REVIEW ARTICLE

Application of CRISPR and Stem-Cell Therapy in a Potential Curative Approach to Familial Alzheimer's Disease

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Abstract

Alzheimer's disease is a neurodegenerative disease that affects millions globally, yet still lacks a definitive cure. However, an emerging cure could lie within two rising technologies: Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and stem-cell therapy. CRISPR is a gene-editing technology derived from a bacterial defense mechanism that allows scientists to edit specific genes. Stem-cell therapy is the use of stem cells (undifferentiated cells) to restore damaged tissue, and is a form of regenerative medicine. There is a focus on using these technologies specifically for Familial Alzheimer's disease, as its genetic cause can be more clearly identified. Therefore, this article aims to understand the potential role of CRISPR and stem-cell therapy in a curative approach for Familial Alzheimer's disease. To fully discover their use, this study compiles existing scholarly articles and clinical trials with specific inclusion and exclusion criteria, utilizing a variety of sources to account for any discrepancies. The exploratory method was utilized to define early areas of initial insight and to better gain an understanding of a relatively new and unknown subject. Through this method, it is found that the use of CRISPR and stem-cell therapy could potentially account for the genetic causes of Familial Alzheimer's disease as well as the symptomatic effects of the disease, thereby presenting itself as a key contender in the field of curative medicine. By utilizing CRISPR for genetic alteration, and stem-cell therapy to restore the damaged neural network, it is possible to mitigate symptoms and the lasting effects of FAD, as well as correct the genetic cause of FAD. This paper advises future researchers to further their research on potential limitations, especially concerning safety issues and potential barriers. Expanding clinical trials of CRISPR on AD patients remains an important next step. This approach could result in a cure for patients with FAD, and even potential applications for general AD with symptom management as well as gene correction.

Keywords

Behavioral and Social Sciences, Neuroscience, Alzheimer's Disease, Familial Alzheimer's Disease, CRISPR.

Introduction

Alzheimer's disease is a form of dementia and a neurodegenerative disease that affects memory, thinking, and judgment. Worldwide, 55 million people are affected by Alzheimer's, and this number is expected to double every two decades.[1] One of the main causes of Alzheimer's is genetic factors, such as mutations in genes like *APP*, *PSEN1*, and *PSEN2*. These genes play a role in the formation of the amyloid beta precursor protein. In people with Alzheimer's, the amyloid beta precursor protein is not properly formed, and there is instead an excess of amyloid beta fragments that clump together, forming plaques. Similarly, the *MAPT* and *APOE* genes influence another protein significant to Alzheimer's, the Tau protein.[2] Patients affected by Alzheimer's will have Tau proteins that are improperly phosphorylated, causing the Tau protein to form clumps that later lead to neurofibrillary tangles. Eventually, the neurons

die due to the toxic Tau tangles.[3] It is important to note that genetic factors are not the only cause of this disease. Environmental and lifestyle factors also play a significant role in Alzheimer's.

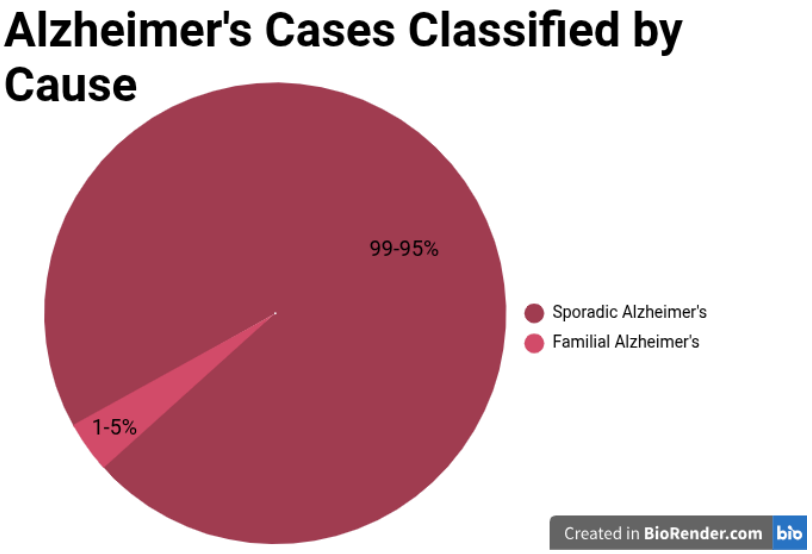

Figure 1. Percentages of sporadic Alzheimer's cases and familial Alzheimer's cases. This chart shows that the vast majority of Alzheimer's diagnoses are sporadic, while familial cases make up only a small fraction (1-5%) of total Alzheimer's cases. This highlights the limited scope of targeted gene therapies like CRISPR, as they can only be applied to a small population of patients.

The two types of Alzheimer's disease are familial and sporadic. Familial Alzheimer's Disease (FAD) accounts for around 1–5% of total Alzheimer's cases and is linked to specific genetic mutations in *APP*, *PSEN1*, *PSEN2*, and other genes.[4] Figure 1 illustrates the prevalence of Sporadic Alzheimer's, as these cases account for the vast majority. Sporadic Alzheimer's Disease (SAD) is thought to have emerged due to a combination of various lifestyle, environmental, and genetic elements.[5] This study aims to examine how CRISPR technology can be used as a curative treatment, specifically focusing on the reduction of the amyloid beta protein.

