RAMATHIBODI
PITCHING CHALLENGE

Utilising the combination of the CRISPR engineering method and iPSC-based Neural Stem Cells as alternatives to current AD therapies to cure Alzheimer's disease.

[Healthcare Innovation]

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Table of contents

Research Question: Can Alzheimer's disease be entirely remedied through biomedical engineering practice associated with genome editing by CRISPR CAS9 and in vitro iPSCs derived neural stem cells reprogramming delivered to the hippocampus by the direct intrahippocampal nano injection?

Introduction:

According to Alzheimer's Association Resource, the number of people who have Alzheimer's disease has risen more every year. The deaths have escalated 145% from 2000 to 2019 [1]. In Thailand, 3-5% of the population experienced this disease, especially the elderly. [2] Nowadays, the estimated total healthcare outcomes for the treatment of Alzheimer's disease is about 305 billion dollars, cost of skilled nursing care, home healthcare, and hospital care.[3] The symptoms of dementia could result in memory loss and confusion, and difficulties in everyday life. Therefore, to solve such obstacles, we have been investigating many possible ways to prevent Alzheimer's disease.

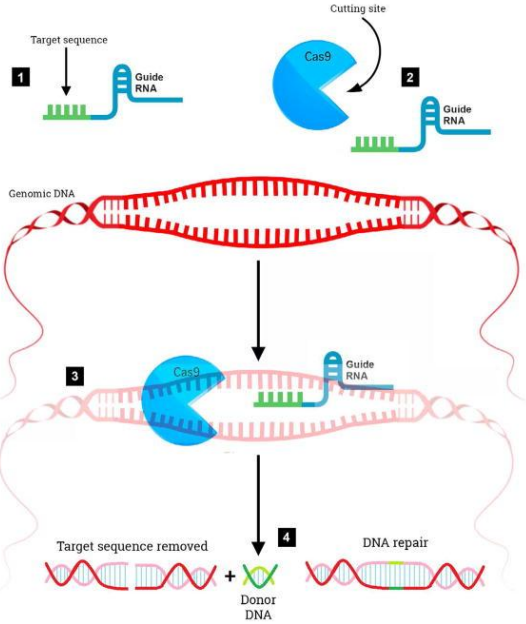
Alzheimer's disease (AD) is a neurodegenerative disorder that destroys memory and thinking skills, resulting in dementia. According to previous studies, The underlying cause of Alzheimer's disease (AD) is associated with the accumulation of extracellular senile plaques (SP) within the hippocampal dentate gyrus that related primarily to learning and memory involved forming, organising, and storing them[4], in which finally leads to AD. Another cause is the breaking of tau, making neurofibrillary tangles that all disrupt neuron-transmitted messages. However, the SP process will be focused on this proposal.

The generation of SP developed from the excess built-up of the neurotoxicity Amyloid-beta (Aβ), Aβ40 and Aβ42, via the amyloidogenic pathway of Beta-amyloid precursor protein (β-APP), essential for neuron growth and repair, found attached to the neuronal membrane; in consequently, aggregation of Aβ oligomers that ultimately form SP of AD which blocks neurotransmitters released at the axon terminal of presynaptic neuron from binding to its receptors at the dendrite of the postsynaptic neuron. Thus, blocking neural communication. More specifically, beta-amyloid is an abnormal protein cell that blocks the neuron's transport system, leading the brain to shrink and die. Nowadays, healthcare can only control the symptoms, such as slowing the worsening of the symptoms and supporting communication among nerve cells. As a result, the researchers attempt to invent a recent treatment to stop the disease by enhancing the beta-amyloid. Not only eating habits that help to minimize the amount of beta-amyloid, but also bioengineering tools, such as CRISPR, zinc-finger nucleases, and transcription activators.

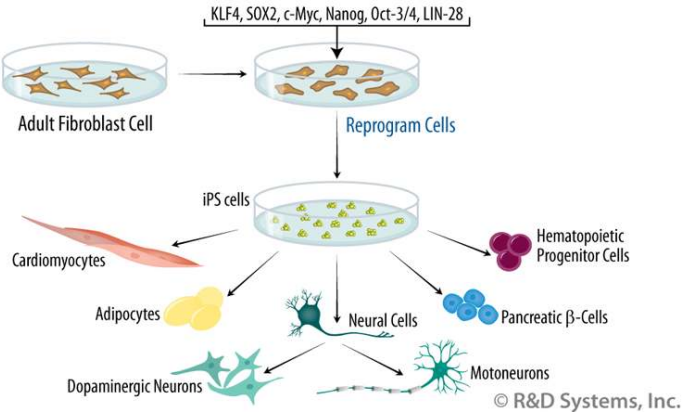
We hypothesise that, in order to prevent the brain from accumulating additional beta-amyloid plaques, the CRISPR method seems to the favourable choice to make

