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Using CRISPR/Cas9 for Therapeutic Approaches to Alzheimer's Disease

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Abstract. This review summarizes the key aspects of the CRISPR/Cas9 system. CRISPR/Cas9, derived from the genomes of bacteria and archaea, is a gene-editing tool consisting of single-guide RNA and the Cas9 enzyme, with Type II being the most commonly used due to its simplicity. It functions by recognizing target DNA via sgRNA, cutting it with Cas9, and repairing breaks through non-homologous end joining (NHEJ) or homology-directed repair (HDR). In practical, it's widely used in gene engineering, including gene therapy and disease modeling. For Alzheimer's disease (AD), it aids in creating cell and animal models, identifying causal genes like APOE and APP. Delivery systems include viral vectors (e.g., AAVs) and non-viral methods (e.g., nanocomplexes, microvesicles), with intranasal delivery showing promise for AD. However, challenges exist: off-target cleavages due to factors like mismatches, sequence similarities, and GC content; vector issues such as stability, target accumulation, and immune responses; and ethical and safety concerns including irreversible edits and limited long-term studies. Despite this, its feasibility and efficiency drive ongoing research, with future work focusing on improving safety and addressing these challenges.

Keywords: Alzheimer's disease, CRISPR/Cas9, gene editing, delivery system

1. Introduction

The most prevalent kind of dementia, Alzheimer's disease (AD), is pathologically defined by the buildup of neurofibrillary tangles (NFTs) and β-amyloid (Aβ) plaques in the brain [1]. Its primary pathogenic characteristics are permanent neuronal loss and a progressive deterioration in cognitive function. A global research from 2019 states that more than 5.8 million Americans have been diagnosed with AD, and by 2050, that figure is expected to increase to 15 million [2]. Since there are currently no effective disease-modifying medications for AD, the biological process underlying the condition is still poorly understood, and present treatments solely address its symptoms. Because of its high efficiency and precision, CRISPR/Cas9, an emerging gene editing technique, has shown significant promise in the study and potential treatment of AD. The protein Cas9, part of the CRISPR system—which consists of Regularly Interspaced Short Palindromic Repeats—works by identifying gene sequences that cause double-stranded breaks, enabling gene inactivation or correction. This has generated considerable excitement over its ability to potentially treat genetic issues related to Alzheimer's disease [3].

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Firstly, the CRISPR/Cas9 technology is widely used in the construction of AD cell models and animal models. For instance, through the CRISPR/Cas9 technique, researchers have successfully introduced AD-related gene mutations, such as APP and PSEN1, into various cell lines, for the study of Aβ metabolism and tau protein pathology [4]. Secondly, the discovery of the genes and gene mutations responsible for sporadic Alzheimer's disease (SAD) has also been greatly aided by the CRISPR/Cas9 technology. For example, researchers used the CRISPR/Cas9 approach to identify the APOE4 gene and APP gene mutations as being linked to Alzheimer's disease. They then used gene editing techniques to repair the APOE4 gene, APP gene and study its effects on tau protein phosphorylation and Aβ metabolism [4]. In addition, an effective delivery method is essential for the successful usage of CRISPR/Cas9 technology. Vectors, both viral and non-viral, are the two primary delivery techniques covered in the article. Viral vectors are highly effective and stable, making them essential tools in gene therapy. However, viral vectors also run the danger of causing insertional mutations and inciting immunological reactions [3]. While, non-viral vectors are thought to be better choices for AD treatment, due to their high cost-effectiveness and good safety [5].

Although there is fresh promise for treating Alzheimer's disease utilizing the CRISPR/Cas9 tool, more study is still needed to determine its safety and effectiveness in the body: (1)Off-target effect: Unexpected gene changes may arise from CRISPR/Cas9 gene editing at non-target locations [2]. (2)Delivery system optimization: Further research is necessary to further develop the targeting as well as stability of the delivery mechanism [4]. (3)Long-term safety and efficacy: Additional research is needed to evaluate the CRISPR/Cas9 technology's long-term effects [2]. (4) Ethical concerns: Before gene editing technology is used in clinical settings, all ethical and legal concerns must be thoroughly examined [5].