CRISPR, short for clustered regularly interspaced short palindromic repeats, is a newly emerged technology for gene editing. CRISPR results from the natural defense mechanism in bacteria. The CRISPR process starts with scientists creating a single guide RNA (sgRNA) sequence that matches the sequence of the DNA they desire to remove. Cas9, an enzyme, is then added to the single guide RNA, creating an RNA-Cas9 complex, which is administered to the patient. Once administered, the single guide RNA will identify its matching DNA sequence, and Cas9 will remove this sequence. With this targeted DNA sequence removed, scientists can now add their desired DNA sequence in its place. CRISPR can also simultaneously edit multiple genes if we provide more than one guide RNA and modify the cleaving behavior of Cas9. The ability to manipulate multiple genes at once is especially useful when focusing on adjoining genes. The use of Cas9 with multiple sgRNAs has allowed for large-fragment deletion and inversion between sgRNA targeting to be possible. Lentivirus-based sgRNA libraries have also been created thanks to the CRISPR-Cas9 system, and these libraries are used for high-throughput functional screening that identifies genes related to a certain biological process.[6]

In addition to CRISPR gene editing, this review also considers the role of stem cell technology. The use of CRISPR as a cure for Alzheimer's involves editing the genes that are associated with the disease, but it fails to account for the neurodegeneration of the brain. By utilizing stem cell therapy in conjunction with CRISPR, scientists can hopefully correct damage done to the brain by Alzheimer's disease. Stem cell therapy is key to regenerative medicine, since they can be differentiated into a variety of different cells and are self-renewing. There are many different stem cell types, such as induced pluripotent stem cells

(iPSCs), mesenchymal stem cells (MSCs), neural stem cells (NSCs), and embryonic stem cells (ESCs). These stem cells are distinguished from traditional non-stem-cell-based therapy in that they can come from multiple locations, such as bone marrow, somatic body cells, adipose tissue, and embryonic tissue. This study will mainly focus on the use of stem cells for cell replacement, where healthy neuronal cells (that were originally stem cells) will replace diseased neurons. Other uses of stem cells include paracrine signaling, immunomodulation, and stimulation of endogenous repair mechanisms. Stem cell use in neurodegenerative disease has shown promise, as preclinical studies in animals demonstrate that stem cells can differentiate into neuronal and glial lineages, integrate into host neural circuits, and promote functional recovery across varied neurodegenerative diseases. There are many obstacles to stem-cell therapy that prevent clinical translation, such as safety considerations, delivery systems, and efficient stem cell production.[7]

CRISPR technology will be mainly effective on Familial Alzheimer's Disease, especially those with early-onset Alzheimer's. Individuals with early-onset FAD are affected by the disease before age 65. Mutations in the *APP*, *PSEN1*, or *PSEN2* cause this form of FAD. Early-onset FAD has autosomal dominant inheritance, meaning there is a 50% chance for a child to inherit it from an affected parent. Many differences besides the age of onset exist between early-onset AD (EOAD) and late-onset AD (LOAD). EOAD requires a more extensive evaluation for diagnosis and has a greater impact on dementia risk factors. Additionally, EOAD accounts for only 5% of Alzheimer's cases. There are also specific psychological effects of EOAD.[8] This study will focus on early-onset FAD, or FAD caused by a genetic mutation, since the use of CRISPR for genome editing in Alzheimer's is limited only to those cases. The cases where genetic mutation causes FAD account for approximately 1% of all FAD cases.[9] This technology is still key to a curative approach to Alzheimer's, as the vast reach of the disease means that a cure that affects 1% of all FAD cases would still be a massive breakthrough that impacts many.

This study is essential as it seeks to evaluate a potential emerging solution to the unsolved problem of Alzheimer's disease. As illustrated previously, Alzheimer's affects a large population, and the absence of a real cure means that many individuals will continue to suffer from the disease without hope. Additionally, the newfound gene editing technology of CRISPR provides a new frontier for Alzheimer's curative approaches, one that must be explored to fully understand the role this key genetic technology will play in the search for a cure. By also incorporating the analysis of stem cell technology in conjunction with CRISPR, this study aims to provide an explanation of the use of these technologies and their role in a curative treatment for Alzheimer's disease. By utilizing gene editing technology, the origins of disease can be cured, instead of current approaches that attempt to cure the effects of the disease. The use of CRISPR in conjunction with stem cell technology offers hope of regeneration of diseased areas, a possibility that has implications for a variety of neurodegenerative diseases. By understanding the usage of CRISPR and stem cell technology in a potential cure for Alzheimer's disease, this study intends to provide insight into emerging Alzheimer's cures and spur the possible development of a future cure utilizing CRISPR and stem cell technology.

Methods

The objective of this study is to evaluate the role of CRISPR technology alongside stem-cell technology in a curative approach to Alzheimer's disease. This study is a review article and will compile information from a variety of sources, primarily scholarly articles and clinical studies. The exploratory method was utilized to define early areas of initial insight and to better gain an understanding of a relatively new and unknown subject. Numerous sources were filtered through to find key information on this emerging cure. The majority of this information was found by utilizing PubMed Central and ClinicalTrials.gov. When selecting this paper's references, the inclusion criteria included peer-reviewed articles, clinical trials, studies focused on familial Alzheimer's disease or early-onset Alzheimer's disease, studies on CRISPR, studies on stem cell therapy, preclinical animal model studies, recent publications, and English sources. The exclusion criteria covered studies focused on late-onset AD, studies that focused on symptom management interventions, non-scholarly sources, studies without a defined and accurate methodology, and cell studies that lack connection to neuroscience. Only online sources were used in this study, and therefore, ethical considerations were not made, as no field research was conducted.

