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Relationship Between ER Stress and CFTR Gene and Its Role in Alzheimer's Disease

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Abstract: This research proposal aims to study the relationship between CFTR gene and ER stress and its potential involvement in the aetiology of AD. Using CRISPR Cas9 technology, we intend to induce the F508del mutation in the CFTR gene of a mouse model in a way that mimics the pathophysiological state of Cystic Fibrosis. It describes a study design, including the assessment of spatial learning capability in the Morris Water Maze experiment, and the analysis of Aβ protein aggregation in the hippocampus. The results may give clues as to the molecular mechanisms of Alzheimer's disease and help identify new targets for therapeutic intervention.

Keywords: Endoplasmic Reticulum stress, CFTR gene, Alzheimer's disease, Unfolded Protein Response, CRISPR-Cas9

1. Introduction

The endoplasmic reticulum acts as an essential membrane organelle in the cell structure. The organellae are thought to carry out several tasks in creating lipids and steroids and ensuring Ca2+ balance and storing as well as aiding carbohydrate metabolism and protein formation [1]. When properly functioning the ER can shape and operate proteins correctly. The ER's internal environment can be disrupted by specific pathological conditions and then activates the ER stress response.

This membranous organelle will react sharply to modifications that can disrupt its stability and ability to work correctly resulting in problems with protein folding. If the correct folding of proteins fails to occur misfolded proteins move to the cytoplasm where they receive ubiquitination followed by proteolytic breakdown known as ERAD [2-3]. When too many unfolded or improperly shaped proteins gather in the cell the ER struggles to regulate the protein inventory which triggers ER stress and influences numerous diseases.

To deal with stress eukaryotic cells use mechanisms that decrease protein synthesis and boost levels of genes transcribed for protective proteins. These cell reactions start with the activation of a linked pathway of intracellular signals labelled the Unfolded Protein Response (UPR).

The three major sensor proteins modulating the UPR are PERK, which stands for PKR-like ER kinase; IRE1α, or inositol-requiring enzyme 1α; and ATF6, which is short for activating transcription factor 6. These proteins are responsible for the maintenance and determination of the proper ER stress response. Under unstressed conditions, these proteins remain in their inactive forms through interactions with BiP or GRP78. It is suggested that the ATPase domain of BiP interacts with both PERK and IRE1α and that upon binding of an unfolded protein to the substrate-binding domain of

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BiP, it causes a dissociation of PERK and IRE1α from BiP [4-5]. During times of ER stress, BiP's release starts the UPR cascade by causing PERK and IRE1α to connect and autophosphorylate.