2. CRISPR/Cas9 system

CRISPR/Cas9, which stands for Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9, is a gene editing tool guided by RNA. CRISPR/Cas9 comes from the genomes of bacteria and archaea. It's part of their natural defense system, helping them recognize and get rid of harmful viruses called bacteriophages, and other dangerous DNA like plasmids [6]. The two primary parts of CRISPR/Cas9 are single-guide RNA, which can be abbreviated sgRNA, and the Cas9 enzyme. The first step is for sgRNA to recognize the target DNA sequence, and to improve specificity, a number of features are considered during the planning phase. Moreover, the Cas9 protein operates as an endonuclease, cutting DNA double strands like a pair of molecular scissors [5].

The "Type II CRISPR/Cas9" is the most commonly used term for the "CRISPR gene-editing" technique, since Type II only uses one Cas protein, which makes it appealing and straightforward for genome editing [7]. Once the Cas9 protein recognizes the target gene sequence, it produces a double standard break. There are then two different approaches that can be used to fix this break: non-homologous end joining (NHEJ) and homology-directed repair (HDR). While the HDR process aids in substituting the right sequence for the mutant or defective one, In order to initiate HDR, the right DNA sequences are integrated into the target region using a donor DNA template. NHEJ results in deletions and insertions, early stop codons, and/or DNA frameshifts, all of which eventually lead to gene deactivation [8]. The utilization of CRISPR/Cas9 in gene editing has been brought into focus for purposes like human disease modeling, gene knockdown into mammalian cells. As a multipurpose carrier, lipid-encapsulated nanomaterials have the potential to significantly boost the CRISPR/Cas9 technique's potential and provide a considerably more potent tumor therapy strategy [9].

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The CRISPR–Cas9 technique is frequently used to both inactivate a gene pair and identify intergenic connection, while it can also be used to examine how cells react to medication exposure. Protein assay methods can be used to independently examine the protein product of this correlation. Finally, understanding the collection of messenger routes implicated in cancer and, consequently, how the intergenic correlation network influences tumorigenicity can be gained by repairing this correlation framework in eukaryotic cells [10]. Regarding the effectiveness of treating Alzheimer's disease, as mutations in the genes account for around 1% of family occurrences of AD, modifying the genome with CRISPR/Cas9 may be beneficial for familial AD (FAD) in general but of little to no benefit for sporadic SAD. Nevertheless, reducing Aβ formation could serve as a useful treatment regardless of the cause, whether familial or sporadic, given the importance of aberrant Aβ conversion in both FAD and SAD [5].

3. CRISPR/Cas9 in AD

Alzheimer's disease (AD) is a progressive neurological disorder with no current cure. People often first notice symptoms like memory loss, language difficulties, confusion, mood swings, and changes in behavior. As the disease advances, it leads to a major decline in mental abilities, eventually resulting in death. The key features of AD include shrinking and dying brain cells, along with the build-up of sticky plaques made of β-amyloid outside brain cells and abnormal tangles of tau protein inside neurons. While scientists haven’t fully unraveled what causes of AD, they do know that many factors increase the risk—things like getting older, a family history of the disease, prior head injuries, Down syndrome, depression, heavy drinking, smoking, oxidative stress, certain metals, protein buildup, mitochondrial issues, brain inflammation, obesity, blood vessel problems, high blood pressure, and even the gut-brain connection via the microbiota [11].

Firstly, CRISPR/Cas9 is utilized in creations of cell models and animal models. Since there are no ethical issues, the experimental time is brief, and the expense is inexpensive, cell models are frequently utilized in research on a variety of neurological conditions, including AD, and many in vitro cell models of AD have been developed in recent decades. However, with help from CRISPR/Cas9, the efficiency of the treatment could be facilitated. In 2016, researchers introduced mutations in the amyloid precursor protein (APP) and PSEN1 into human induced pluripotent stem cells (iPSCs), aiming to study Alzheimer's disease (AD) mechanisms [12]. The study then revealed elevated Aβ levels in transformed neurons derived from mutated homozygous and heterozygous human iPSCs, compared to isogenic controls. This finding underscores the potential of CRISPR/Cas9 technology to effectively model Alzheimer's disease mutations in human brain cells [12].