Overview of Alzheimer's Disease

Alzheimer's disease (AD) is a neurodegenerative and dementia-related disorder that is increasingly prevalent in society today. The global burden of Alzheimer's disease is projected to continue its increase, with a significant spike expected in 2040.[10] As the disease continues to spread, its impacts are not only limited to those diagnosed, but also include the global economy. The worldwide costs of dementia have continued to increase, with projected costs reaching an estimated US $2 trillion in 2030.[11] The costs of Alzheimer's disease negatively impact the economic state of the world, and these threats are expected to grow. Alzheimer's disease harms the health of many individuals, with an estimated 415 million suffering from Alzheimer's disease.[12] This disease has implications for all aspects of the world, and its projected growth adds to the necessity of a cure. The far-reaching spread of Alzheimer's disease is necessary to understand the key importance of this study.

Alzheimer's can be classified into two main categories based on cause: Familial AD (resulting from genetic causes) and Sporadic AD (resulting from environmental/genetic causes). Familial AD is caused by mutations in three main genes: the presenilin 1 (*PSEN1*) gene, the amyloid precursor protein (*APP*) gene, and the presenilin 2 (*PSEN2*) gene. On the other hand, Sporadic AD results from a variety of environmental and genetic factors that have not been fully determined.[13] Some of these risk factors for Sporadic AD could include hypertension, chronic high blood pressure, type 2 diabetes, smoking, and hypercholesterolemia.[14] Alzheimer's disease can also be classified as Early-onset Alzheimer's disease or Late-Onset Alzheimer's Disease based on age of onset, with 65 years typically used as an age cutoff.[15] Early-onset Alzheimer's disease (EOAD) accounts for approximately 5% of total AD cases. EOAD cases are usually sporadic, but around 10–15% of EOAD cases are familial. Familial EOAD commonly starts around ages 30 to 40 with an aggressive progression, while sporadic EOAD usually begins after age 50 and has a temporal profile similar to that of Late-Onset Alzheimer's Disease (LOAD).[16] This study will mainly focus on using CRISPR and stem cell technology as a curative approach for early-onset FAD, since it results directly from genetic mutations.

Pathophysiology and Molecular Mechanisms

Cholinergic Hypothesis

The development of Alzheimer's disease is characterized by the accumulation of amyloid beta plaques and the formation of tau tangles. These developments result in neurodegeneration, specifically, the loss of cholinergic neurons in the basal forebrain and the neocortex. There exist two main pathophysiological hypotheses based on these understandings. Firstly, the Cholinergic Hypothesis suggests that AD development emerges from decreased levels of acetylcholine (ACh) in the brain due to neuron death in the Nucleus Basalis of Meynert. This hypothesis is derived from the early degeneration of cholinergic neurons in AD. It is thought that Beta-amyloid causes cholinergic synaptic degeneration and decreases ACh release. Clinically, the use of Anticholinergics in elderly patients negatively impacts their memory, further supporting this hypothesis.[17]

Amyloid Hypothesis

The second hypothesis is the Amyloid Hypothesis, which is currently widely established as the pathophysiological mechanism of AD. This hypothesis proposes that the activity of β- and γ-secretase enzymes results in the formation of the amyloid beta (Aβ) peptide from the amyloid precursor protein (APP). Normally, the protein fragments that are created when APP is cleaved by alpha or beta-secretase are not harmful to neurons, but the sequential cleavage of amyloid precursor protein (APP) by beta-secretase and then gamma-secretase produces 42-amino-acid peptides (Aβ42). Increased levels of Aβ42 will lead to the formation of aggregated amyloid plaques, as Aβ42 supports the dangerous buildup of amyloid fibrils instead of the usual APP decomposition.[17] This accumulation of Aβ eventually results in the aggregation of hyperphosphorylated tau, the incitement of neuronal death mechanisms, and finally, dementia.[18] Normally, in an environment without AD, tau modulates the structure of microtubules. In a brain affected by AD, tau becomes hyperphosphorylated, resulting in the dismantling of microtubules and an aggregation of free tau.[19]

Effects of AD

The changed physiology of a brain with AD can be seen through the formation of Aβ neuronal plaques and neurofibrillary tangles, which are the two key pathological hallmarks of AD. They begin in the hippocampus and cerebral cortex regions and spread throughout the brain. The brain of an AD patient commonly features neurodegeneration in the frontal, temporal, and parietal lobes of the cerebral cortex. The area of the brain will also be reduced, and the sulci in the depressions of the cerebral cortex are disproportionately enlarged.[20] The most common symptom associated with AD is episodic short-term memory loss. Individuals with this symptom will be able to recall long-term memories, but will be unable to retain newer information. This short-term memory loss also commonly weakens judgment, problem-solving, executive functioning, and organizational skills. Executive functioning impairment often affects key daily activities, such as activity planning, driving, financial management, and cooking. Cognitive deterioration in AD patients is often followed by weakened visuospatial skills and language disorder. During the moderate to late stages of the disease, many neuropsychiatric symptoms such as apathy, social withdrawal, and disinhibition are observed. [17]