because of its effectiveness and higher quality to genetically delete the BACE1 gene that is the cause of Amyloid aggregation that leads to the formation of senile plaques. To promote such a process to completion, we also introduce neural stem cells (NSCs) to this solution in which NSC transplantation could, at the same time, reduce neurotoxicity. Thus, our proposal aims to provide an innovative solution by utilising the combination of CRISPR/cas9 and stem cell technology as alternatives to modern mechanism AD therapies as a means to eliminate the production of beta-amyloid protein within the hippocampal region in the brain, thus reduce neuroinflammation.

CRISPR/CAS9 method



CRISPR cas9 is an advanced technology that operates to add, correct, and remove genetic sequences. It contains the cas9 enzyme and guided RNA by the guided RNA located at particular genes and shares the homologous sequence with the target DNA. Next, the enzyme would act like scissors, cutting the strands of DNA. This system was naturally adapted from the function of bacteria that capture the DNA of invading viruses and create DNA segments to remember the viruses. This mechanism has become very successful in genome engineering because of its accuracy in editing, unharmed to human, and inexpensive compared to other editing tools.



Induced Pluripotent Stem cells (iPSCs)

Induced pluripotent stem cells are the pluripotent stem cells that do not require eggs to create but genetically reprogram the normal adult somatic cells, such as skin or blood cells, to behave similarly to embryonic stem cells. By induced pluripotency, iPSCs potentially enable to differentiate into any types of cells, such as neurons. This system has improved the health treatment because it considers ethical issues and efficiency suits the tissue transplantation therapies and generates perfectly matched cells.

Literature review:

Based on the current studies on Alzheimer Disease (AD), Transplantation of induced NSCs from, in vitro, Human-induced pluripotent stem cells (hiPSCs) - derived neural cells have shown promising potential and has been widely recognised for their benefit and uses in neurodegenerative disease therapies including AD due to their self-renewable capacity and potency to differentiate into specialised cell types as such neuron cells. Not to mention its recent achievement as a human disease modeling for Parkinson's disease(PD), hiPSCs serve as a probe to explore the mechanism of the disease and as an 'in vivo' platform for potential drug and medication screening.[6] Additionally, other than its favorable capabilities, the research on hiPSCs and PD published by BMJ journal [6] displays the critical practice of hiPSCs as an alternative to human embryonic stem cells (hESCs). hiPSCs are circumnavigating ethical issues associated with the destruction of the human embryonic blastocyst entailed by the harvesting of hESCs. This way of practice results in the death of the human embryo; therefore, it runs a major ethical issue with the onset of human personhood. Thus, hiPSCs hold great promise for regenerative medicine.

Likewise, in the “Stem cells therapy for Alzheimer’s disease” written by Liu et al., this literature review examines the transplantation of hiPSCs derived choline-rich NSCs could reduce AD symptoms in rats and shows a significant improvement in cognitive function.[7] “Despite these successful studies, autologous hiPSCs may show genetic instability and phenotypic neuropathology, such as significant Aβ load rates, shortened axon lengths, and increased tau phosphorylation, hindering their clinical application in AD” [7] To settle this problem, the researchers suggest using CRISPR/CAS9, as a part of the gene-editing process in neuron transplantation to increase qualities and stabilities of the hiPSCs itself.

However, rather than regrowing new neurons by implanting hiPSCs derived neural stem cells, we should consider the root cause of the disease rather than attain some superficial solutions to the problem. By this means, without getting cleared of the Aβ fragment cleaved from β-APP located within the neuron's membrane that broken down via β-secretase, no matter how stabilised the newly rejuvenated hiPSCs derived neurons was, it will finally encounter neurodegenerative progress results from the accumulation of Aβ that eventually leads to extracellular (SP).