Additionally, numerous animal models of AD have been produced in recent years by overexpressing human genes that have been modified to produce the tau and Aβ proteins without utilizing CRISPR/Cas9. A significant number of AD mouse studies do not truly demonstrate neurodegeneration, therefore even while all of these models have shown a high degree of efficacy in studying various elements of AD disease progression, their utility is limited [13]. In order to better imitate the illness phenotype, investigate pathophysiology, identify harmful genes, and eventually find an appropriate therapeutic method for the harmful disorder like AD, novel models of AD have recently been generated using CRISPR/Cas9 technology [2]. By employing the CRISPR/Cas9 technique to produce a humanized Aβ sequence (G676R, F681Y, and R684H) in the mouse and rat APP genes, Serneels developed a model in 2020 named mouse and rat Apphu/hu. Beyond that, compared to the initial WT strain, the quantity of Aβ rose greater than three times after three amino acids which are G676R, F681Y, and R684H, were added to the rodent Aβ sequence [14].

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Secondly, the CRISPR/Cas9 technique has also proven to be important in identifying the genes that cause sporadic Alzheimer's disease (SAD). The APOE gene has been currently recognized as a gene that predisposes people to SAD. In 2019, Wadhwani employed CRISPR/Cas9 technology to convert the E4 allele to the E3/E3 genotype in iPSCs from Alzheimer's disease patients. This conversion resulted in E3 neurons showing enhanced resistance to ionomycin-induced neurotoxicity. Nevertheless, tau phosphorylation was elevated in E4 cells, which led to calcium dysregulation and, eventually, cell death [14]. The author's comprehension of the connection between APOE4 and the pathophysiology of SAD is strengthened by this discovery. Furthermore, Mutations in the APP gene lead to early-onset Alzheimer's disease, primarily because they cause more of the amyloid-beta precursor protein to be cut by β-secretase, which contributes to the buildup of amyloid plaques [4]. Back in 2018, Gyorgy found that reducing Aβ protein levels happens when APP alleles are knocked out using CRISPR/Cas9. This suggests that CRISPR/Cas9 could be a promising gene therapy approach for treating Alzheimer's patients who have mutations in the APP gene [15].

4. CRISPR/Cas9 delivery system

Various CRISPR/Cas9 elements are available according to the intended modification of DNA. The Cas9/sgRNA pair is adequate for the most basic usage of interrupting genes; however, more complex bio-functions such as insertion or gene repairing necessitate an additional segment of DNA [2]. CRISPR/Cas9 can be delivered into cells in a variety of methods, including viral and non-viral approaches. Viral vectors are a conventional method in laboratory specimens such as lineages of cells and mammals, and they represent one of the most flexible approaches to directing the spread of plasmid-based CRISPR/Cas9. Plus, they might contain variants with detrimental effects. Since of their high infection rate, minimal immunogenicity, and little incorporation into the genome of a person, adeno-associated viruses (AAVs) are often used vectors [5]. Additionally, alternatives to viruses have shown promise in delivering CRISPR/Cas9, because they are more affordable, easier to use, feasible, and flexible. They are hence very suitable for usage in AD. Making nanocomplexes is comparatively easy when positive-charge CRISPR/Cas9 peptides are combined with a negative-charge nucleic acid cargo. Compared to viral vectors, they are less likely to trigger an immune response [5].

The application of microvesicles for CRISPR/Cas9 therapeutic delivery has emerged in recent years. Microvesicles are 100–1000 nm diameter extracellular vesicular objects that are produced and discharged into the medium during cell membrane budding [2]. Giving medication by mouth is fast, nonsurgical, and straightforward for recipients, but it is exceptionally challenging to administer due to the numerous hurdles the delivery system must conquer to get its genetic modification package inside the blood [2]. However, the intranasal method has garnered a lot of interest because it is believed to provide a quick and painless way to get past the blood-brain barrier. This raises the possibility of using nose-to-brain transfer to advance CRISPR/Cas9 therapies for AD into research studies. Other techniques including subcutaneous and intraperitoneal injections might be employed, but they would probably only be utilized as a last resort due to their pharmacokinetic restrictions [2]. Owing to the wide range of A-β pathology in AD, stereotaxic microinjection surgery to deliver medicine intrathecally and intracerebroventricularly can be challenging [16]. Because it can get beyond the blood-brain barrier, the intranasal route is an intriguing tactic that should be investigated. To determine the therapeutic safety of the method, however, more clinical studies on the nasal delivery of CRISPR/Cas9-based therapeutics are needed [2].