Genetic and Environmental Factors Influencing AD

Genetic Predispositions

Although the main cause of Alzheimer's disease is not currently known, various risk factors include old age, genetic components, head injuries, vasculopathy, and environmental factors (such as heavy metals, trace metals, etc.). Genetic factors relate to 70% of the AD cases, and the majority of EOAD cases have autosomal dominant inheritance. Specifically, mutations in the Amyloid precursor protein gene (*APP*), Presenilin-1 gene (*PSEN1*), Presenilin-2 gene (*PSEN2*), and apolipoprotein E gene (*APOE*) are related to the onset of AD.[21] These mutations usually relate to early-onset AD, and they are not as common as they are associated with around 5% to 10% of all AD cases.[17]

As mentioned previously, the improper cleavage of APP results in an increase in Aβ, and this is encoded by the *APP* gene. Out of thirty mutations in the *APP* gene, twenty-five are associated with AD, as they increase the buildup of Aβ. However, this gene also has a beneficial mutation. The *A673T* mutation results in decreased Aβ, Aβ40, and Aβ42 secretion, leading to protection against the development of AD. The mutations on the APP gene have different effects, but all mutations encircle the gamma-secretase cleavage site in APP. In murine models with the *KM670/671NL* mutation, there is an absence of NFTs, but an increased number of amyloid plaques in the hippocampus and cortex. Furthermore, the *E682K* mutation relates to hippocampal atrophy, while the *A673V*, *D678H*, *D678N*, *E682K*, and *K687N* mutations are associated with cortical atrophy. Mutations like *E693G*, *E693K*, *D694N*, and *A692G* impact the α-secretase cleavage site and create polymorphic aggregates that can disrupt bilayer integrity, while mutations such as *T714I*, *V715A*, *V715M*, *V717I*, *V717L*, *L723P*, *K724N*, and *I716V* impact the γ-secretase cleavage site and increase the ratio of Aβ42/Aβ40.[21]

Mutations to the Presenilin-1 gene (*PSEN1*) and Presenilin-2 gene (*PSEN2*) disrupt the processing of gamma-secretase, resulting in the accumulation of Aβ.[17] Both mutations cause an increase in the production of Aβ42 peptides.[22] The most common mutation relating to AD is the *PSEN1* mutation, as it accounts for around 5% of AD cases.[17] To that end, genetic surveying recognized close to 75 missense mutations in *PSEN1* and three in *PSEN2* as causes of EOAD. The earliest and most aggressive variant of AD is caused by *PSEN1* mutations, and these mutations usually cause onset before age 50 and death around age 60.[22]

While mutations in the *APP* gene, *PSEN1* gene, or *PSEN2* gene are usually associated with early onset AD, mutations in the apolipoprotein E gene (*APOE*) are a key genetic risk factor for late onset AD. This gene is located on chromosome 19q, an area which was previously known to be linked to late-onset AD. Genetic analysis shows that the inheritance of the ε4 allele of *APOE* is an indicator of risk for AD, as the presence of one or two ε4 alleles increases the probability of developing AD and makes the age of onset earlier compared to those with ε2 and/or ε3 alleles. Those with one or two copies of the ε4 allele of *APOE* are termed individuals with the *APOE4* polymorphism. However, many humans without the ε4 allele develop AD, and there are those with the allele who do not develop AD, meaning the ε4 allele of *APOE* is

not a cause for AD, but merely a risk factor. *APOE4* polymorphism is associated with an increased density of Aβ plaques and vascular deposits.[22]

Environmental & Lifestyle Contributors

Alzheimer's is a complex disease that is influenced not only by genetics but also by environmental and lifestyle factors. Environmental factors such as sleep disturbances or air pollution are key contributors to the disease. For example, children and young adults affected by Mexico City's air pollution displayed improper folding and abnormal aggregation of p-tau and Aβ in the brain stem. Additionally, even an unfavorable acoustic environment could cause hearing loss, a risk factor for Alzheimer's. Lifestyle factors include diet, depression, stress, and smoking.[23] Data from international cohort studies (not affiliated with the tobacco industry) suggest that smoking causes at least 1.7 times (70%) greater risk for AD, and this risk increases with increased cigarette exposure.[24] Midlife obesity is a key risk factor for Alzheimer's, and beneficial dietary methods such as the Mediterranean diet, the DASH (Dietary Approaches to Stop Hypertension) diet, and the MIND (Mediterranean-DASH Intervention for Neurodegenerative Delay) diet were found to have an inverse relationship with the risk of AD. Other protective factors include coffee and green tea, as common consumers of green tea were found to have decreased cerebrospinal fluid (CSF) levels of total tau protein, and it has been proposed that a low but not high coffee intake is related to an 18% reduction of AD risk.[23]