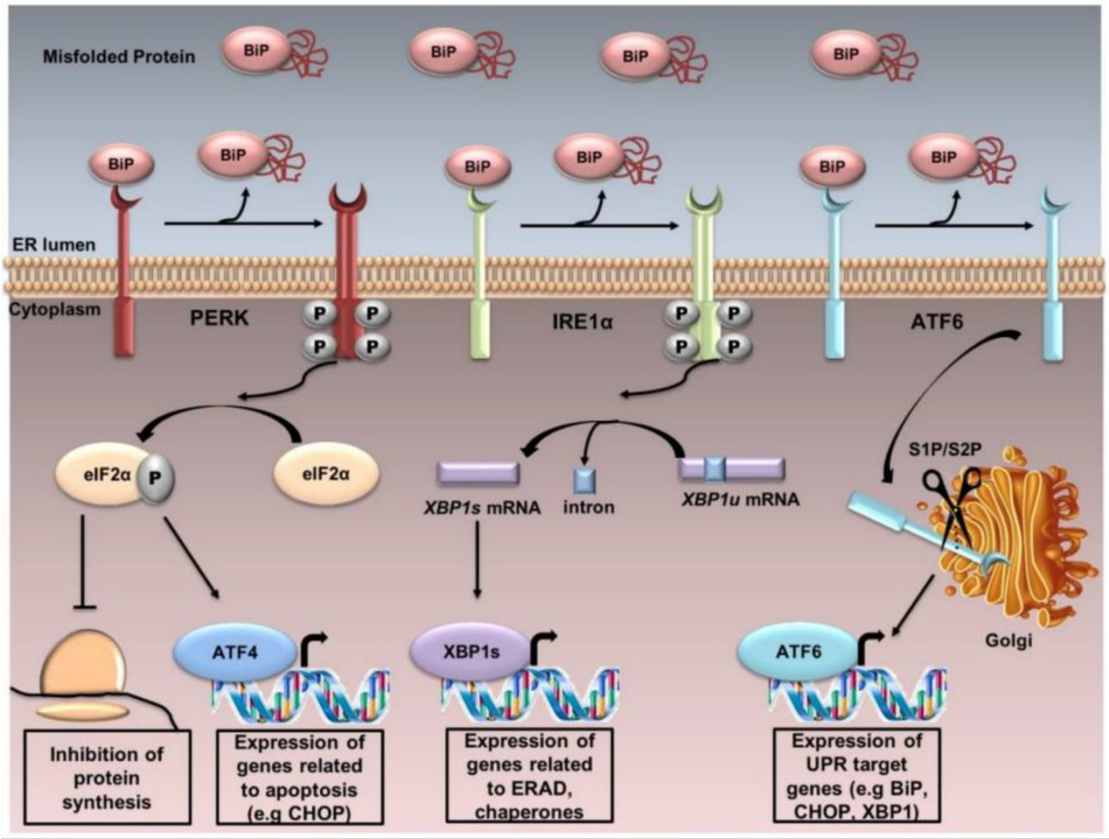

Figure 1: Three UPR sensor proteins [6]

In disorders such as Alzheimer's disease the sustained pressure on the ER and the UPR's imbalance may trigger harm to cells and neurons. This disorder of ER stress and UPR regulation ranks among the primary causes of Alzheimer's disease.

Since there are no reports of systemic damage after the direct injection of Thapsigargin, we utilized the CFTR gene that expresses Cystic Fibrosis Transmembrane Conductance Regulator protein. It is an ATP-binding cassette transporter family protein, which is unique for its chloride channel activity. It plays an important role in the regulation of ion secretion and water absorption through epithelial tissues. The activation of this channel requires a complex process of phosphorylation of the regulatory domain, ATP binds to the nucleotide-binding domains, and also hydrolysis of ATP. Mutations in the gene coding for the CFTR protein cause cystic fibrosis. It is a genetic disorder and most frequently affects individuals of Northern European origin. The most common mutation leads to impaired folding and trafficking of the resulting protein and it has been termed DeltaF508 [7-10].

This research will look into the possibility that mutations in the CFTR gene cause ER stress and may be part of Alzheimer's disease aetiology. Various genetic analyses and biochemical examinations will allow us to evaluate the consequences of CFTR-induced ER stress on hippocampal capability and A-beta protein formation. Our data may shed light on the challenging dynamics of ER stress in relation to Alzheimer's disease.

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2. Material and methods

2.1. Mutation of gene coding CFTR

To target the F508del mutation in exon 10 of the CFTR gene, we designed a guide RNA (gRNA) that specifically binds to a critical 20-nucleotide sequence adjacent to the mutation site. This selection was based on computational predictions to minimize off-target effects while ensuring high on-target efficiency. We accompanied the gRNA with a single-stranded DNA template for homology-directed repair (HDR), optimizing the sequence to improve repair efficiency and fidelity.

The CRISPR-Cas9 system was packaged into adeno-associated virus serotype 9 (AAV9) vectors, chosen for their high transduction efficiency in neuronal tissue and low immunogenicity. These vectors were prepared following standard viral production protocols. For in vivo delivery, the AAV9 containing CRISPR-Cas9 was administered into the hippocampus of 6-8 week-old mice via stereotaxic injection, with a precise delivery of 0.5 to 1 microliter of the vector solution at a rate of approximately 100 nanoliters per minute.

Following an incubation period of 6-8 weeks to allow for gene editing and expression, hippocampal tissue was harvested for analysis. Genomic DNA was extracted and the targeted region of the CFTR gene was amplified by PCR, followed by Sanger sequencing to confirm the introduction of the F508del mutation and assess for any indels. Protein expression levels of CFTR in the hippocampus were quantified using Western blot analysis to evaluate the success of the mutation and its expression.

2.2. AAV packaging

Construction of a plasmid with the CFTR gene coupled with the desired mutation marked the start of packaging AAV. In addition to the plasmid with the CFTR gene mutation for AAV generation other plasmids encoding CAP and REP were also developed. Neuro-2a cells received a mixture of the plasmids via transfection using RFect293 reagent in an unsupplemented medium to promote the uptake of the DNA complexes.

After transfecting the necessary plasmids into the cells they were placed into incubation to facilitate the formation of the virus. A sample of cell extracts and media was taken for Western blot evaluation to locate ER stress biomarkers including p-eIF2α and BiP. At the same time qPCR analyzed mRNA levels of these markers by adjusting for housekeeping gene expression.

To examine and quantify protein location and expression levels through visualization techniques; immunocytochemistry (ICC) was conducted. Fluorescence microscopy was used to analyze the localization and concentration of ER stress-related proteins after cells were stained with DAPI and fixed.

2.3. Construction of viral vector

We started by isolating the CRISPR-Cas9 elements containing the Cas9 enzyme and the guide RNA directed by a neuron-active promoter into a plasmid structure that works with AAV. We included repair templates to support homology-directed repair (HDR), which is crucial for repairing DNA that Cas9 has cut.