In light of these studies, we would like to introduce an innovative approach and idea regarding regenerative medicine for AD patients via using CRISPR/CAS9 to knock out the specific gene produced B secretase, and the reprogramming hiPSCs derived neural stem cells through the use of CRISPR/CAS9 to stabilise and to ensure no gene would potentially arise Aβ in their the mature functional stage. Additionally, as a

tool to prevent the risk of teratoma formation. This will inhibit the action of the B secretase enzyme and violate the formation of Aβ, which is the primary cause of neurons malfunctioning. Correspondingly with the regrowing hiPSCs derived NSCs that replenish the neuronal loss from the SN.

Research Methodology and Innovation:

Since there is completely no cure for Alzheimer's that causes damage to the brain due to the accumulation and aggregation of Aβ40 and Aβ42 results in the formation of SP appears to be the primary cause of AD. We propose to test the hypothesis that SP within the brain is needed to be removed by prior cutting off of Amyloid-beta (Aβ) and Aβ40 and Aβ42, along with the introduction of neural stem cells for a reduction in neurotoxicity.

Therefore, the innovative solution to this principle causation can be denoted in a three main steps approach via the potential utility of CRISPR/CAS9 (Clustered regularly interspaced short palindromic repeats) by targeting and knocking out specific gene producing β site amyloid precursor protein cleaving enzyme 1 (BACE1) and, in vitro, induced pluripotent stem cells (iPSC) derived neural stem cells (NSCs) to grow and replace the death neuron ascribable to SP deposition as a treatment option for AD. These can be explained as follows;

1. **Targeted deletion of BACE1 gene within the hippocampus region of the brain using CRISPR-CAS9 with sgRNA loaded into the nanocomplex delivery**

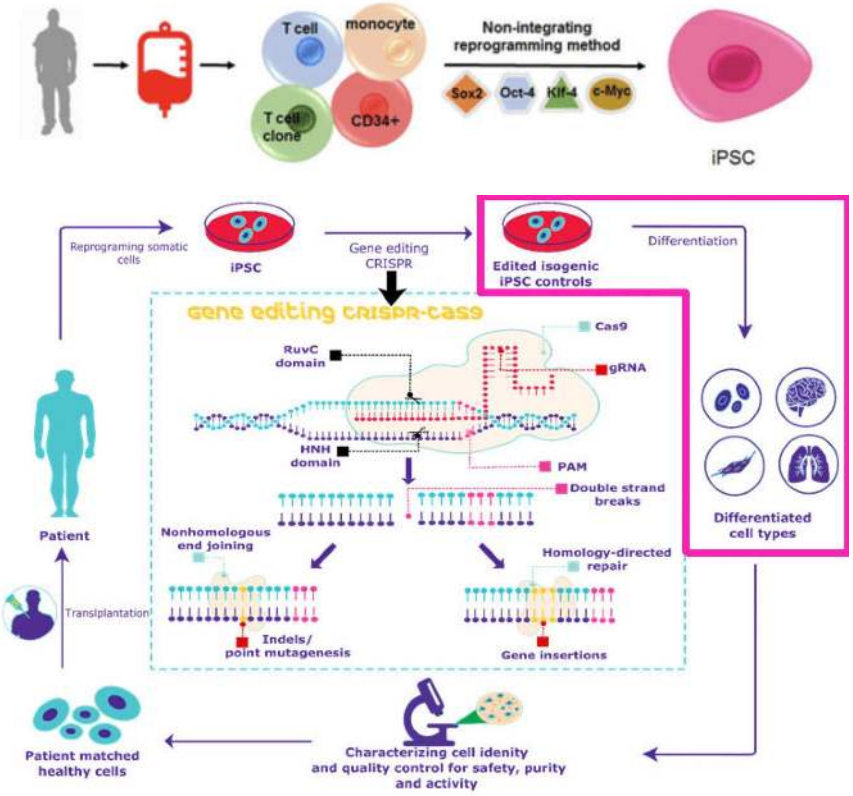
   Per our expectation to disrupt the BACE1, a modified CRISPR-CAS9 method would be possible to knock out such BACE1 gene to eliminate Aβ protein production. Our aim, therefore, is to examine if modified CRISPR could be possibly injected via nanocomplex delivery into the brain, thus inhibiting amyloid plaques. In vivo gene targeting in post-mitotic neurons in the Hippocampus region using CRISPR-CAS9 based diseases altering can be done by loaded CAS9 and simplify single-guide RNAs (sgRNAs) forming a Ribonucleoprotein complex (CAS9:sgRNA complex) for directed CAS9 protein to undergoes the bind and cleave at a site-specific gene targeting the BACE1 gene, into a stabilised lipid-soluble nanocomplex. [8]

   Therefore, to determine if the formation of CAS9 with sgRNA CRISPR inside the nanocomplex is successful, Size Exclusion Chromatography (SEC) Assay and Electrophoretic Mobility Shift Assay (EMSA) could be performed to analyse the interaction between Cas9–sgRNA–DNA complex [9]. Such assays could label fluorescently on DNA oligonucleotides to detect the sgRNA and DNA structures. Lastly, we also plan to examine the genetic stability within the pool of post-mitotic neurons.