Current Treatment Approaches

Alzheimer's Drug Treatment Process

AD is commonly managed with a multifaceted approach that accounts for the impact of the disease on all aspects of the patient's life. Open communication with physicians is a key foundation of disease management and can ensure the best results. Behavioral changes due to the disease are accounted for by streamlining the environment and creating consistent routines. Those around the patient should use calm, simple language and avoid saying no (except if it affects the patient's health). Financial and medical planning is also an essential step in disease management. A variety of therapy forms, such as behavioral therapy, exercise therapy, light therapy, and music therapy, are also used in behavioral management. Providing caregiver support is also essential after diagnosis. Caregiver support can include planned resting periods, proper education on the disease's effects and how to care for these changes, and using support networks for the caregiver.[25]

Symptom Mitigating Drugs

Currently, many FDA-approved drugs targeting those afflicted by Alzheimer's exist, but they come with various side effects and benefits. Symptom-mitigating drugs include those that target behavioral and psychological symptoms, cognitive symptoms, and glutamate regulators.[26]

Brexpiprazole (Rexulti®) is a behavioral symptom-mitigating drug that is used to alleviate agitation associated with dementia. The drug acts on both dopaminergic and serotonergic receptors, reducing neuronal excitability and improving the patient's mood. Mild to moderate side effects include nasopharyngitis, headache, dizziness, urinary tract infection, insomnia, and somnolence. A few patients observed serious side effects of agitation and QTc interval prolongation.[26]

Donepezil (Aricept®) is an acetylcholinesterase (AChE) inhibitor for the mitigation of cognitive symptoms. In AD patients, studies show that 5 mg and 10 mg once-daily doses were able to effectively reduce tau-protein expression, decrease hippocampal atrophy, lower serum Aβ levels, and improve cognitive performance. Donepezil (Aricept®) acts by binding with AChE, reducing the hydrolysis of ACh in the synapse and ameliorating cholinergic neurotransmission. The drug is usually well-received, but side effects include nausea, vomiting, diarrhea, weight loss, fatigue, insomnia, anorexia, and asthenia.[26]
Memantine (Namenda®) is a glutamate regulator for those with moderate-to-severe AD. The drug acts by obstructing current flow through channels of the glutamatergic NMDA receptors and impeding the inflow of calcium into cells that exists due to chronic, glutamate-induced NMDA receptor activation. The drug is

usually well-received, but side effects include agitation, dizziness, falls, accidental injury, influenza-like symptoms, headache, and diarrhea. Those with sensitivity to memantine hydrochloride should avoid the drug.[26]

Disease-Modifying Drugs

Aside from symptom-managing drugs, FDA-approved disease-modifying drugs also exist. Aducanumab (Aduhelm®) is one such drug, and it attempts to reduce Aβ-burden. The drug is a monoclonal immunoglobulin gamma (IgG)-1 monoclonal antibody that acts by traversing the blood-brain barrier and selectively targeting soluble oligomers and insoluble fibrillary aggregates of Aβ-plaques in the brain, thereby slowing disease development. The most common side effects are amyloid-related imaging abnormalities (ARIA). Those who take the drug will need routine magnetic resonance imaging (MRI) to check for ARIA.[26]

Current Stem-cell/CRISPR Clinical Trials

Although the definitive role of CRISPR and stem-cell technology in a cure for Alzheimer's has not been established, various clinical trials are attempting to gain a better understanding of their role. For example, a clinical trial from the University of Miami aims to understand how the administration of allogeneic hMSCs will impact those afflicted by AD. The study uses an experimental hMSC Treatment group, which will receive 4 doses of the hMSC intervention (with each dose being administered every 13 weeks). The allogeneic hMSCs will be administered intravenously, and each dose will have about 100 million cells per infusion. The study will primarily measure the number of Treatment-Emergent Serious Adverse Events. These events are defined as life-threatening events, require inpatient hospitalization, result in disability, result in clinically notable symptoms, or result in death. The study will also measure a variety of secondary outcomes, including cognitive function over time, depressive symptoms over time, participant quality of life over time, and other indicators of effectiveness. This clinical trial will provide insight into the variety of effects allogeneic hMSCs have on Alzheimer's patients and their promise as a possible cure to AD.[27]

Although clinical trials on the use of CRISPR in AD have not yet been conducted, animal models offer some insight into its effectiveness. One key study has used an animal model to investigate how the knockdown of the *CysLT1R* gene using CRISPR would impact APP/PS1 mice. The researchers had previously determined that cysteinyl leukotriene receptor 1 (CysLT1R) antagonists were able to improve exogenous Aβ-induced memory damage. By using CRISPR to delete the *CysLT1R* gene, the researchers concluded that *CysLT1R* knockout or knockdown may help to preserve synaptic structure and plasticity, and improve cognition in APP/PS1 mice. These changes are linked with concurrent decreases in amyloid processing, reduced neuroinflammation, and suppression of the kynurenine pathway. This study presents the role of CRISPR as a tool to improve the neurological environment of those with AD, as stem-cell therapy needs a supportive environment to be effective. The researchers discovered that using CRISPR to modify genes (other than the genes directly related to causing AD) can reduce brain damage.[28] This has serious implications for this study as stem-cell therapy requires a healthy environment for neuronal replacement to succeed. Additionally, the possibility of utilizing CRISPR to directly modify genes causing AD is made futile if neuroinflammatory signaling can still damage neurons and prevent recovery.