Then we added bacteria with this plasmid to allow replication and generate numerous copies. We developed bacterial colonies and examined the plasmid to confirm the proper positioning of Cas9 and repair components.

After validating the sequence we transferred HEK293T cells with the CRISPR-Cas9 plasmid along with crucial helper and packaging plasmids. During this procedure the cells generated AAV particles laden with our CRISPR-Cas9 equipment.

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In 48 to 72 hours we obtained the AAV particles from cells and from the culture medium. We retrieved the supernatant from lysed cells as well as the broth in which they were maintained. We purified the AAV particles by using iodixanol gradient ultracentrifugation. This method rapidly spins the mixture to distinguish particles according to their density. And to determine the number of viral genomes in our sample we conducted qPCR analysis.

To administer the AAV solution of 0.5 to 1 microliter to both hippocampal areas in mice we performed stereotaxic surgery at a rate of about 100 nanoliters per minute. In mice the hippocampus plays a vital role in learning and memory. By placing the virus here; the CRISPR-Cas9 system can modify genes within these brain cells.

2.4. Morris water maze experiment

Analysis of data requires the grading of the recorded delays and the durations in each quadrant by drawing on tracking information. Evaluating the performances of two groups allows for the discovery of variations in their spatial learning capacity.

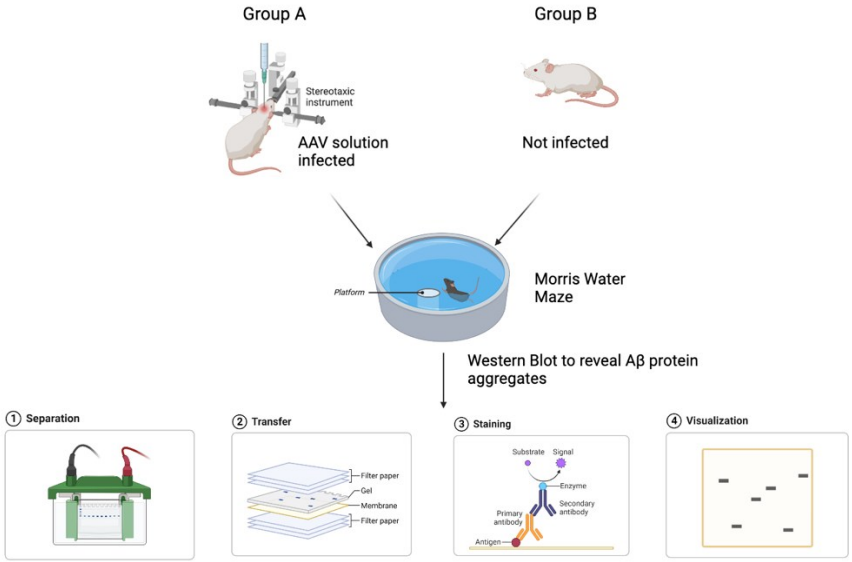

Figure 2: Experiment procedures

2.5. Aβ protein tangle analyzing

Mice receiving treatment had their hippocampal tissue examined using immunohistochemistry to reveal Aβ protein aggregates. Sections underwent staining with the 6E10 antibody to evaluate plaque characteristics and markings. A reliable examination of staining intensity and area was possible through image analysis tools.

The evaluation of total hippocampal Aβ protein contents was carried out through Western blotting to compare the results in CFTR-mutated mice to unaffected controls and interpret the mutation's role in Alzheimer's disease.

3. Expected results

We examine CFTR mutated mice samples to verify that the CFTR gene has indeed undergone successful changes. It's essential to ensure that the mutation is seen and functioning within the mice.

If the CFTR gene is altered correctly it triggers stress within cellular endoplasmic reticulum. When genes are altered in certain ways it results in stress within the cells related to improper protein folding.

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We expect to detect heightened levels of markers related to Alzheimer's disease in the CFTR mutated mice over a control group lacking the mutation.

We place great importance on measuring the Aβ40 levels since they accumulate in those with Alzheimer's. The mice with the mutation show elevated Aβ40 levels which point to a relationship between ER stress and Alzheimer's signs. If we see these symptoms in mice undergoing the CFTR mutation this would strongly suggest a tie between ER stress and Alzheimer's development.

Understanding this link between the CFTR mutation and increased Aβ40 levels is essential for recognizing how genetic elements might shape Alzheimer's and could identify new medication targets.

4. Discussion

While this study represents a first step toward understanding how ER stress might be linked to Alzheimer's disease, we have to admit that there are challenges that we have yet to be addressed in future studies. There is currently a real effort in the CF field about perhaps how alterations in CFTR gene could be linked to ER stress. However, Alzheimer's is an extremely complex disease, it is not only about genes but also about lifestyle and environment. Therefore, it might be oversimplistic to claim that these are the causes of Alzheimer's, the same as saying CFTR gene changes cause cystic fibrosis.

