapeutic set-up, which can be minimized by utilizing algorithms specifically designed for the section of guide RNAs[42]. The scientific consortiums to standardize and quantify the outcomes of the development of genome-editing technique can be helpful in tackling the situation alongside the research on the potency, delivery, and precision of CRISPR-Cas technique[43].

Overall, several animal models and methods have been used to successfully explore the potential and probable uses of "CRISPR-Cas9" in the area of AD. To completely evaluate the existence or absence of genome changes (off-target) that can be materialized after the use of CRISPR-Cas9 technology, more research is nonetheless required.

Conclusion

CRISPR/Cas9 is a promising gene-editing technique since it can fix certain gene sequences and has a lot of potential for treating AD and other human disorders. Regardless of hereditary considerations, an altered Aβ metabolism is frequently seen in familial and sporadic AD. Therefore, since mutations in the PSEN-1, PSEN-2 and APP genes are a contributing factor to familial AD, CRISPR/Cas9 technology could address excessive Aβ production or mutations in these genes. Comprehensively, the potentiality of CRISPR-Cas9 technology outweighs as currently the greatest gene-editing tool available for researching on the neurodegenerative diseases like AD.

Ethical approval

Not Applicable.

Consent

Informed consent is not required for this review.

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N.S.: study concept, drafting and editing the paper. I.K.: drafting and editing the paper. J.D.N.: drafting and editing the paper. S.K.S.: drafting and editing the paper. L.K.: drafting and editing the paper. N.M.: drafting and editing the paper. M.P.K.: drafting and editing the paper. M.H.C.: drafting and editing the paper. V.A.D.: drafting and editing the paper. L.K.: drafting and editing the paper. L.A.: drafting and editing the paper. V.G.: drafting, editing and submitting the paper.

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There are no conflicts of interest.

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