CRISPR-Cas9 Technology as a Gene-Editing Solution

The mechanism of CRISPR-Cas9 has three key steps: recognition, cleavage, and repair. First, the sgRNA directs Cas-9 and uses its 5'crRNA complementary base pair component to identify the target sequence in the gene of concern. The Cas-9 protein will be inactive without the presence of sgRNA. Next, the Cas-9 nuclease creates double-stranded breaks (DSBs) at a certain location 3 base pairs upstream of the PAM. Downstream of the cut site is the PAM, a short conserved DNA sequence whose size fluctuates based on the species of bacteria. The Cas-9 protein (the most frequently used nuclease in the genome-editing tool) will identify the PAM sequence at 5'-NGG-3' (N can be any nucleotide base). Cas-9's recognition of a target site with the correct PAM will then trigger local DNA melting, followed by the formation of an RNA-DNA hybrid. However, exactly how the Cas-9 enzyme melts the target DNA sequence is not yet

understood. Subsequently, the Cas-9 protein will be activated for DNA cleavage. As the RuvC domain cleaves the non-complementary strand of target DNA to produce mainly blunt-ended DSBs, the HNH domain will cleave the complementary strand. Lastly, the host cellular machinery will mend the DSB.[29]

A key advantage of CRISPR technology is its duration. In fact, a single gene edited by CRISPR has the potential for lifelong knockdown, not just long-term knockdown. This feature is particularly useful for the treatment of monogenic diseases (such as early-onset FAD). However, this also presents safety concerns due to off-target edits and unintentional DNA changes. CRISPR technology is also highly effective, as CRISPR/Cas9 can be used to reverse mutations that cause diseases in animal models. CRISPR/Cas9 uses deletion and targeted correction, strategies that are key to the reversal of any gene mutation related to neurodegenerative disorders. Additionally, CRISPR technology is very efficient, as it has the potential to target any gene in any type of cell (allowing CRISPR/Cas9 to directly target the genome of embryos in large animals).[30] The viability of CRISPR in a curative approach to Alzheimer's is beginning to be confirmed. For example, a study where researchers used Tg2576 mice (an animal model of AD expressing mutant human APP) showed that CRISPR-Cas9-mediated disruption of the APP mutant gene was adequate to lower AD-like pathology.[31] Whether this result will translate to humans is yet to be determined, but it provides a powerful foundation as proof of concept for the role of CRISPR in a potential cure to AD. As previously mentioned, CRISPR also plays a key role in improving the brain environment in AD, giving CRISPR two main functions in this paper: correction of the cause and improvement of the brain environment.

Limitations of CRISPR technology

A key concern in the use of CRISPR technology for gene editing is the possibility of off-target effects (OTEs). This frequency is relatively high, at ≥50%. Fortunately, methods to reduce this likelihood are being developed, such as engineering different Cas9 variants that have a lower frequency of OTEs. One particular method involves utilizing a sgRNA pair that produces a DSB by engaging both strands of DNA at the selected site and incorporating Cas9 nickase (Cas9n), a variant that creates single-strand breaks. Despite these upcoming solutions, OTEs remain an important risk when utilizing CRISPR technology. Another key limitation is the necessity for a PAM close to the selected target site. One of the most substantially used Cas9s is Cas9 from the *Streptococcus pyogenes* bacterium (SpCas9). This Cas9 has a comparatively short canonical PAM recognition site and is also comparatively large, making it difficult to package in AAV vectors, which are the most common delivery tool for gene therapy. However, another Cas9, *Staphylococcus aureus* Cas9 (SaCas9), is smaller, making it easier to package in AAV vectors, but its longer PAM sequence limits the span of therapeutic targeting sites. Another option is engineered SaCas9 variants, namely KKH SaCas9, which has a broader span of therapeutic targeting sites. It is important to note, though, that the wildtype SaCas9 is associated with OTEs.[32] It is important for future researchers to understand the best Cas9 variant for this therapeutic approach to best mitigate the likelihood of OTEs and cover the required therapeutic targeting sites.

Limitations of Stem Cell Therapy

One of the largest limitations to the use of stem cell therapy is transferring the mechanisms of stem cell therapy in animal models to human application. To create the same results in human use, researchers must properly understand how the stem cells function in the injured microenvironment in animal models. Challenges specifically associated with the use of embryonic stem cells (ESCs) include rejection by the recipient's immune system and ethical concerns (as they result from human embryos and can result in malignant tumors in mouse models). Immunorejection may be circumvented with induced pluripotent cells from the patients, and this could resolve the ethical issues associated with ESCs as well.[33] The importance of proper translation from animal models to human application, the possibility of immunorejection, and ethical issues all present challenges that must be considered in the use of stem cell therapy for the symptomatic treatment of FAD.