2. **Reprogramming of human peripheral blood cells and genetic knock-out and examining the nano delivery intrahippocampal injection of the nanocomplex.**

Utilizing the potential of human-iPSC (hiPSC) based technology to induced pluripotency in human peripheral blood cells by reprogramming and treated with defined factors [10] into active NSCs, seeing as this approach is already proven to be effective in the past study by Jing Zhao et al. [11]. To successfully obtain the modified hiPSCs derived NSCs, the CRISPR knock-out technique must be performed in order to get rid of the gene that produced BACE1 to ensure the regrowing NSCs from blood cells derived-hiPSCs. Such blood cells do not contain any gene that will generate additional Aβ and possibly lead to SP in the future. Then, we load the modified hiPSCs derived NSCs into the nanocomplex along with the nerve growth factor (NGF) to increase the proliferation of the NSCs [12].

Succeedingly, we are introducing it to target BACE1 in vivo in the neurons by direct nano delivery intrahippocampal injection into the cerebral fluid within the hippocampus region, without passing the blood-brain barrier (BBB) blockage will ensure the circumnavigation of the nanocomplex into the membrane of the hippocampus.

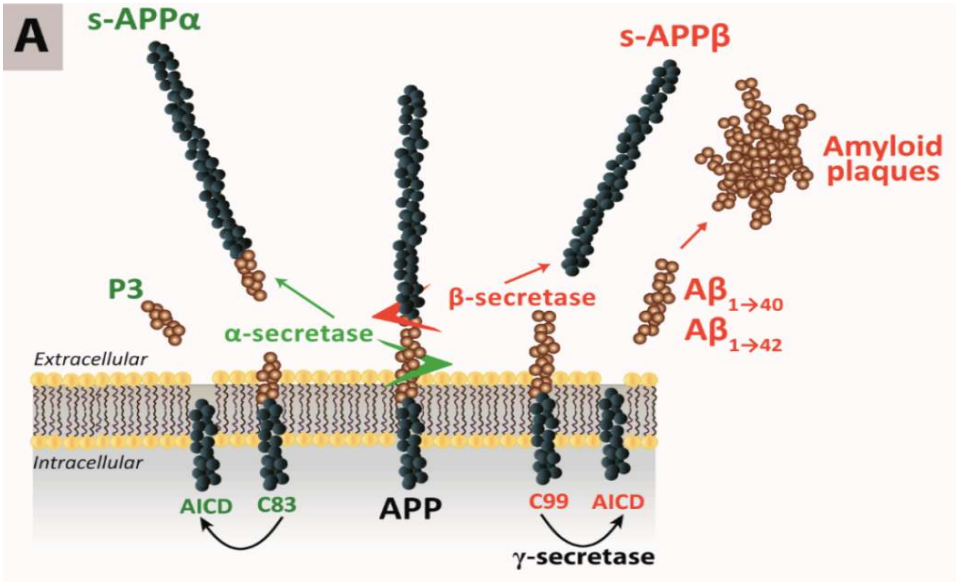


(source: https://www.sigmaaldrich.com/technical-documents/protocols/biology/cell-culture/human-ipsc-crispr-protocol.html)

3. **Monitoring the regrowing hiPSCs derived NSCs through MRI and the clearance of senile plaques accumulation by an amyloid PET scan.**

The final step will be to monitor the efficacy of the differentiation and distribution of the hiPSCs implantation in the hippocampal region, which can be determined through Magnetic resonance imaging or MRI [5] by measuring the brain function.

At the same time, we can use a Positron Emission Tomography (PET) imaging to visualise an in-vitro detection of the AB Plaques deposition present in the regrowing area [13] that three FDA had approved as an amyloid radiotracer used in clinical practice. The envision of the amyloid PET scan can be observed by labelling the ligand that is attached to SN as a targeted substrate with 18F-labelled radiopharmaceuticals (radioisotope Fluorine F18). As 18F is a short radioactive half-life compound, it crosses the BBB and selectively binds to SN via emitting its positron after it undergoes a decaying process. Then, use the PET's positron scan tomography to measure the decaying of the injected radiopharmaceutical within the hippocampus.