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5. The challenges and future of CRISPR/Cas9 technology

Compared to other editing techniques, CRISPR/Cas9 technology offers enhanced feasibility, efficiency, and multi-role clinical application, which advances molecular engineering through genome engineering. However, the possibility of Cas9 off-target cleavages could make this method more difficult to deploy

Firstly, for the weight to be transported to the intended position, the vectors should preferably be stable and efficient. In order to target the nucleus and avoid lysosomal degradation, when the viruses reach to touch with the targeted cells, they ought to internalize [17]. The protospacer adjacent motif, or PAM for short, is a crucial short DNA sequence that follows the targeted DNA region for cleavage by the CRISPR system, particularly in CRISPR/Cas9 applications, facilitating precise gene editing. Nevertheless, the CRISPR/Cas9 system may bind to and cut a different spot than expected on occasion. To a certain degree, Cas9 can accept the mismatches between the target DNA and the sgRNA. According to the research results, when Cas9 tolerates the mismatches in the target seed region—which contains 8–12 sequences nearest to the PAM—it may result in the incorrect cleavage[18]. Also, Off-target editing is common in genomic areas with comparable sequences. As a result, sgRNAs targeting conserved or repeated regions are prone to cause accidental cuts [18]. And the guanine-cytidine (GC) composition of the target sequence may make off-target editing more likely. Although the CRISPR/Cas9 structure must be stabilized by a certain amount of GC content, too much GC content (such as poly-G sequences) might lead to Cas9 misfolding and the possible cleavage of Cas9 at the incorrect location [19].

Secondly, after viral vectors are introduced into the body, extended contact with them can result in alterations, off-target splitting, and potential in vivo immune reactions. Plasmids can be swapped out for mRNA in the form of RNP to solve these issues. This decreases the duration that viral vectors remain in the host and the potential for off-target action, thereby increasing efficacy [19]. Non-viral vectors still face a number of difficulties, though. These include cytoplasmic movement, phagocytic clearance after delivery, cytoplasmic migration, protease or nuclease destruction, and inadequate deposition in target tissues. First off, a lot of study has been done to enhance the buildup of non-viral carriers at the target location. One strategy is to encourage the clustering of nanocarriers into tumors by taking use of the enhanced permeability and retention (EPR) effects [20]. The EPR effect can also be increased by applying physical external elements as electromagnetic radiation, ultrasound, thermotherapy, and photodynamic healing [21].

Finally, compared to germline cells, somatic cell populations are considerably impacted by the CRISPR/Cas9 technology. Gene editing would therefore only be useful for the patients who receive the treatment and would not be passed on to subsequent offspring [22, 23].

6. Conclusion

To sum up, CRISPR/Cas9 system represents a groundbreaking advancement in genetic engineering, offering unprecedented precision and versatility in gene editing. This revolutionary tool, with its origins in microbial defense mechanisms, takes part in gene expression activation and inactivation, which makes it possible to figure out the difficulty in cancer by repairing frameworks in eukaryotic cells. As the author delves deeper into its applications, particularly in addressing complex diseases such as Alzheimer's, it becomes evident that CRISPR/Cas9 is not just a scientific marvel, but a beacon of hope for those affected by genetic disorders. By building cell models and animal models, a variety of neurological conditions could be studied further. And CRISPR/Cas9 has a role in reducing Aβ production by using gene editing techniques to repair related genes. It is also essential

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to consider the delivery methods during the treatment of Alzheimer's disease. Viral vectors have higher risk of leading to mutations and negative consequences, while non-viral vectors are less immunogenic and more affordable according to the study.

However, as the author continues to explore the full potential of this technology, the author must also remain vigilant regarding the challenges it presents, such as off-target effects caused by mismatches of DNA, and unimproved delivery mechanisms, which may result in mutations and immune responses. The path forward will require a delicate balance between innovation and ethical considerations, ensuring that the author harnesses the power of CRISPR/Cas9 responsibly and effectively, since there are still risks using this technology compared to traditional methods. Ongoing research will undoubtedly unveil new methods to enhance its efficacy and safety, paving the way for a future where genetic diseases can be treated or even cured through targeted gene editing. As the author stands on the cusp of this exciting frontier, it is crucial to foster collaboration among scientists, ethicists, and policymakers to navigate the complexities of this powerful technology of CRISPR/Cas9.

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