Integration of Stem Cell Therapy with CRISPR Technology

In this paper's curative approach for AD, one pillar of treatment involves using CRISPR to correct the genetic cause of FAD. The second pillar involves replacing lost neurons using stem cell therapy, thereby mending the damage caused by the disease. Recent preclinical studies have provided a promising outlook on the use of stem-cell therapies as a replacement or regeneration strategy.[34] Therapeutic effects of stem cells in treatment for AD mainly arise from their capability to differentiate into functional neurons or glial cells. This allows them to replace damaged neurons and cells from the effects of AD, restoring the neural network. Additionally, stem cells can regulate and mitigate neuroinflammation and support the removal of β-amyloid (Aβ) deposits, helping lessen disease progression. However, key limitations include immune rejection, traversing the blood-brain barrier, tumorigenic risks, and low cell survival rates.[35]

Neural stem cells

Researchers have used animal models to investigate the use of neural stem cells (NSCs). One such example is shown by Qu *et al.*, who injected human undifferentiated NSCs into the brains of 6-mo-old and 24-month-old rats, thereby uncovering a substantial improvement in cognitive function. Additionally, Wu *et al.* discovered that human fetal brain–derived NSCs transferred into adult rat brains could create cholinergic neurons in specific regions. Furthermore, Blurton-Jones and others injected NSCs into aged transgenic mice expressing mutant presenilin, tau, and APP. They discovered that transplanted NSCs may help improve spatial learning and memory function in mice with dementia without changing the pathology of Aβ, providing a foundation for NSCs as part of the symptomatic management of AD.[34]

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) have three main functions in AD treatment: immune regulation; lowering of Aβ plaque burden through internalization and Aβ degradation of endosomal–lysosomal pathway oligomers; and regenerative potential. For this paper, we will primarily focus on regenerative potential. Studies show that after transplanting MSCs in AD animal models, NPCs were stimulated to differentiate into mature hippocampal neurons via activation of the Wnt pathway, giving justification for MSCs aiding the development and differentiation of local stem and progenitor cells. To this end, another study has discovered that human MSCs injected into mature rats have been found to migrate to the brain and differentiate into nerve cells, thereby reinstating motor and cognitive activity.[34]

Embryonic stem cells

Embryonic stem cells (ESCs) are totipotent and self-renewing, capable of differentiating into NPCs *in vitro*. In animal models of AD, mESCs-derived NPCs were transported to unilateral Meynert basal nuclei with and without pretreatment, leading to improved learning and memory abilities. Human embryonic stem cells (hESCs) are another possibility in the treatment of AD. However, before using hESCs in AD clinical trials, key ethical considerations must be made, as they are derived from pre-implantation human embryos. Another obstacle is the chance of immune rejection in ESC-based AD cell therapy.[34]

Induced pluripotent stem cells

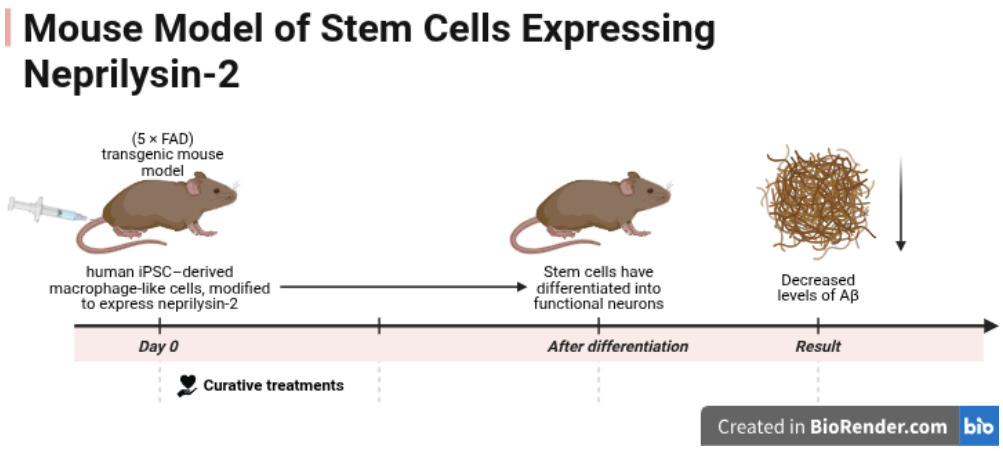

Figure 2. Timeline observed in a mouse model showcasing the effect of stem cells expressing neprilysin-2. This timeline shows that in mouse models, after differentiation, stem cells reduce accumulated levels of Aβ. This highlights the therapeutic potential of pairing gene modification with stem cell therapy to help reduce pathological hallmarks.

Some AD models have been able to replace lost neurons through iPSCs. For instance, Figure 2 illustrates a five familial AD (5 × FAD) transgenic mouse model, which showed that human iPSC–derived macrophage-like cells that were genetically modified to express neprilysin-2 (an Aβ-degrading protease) would differentiate into functional neurons and therapeutically reduce Aβ levels. Additionally, a recent study shows that the use of protein-induced iPSCs and ferritin released by mESCs strongly encouraged the differentiation and maturation of oligodendrocytes. However, genetic instability and phenotypic neuropathology can be shown in autologous iPSCs, such as significant Aβ load rates, shortened axon lengths, and increased tau phosphorylation, thus limiting their practical implementation in AD.[34]

Results and Discussion

This paper has evaluated whether the combined use of CRISPR and stem-cell therapy can be used as a curative approach for familial AD, and provided future insights into these two emerging technologies.

CRISPR for Gene Correction

In the utilization of CRISPR to correct familial AD caused by genetic issues, the studies presented in this paper lead to the conclusion that CRISPR may be able to successfully correct the *APP*, *PSEN1*, and *PSEN2* in animal models. Whether this result will be mimicked in humans is unclear and needs further exploration. The literature presented has shown that this correction via CRISPR results in reduced AD-like pathology specifically for the correction of the *APP* gene, implicating that correction of the *PSEN1* and *PSEN2* genes could result in a similar benefit. CRISPR's effectiveness as a tool has also been established, as these beneficial corrections are proven to last as long as a lifetime. The primary use of CRISPR technology in AD is limited to gene correction, meaning neurodegeneration resulting from the symptomatic effects of AD will not be alleviated by gene correction.

