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Investigating ER Stress as A Potential Cause to Alzheimer's Disease

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Abstract: Through the purposed series of experiment, this study is targeted to determine the correlation between ER Stress and AD. The gene that the proposed series of experiment will utilize is the CFTR gene as it the mechanisms are well studied, and it could be incorporated into mouse cells and tissue. The mutated CFTR gene, specifically the most common △F508 mutation that is clinically associated with cystic fibrosis would cause misfolded CFTR protein. The degraded mutant protein would be retained in the ER of the cell, and eventually the accumulation of the misfolded protein would lead to ER stress [3,4]. ER stress is induced by injecting CRISPR-Cas9 edited CFTR coding gene into mouse hippocampus. The mutant gene is delivered by a viral vector that is directly injected into mice.

Keywords: Alzheimer's Disease, ER Stress, Viral Theory.

1. Introduction

Over 50 million individuals worldwide suffer with Alzheimer's disease (AD), a progressive neurological disease. Although prevalent, the exact mechanism that causes AD remains unknown due to the variety of risk factors that contribute to the risk of AD. One major characteristic of AD is the β-amyloid protein in the cerebral cortex. A study by Lee, et al. demonstrated that viral infections like the Zika virus could promote differential expressions of proteins that are linked to AD. Zika virus proteins are found close to the endoplasmic reticulum, causing unfold protein response (UPR) and eventually leads to ER stress within host cell [1]. Furthermore, a study published by Ajoolabady, et al. examined the relationship between ER stress and UPR in Alzheimer's disease and concluded that ER stress plays a role in the onset and development of AD. [2]. Thus, the correlation between ER stress and AD as whether ER stress can be a potential cause to AD is worth investigating.

2. Experimental design

2.1. Mutation of gene coding for CFTR

Identify an area-specific 20 nucleotide sequence that flanks the mutation site near exon 10 of the CFTR gene where the F508del mutation occurs. This sequence is used to design a gRNA that targets exon 10 of the CFTR gene. The gRNA can co-deliver a single-stranded DNA template for homology-directed repair (HDR) for CRISPR-Cas9 to introduce the desired point mutation. The CRISPR-Cas9 components are packaged into AAV9 (adeno-associated virus) vectors using standard virus production protocols. 0.5 to 1 μL of AAV9 vector solution are then injected to both sides of the

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hippocampus in mice at a rate of 100 nL/min using stereotaxic injection methods [5]. Mouse used in this study should be between 6 to 8 weeks old and another group of mice with no treatment is prepared to serve as the control group of this experiment.

2.2. Construction of viral vector

Take the gRNA under U6 promoter and Cas9 under neuronal promoter for cloning, and after cloning include the repair template to enable HDR. Grow and transform bacterial colonies and sequence the plasmid to confirm the successful insertion of Cas9, gRNA, and the repair template. Transfect HEK293T cells with the previously assembled CRISPR-Cas9 plasmid, helper plasmid, and packaging plasmid. Harvest AAV material after 48-72 hours by collecting cell lysate and culture medium. Purify the collected cell lysate and medium using iodixanol gradient ultracentrifugation.

2.3. AAV packaging

The transfection complex is composed of the vector plasmid, helper packaging plasmid, AAV-RC serotype plasmid in a RFect293 transfection reagent mixed with serum free medium. The complex is then transfected to a Neuro-2a cell line.

2.4. Assessment of CFTR mutation efficiency

Extract and prepare DNA samples from the infected mice group after 6 to 8 weeks from the initial viral infection. The samples are then taken under PCR to amplify the region that was targeted by CRISPR-Cas9. Then perform Sanger sequencing to confirm the presence of F508del mutation and or any insertions or deletions in the CFTR gene in the samples. After sequencing, perform a western blot to assess the levels of CFTR protein in the mouse hippocampus. A reduced or absent CFTR expression would indicate a successful mutation [6, 7].

2.5. Assessment of ER stress

In CFTR mutant and control samples, perform western blot to detect ER stress markers (biP, CHOP, p-eIF2α, ATF4) and use Image J to quantify resulting protein levels. Conduct qPCR on both samples to measure the mRNA expression of previously mentioned ER stress markers, then normalize on a housekeeping gene to analyze the results using ΔΔCt. Then quantify and visualize the markers using immunocytochemistry (ICC), and stain sample with DAPI to run fluorescence microscopy. Use XBP1 Splicing Assay to detect spliced XBP1 via RT-PCR. Run PCR products on an agarose gel to confirm XBP1 splicing. Analyze Data by comparing ER stress marker levels between CFTR-mutant, control, and ER stress-induced cells.

2.6. A-β protein tangle analyzing

Prepare live tissue samples of both mutant and control mice hippocampus tissue. The samples are then stained using immunohistochemistry with antibody 6E10 for Aβ plaques and tangles [8]. After staining, use Image J or similar biological image processing software to quantify the number, size, and distribution of Aβ tangles in the hippocampus tissue for both control and mutant samples. Compare the difference between the control and the mutant group sample. Then, homogenize both tissue samples and conduct western blot to quantify the levels of Aβ40 peptides. In the end, use anti-Aβ antibodies and ELISA assay to quantify the protein in both samples. Compare the results of both samples and use a T-test to analyze the statistical significance of the difference in both samples.

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3. Evaluation

From the designed series of experiments, the mice samples should demonstrate successful CFTR gene mutant expression. From the successful CFTR mutant gene expression, the accumulation of improperly folded protein within mice cell should eventually lead to ER stress in cell. The ER stress in cell would then cause AD markers to rise in the mutated mice group. The specific rise of Amyloid beta 40 (Aβ40) peptide would ultimately contribute to Alzheimer's Disease in mice and confirming the link between ER stress and AD.

Although the series of experiments in theory could lead to promising results on the hypothesis of ER stress as a possible cause of Alzheimer's Disease, the induced ER stress onto viral vector and eventually into mouse is dependent on the CFTR encoding gene. Yet Alzheimer's Disease is not commonly considered to have any correlation between CFTR gene and its mutations. Furthermore, the clinical treatments of cystic fibrosis and other CFTR related diseases do not involve reliving ER stress.

To further investigate the correlation between ER stress and AD and to validate the results of the purposed study, different methods of inducing ER stress to mice sample including using different gene mutations or other types of viruses. Other model systems like brain organoids can serve as an alternative to mice to model the effect of ER stress and later developed AD. Since the purposed study utilized the CFTR gene as the initial approach into inducing ER stress into cell and the CFTR gene is heritable, longitudinal studies can be done on the descendants of the mutated mice group to observe the heritable aspects of CFTR mutation induced ER stress and the rate of AD on later generations. Clinical observational studies are also highly recommended to determine the link between ER stress and AD. If all the purposed studies provide promising results on ER stress and its positive correlation with AD, then the effect on therapeutic methods on reliving ER stress and its effects on patients with early stages of AD is also on the horizon.

4. Conclusion

This paper purposes a series of experiments that aim to prove a correlation between ER Stress and the onset of Alzheimer's Disease. The series of experiments also referenced the viral theory of Alzheimer's Disease. If the series of experiments are to be conducted and the results show statistical significance towards proving ER Stress and Alzheimer's, then it would provide more insight into the mechanisms of Alzheimer's Disease as well as a new approach towards clinical treatment and prevention of Alzheimer's Disease.

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