Apart from amyloid PET scanning, Aβ could possibly be identified by using a developed sensitive assay and western blot analysis to trace C99, the direct precursor of Aβ [14]. The image above depicts the amyloidogenic pathway of the Aβ biogenesis from both α and β secretase. By looking at the cleavage of the embedding APP as a result of β-secretase (BACE1) fragmentation, soluble APP β protein (s-APPβ), and C99 produced. γ secretase then cleaves C99 to generate Aβ and amyloid precursor protein intracellular domain (AICD). According to Pulina et al. and their research on the "C99 selectively accumulates in vulnerable neurons in Alzheimer's disease", had shown that C99 levels are correlated with the degree of cognitive impairment in patients

suffering from AD. Therefore, detecting C99 would be an alternative option to determine the built-up of Aβ.

Trial study on non-human primates:

For this experiment based on AD patients suffering from neuronal degeneration, we decide to bring in a large animal model such as Guania pigs to mimic the disease. Since the genetic tool (i.e. CAS9) can be used to modify the genome in experimented animal to create a loss- or gain-function model, this genome editing was successfully done in transgenic mouse and pig models of Huntington's disease (HD) [15] that finalise the conclusion that the larger animal show more accurate result similar to the patients suffering from HD.

Limitations:

Genetic deletion of BACE1 could influence the remyelination of nerve cells. In spite of the fact that the BACE1 gene is the preventive target for AD therapies as is necessary for reducing AB deposition. However, according to Hu et al., had investigated 'Bace1 modulates myelination in the central and peripheral nervous system' in the non-primate animal during the preclinical trial, which shows that genetic deletion of BACE1 results in hypomyelination. Where there is an abnormally limited amount of myelination around nerve cells that have the likelihood to decrease the efficiency of electrical transmission, therefore, this must be investigated further to promote the mechanism of curing AD in the future.

Expected outcomes:

Since the curing treatment can't stop Alzheimer's disease from progressing, it inspired us to study the possible solutions to treat this disorder in long-term conditions. The formation of CAS9 with sgRNA CRISPR inside the nano complex could directly solve the main complication of the issues because the BACE1 gene that produced the Aβ protein would get eliminated by utilising CRISPR cas9 to detect the targeting gene. Then, reprogramming the blood cell turns into hiPSCs that do not contain any gene that generates Aβ, inserting through the hippocampus. The success of this technology significantly benefits healthcare, patients, and the world because this advanced technology will improve the quality of Alzheimer's treatment, so patients and healthcare, instead of paying the personal care costs, rather spend it on the CRISPR cas9 methods that are cheaper, safer, and more efficient. Also, the number of Alzheimer's patients and the expansion from this disease will decline, significantly support the growth of the economy, and contribute the fund to the other treatment that needs it. Besides, the hiPSCs can regenerate the tissue, especially still relying on medical ethics; therefore, it could be involved in the therapy of various diseases and might be satisfied with medical methods in the future. Nevertheless, if the treatment can only control the symptoms, the harshness of the disease would be more dangerous, and more people have to struggle with dementia. Hence, the combination of CRISPR cas9 and hiPSCs based neural stem cells can be the choice for the innovation of Alzheimer's treatment.

Conclusion:

As technologies have improved, scientists have been investigating the innovation of medicine and discovered the possible solution to stop AD. The combination of CRISPR cas9 and iPSCs derived NSCs carried in the nano complex removes the BACE 1 gene that cleavage the APP protein generates beta-amyloid and the insertion of the new NSCs in the replacement; as a result, according to the Journal of Alzheimer's disease had proved that deletion of BACE1 reduces 60%of the beta-amyloid production.[16] This, therefore, would help the neurotransmitter flowingly transport throughout the neuron system. Moreover, the reduction in Aβ can be monitored via PET scan which the easy flowing of neurotransmitters is inverse proportional to the amount of with the Aβ. The iPSCs-delivered NSCs automatically differentiate into neurons and enhance the speed of neurotransmitters. If the scientists study more about this engineering tool, CRISPR cas9, and iPCSs NSCs, the hope of using this clinical method in the future will remain, helping to reduce the AD patients population in the long term. Plus, promote the advancement in the field of biomedical engineering and its research to another step.

(Words count: 2703)

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