Stem-cell Therapy for Symptom Management

As for the use of stem-cell technology in familial AD, it can be deduced that the use of stem cells does not solve the root of AD (as it does not change the pathology of Aβ), but can instead help with the symptomatic effects of the disease, helping improve spatial learning and memory function in animal models. Therefore, this paper defines the role of stem cells as symptomatic and supportive instead of curative. However, stem-cell technology may be largely ineffective without a supportive environment, giving an alternate purpose to CRISPR as not just a gene correction tool for *APP*, *PSEN1*, and *PSEN2* but also as a gene editing tool for the purpose of modifying the disease environment, helping stem-cell

technology be effective. The studies presented show that editing of certain genes, such as *CysLT1R*, can help support the brain environment, a key factor for the use of stem-cell technology. Although this potential use is impactful, stem-cell therapy alone cannot account for the root genetic causes of familial AD.

Combined Curative Potential

This paper thereby presents the use of CRISPR in Familial AD for genetic correction of *APP*, *PSEN1*, *PSEN2*, and support of a favorable brain environment. The use of stem-cell technology has been identified as a supportive approach to deal with symptomatic effects, accounting for the changes individuals face from AD. The combined use of stem-cell therapy and CRISPR is thereby deemed to have a higher potential in a curative approach together, compared to solely utilizing one approach, due to the combined approach's ability to address the root genetic causes of familial AD, as well as account for symptomatic effects. By utilizing CRISPR for genetic alteration, and stem-cell therapy to restore the damaged neural network, it is possible to mitigate symptoms and the lasting effects of FAD, as well as correct the genetic cause of FAD. However, these technologies are accompanied by several side effects and challenges that make their use in a curative therapy more difficult. General limitations of stem-cell therapy must be considered, such as immune rejection and cell survival rates. Both technologies face the difficulty of traversing or circumventing the blood-brain barrier. Despite these challenges, their potential in a combined curative approach for FAD is quite promising.

Limitations

Despite thoroughly analyzing existing research, there exists a variety of limitations that must be considered. Firstly, the majority of the studies reviewed only included preclinical animal models, either *in vitro* (stem-cell) or *in vivo* (rodent models), which prevents these results from being directly translated to human impact. To fully understand the effects of these therapies, future human clinical trials will be needed. Secondly, the application of stem-cell and CRISPR technology in the method examined in the paper is only applicable to those with Familial Alzheimer's disease. Familial Alzheimer's disease only accounts for 1–5% of total Alzheimer's cases, with the majority of cases coming from Sporadic Alzheimer's disease, so it should be understood that a solution that targets the genetic root cause (through CRISPR or stem-cell therapy) can only be utilized for Familial Alzheimer's cases. Another key limitation is the difficulty of traversing the blood-brain barrier. It is difficult to deliver CRISPR components across the blood-brain barrier, so even the positive benefits shown in this paper may not be applicable if an effective transportation system is not developed. Future researchers should strive to create a transportation system that accounts for this and consider the emerging solution of nanocapsules. Nanocapsules encompass the single Cas9/sgRNA complex by using a glutathione-sensitive polymer casing that integrates a dual-action ligand to allow penetration of the blood-brain barrier.[36] This solution should be analyzed further, alongside other potential solutions. The potential for off-target effects is another concern associated with the use of CRISPR. Future researchers should thoroughly understand and develop methods for reducing these effects, and also understand if the risk of off-target edits outweighs the potential solution. The aforementioned technology of nanocapsules also claims to reduce off-target effects, with editing a brain tumor resulting in fewer than 0.5% off-target gene editing in high-risk tissues.[36]

Conclusion

This paper has helped define the role of CRISPR technology as a platform for genetic correction of specific genes causing familial AD. This paper not only defines the role of CRISPR in AD as curative for the root cause, but it also understands its many contributions in creating a supportive environment of the brain. This secondary purpose of CRISPR lends itself well by aiding the effectiveness of stem-cell technology. Stem-cell technology in AD has many implications, and its role in managing symptomatic effects of the disease makes it a crucial approach to be used in conjunction with CRISPR, allowing these technologies to account for the root cause of the disease as well as symptomatic effects. While this paper has examined certain limitations of these technologies (the importance of a supportive brain environment, the limited effectiveness of stem cells in correcting disease pathology, and the lack of human models),

those who seek to dive further into this field should consider the delivery issues involved with stem cells. It is also recommended to examine whether ex vivo CRISPR editing of patient-derived iPSCs is truly effective as a possible solution to bypass these delivery issues. Another key area of consideration is the use of multiplexed CRISPR technology to simultaneously target disease-causing genes like APP, PSEN1, PSEN2, and those related to inflammation, like the CysLT1R gene. This method could increase the efficiency of treatment, but also requires further examination and evaluation as a viable option. Ethical concerns of future clinical trials should also be considered. Lastly, additional long-term safety studies are necessary to identify and mitigate risks with these technologies, such as tumor concerns, lack of genetic diversity, and immune rejection.

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