Also, we've seen some signs that ER stress and Alzheimer's might be connected in mice, but we're not sure if that's the same for people. On the other hand, this study mostly looked at what happens when we make ER stress happen, not what happens if we try to reduce it.

To get past these problems and figure out what's going on, a few things can be done in future studies. First, research with more people to see if there's a real link between CFTR gene changes and Alzheimer's. Also, looking for other gene changes that might be related to ER stress. Second, use other ways to repeat the experiments, for example, using brain organoids, and testing on different kinds of animals to see how ER stress might play a role in Alzheimer's. Third, we can try giving some medicines or doing other things to change how much ER stress there is, to see if it helps with Alzheimer's. For instance, check if medicines that reduce ER stress can slow down the disease or help people think more clearly. Also, because changes in the CFTR gene can be passed from parents to their babies, we can watch mice with these changes over a long time. This can help us see how ER stress might be linked to Alzheimer's in the long run.

5. Conclusion

The objective of the proposed research is to relate ER stress caused by the CFTR gene mutations to Alzheimer’s disease. We think that manipulating the CFTR gene in a mouse model will give us a better understanding of the role of ER stress in neurodegenerative processes. Results of the proposed study may suggest how CFTR mutations affect cognitive function and Aβ protein accumulation, as new paths for therapeutic strategies. The translation of these findings to clinical applications and future research directions exploring ER stress modulators as potential treatments for Alzheimer's disease is highly possible.

References

[1] *Schwarz D.S., Blower M.D. The endoplasmic reticulum: Structure, function and response to cellular signaling. Cell. Mol. Life Sci. 2016;73:79–94. doi: 10.1007/s00018-015-2052-6*

[2] *Lopata A, Kniss A, Löhr F, Rogov VV, Dötsch V. Ubiquitination in the ERAD Process. Int J Mol Sci. 2020 Jul 28;21(15):5369. doi: 10.3390/ijms21155369. PMID: 32731622; PMCID: PMC7432864.*

DOI: 10.54254/2753-8818/2025.24216

[3] Zou L, Wang X, Zhao F, Wu K, Li X, Li Z, Li Y, Chen W, Zeng S, Liu X, Zhao M, Yi L, Fan S, Chen J. Viruses Hijack ERAD to Regulate Their Replication and Propagation. Int J Mol Sci. 2022 Aug 20;23(16):9398. doi: 10.3390/ijms23169398. PMID: 36012666; PMCID: PMC9408921.

[4] Anderson NS, Haynes CM. Folding the Mitochondrial UPR into the Integrated Stress Response. Trends Cell Biol. 2020 Jun;30(6):428-439. doi: 10.1016/j.tcb.2020.03.001. Epub 2020 Apr 2. PMID: 32413314; PMCID: PMC7230072.

[5] Carrara M., Prischi F., Nowak P.R., Kopp M.C., Ali M.M.U. Noncanonical binding of BiP ATPase domain to Ire1 and Perk is dissociated by unfolded protein CH1 to initiate ER stress signaling. Elife. 2015;4:e03522. doi: 10.7554/eLife.03522.

[6] Ghemrawi, R., & Khair, M. (2020). Endoplasmic Reticulum Stress and Unfolded Protein Response in Neurodegenerative Diseases. International Journal of Molecular Sciences, 21(17). https://doi.org/10.3390/ijms21176127

[7] Dean, M., Santis, G. Heterogeneity in the severity of cystic fibrosis and the role of CFTR gene mutations. Hum Genet 93, 364–368 (1994). https://doi.org/10.1007/BF00201659

[8] C. Bareil, A. Bergougnoux. CFTR gene variants, epidemiology and molecular pathology. Archives de Pédiatrie, 2020, 27, pp.eS8-eS12. ⟨10.1016/S0929-693X(20)30044-0⟩. ⟨hal-03477908⟩

[9] Lopes-Pacheco M. CFTR Modulators: The Changing Face of Cystic Fibrosis in the Era of Precision Medicine. Front Pharmacol. 2020 Feb 21;10:1662. doi: 10.3389/fphar.2019.01662. PMID: 32153386; PMCID: PMC7046560.

[10] McDonald EF, Meiler J, Plate L. CFTR Folding: From Structure and Proteostasis to Cystic Fibrosis Personalized Medicine. ACS Chem Biol. 2023 Oct 20;18(10):2128-2143. doi: 10.1021/acschembio.3c00310. Epub 2023 Sep 20. PMID: 37730207; PMCID: PMC10595991.