Effects of C-Terminal Mutations on Tau Protein Function and Cell Viability

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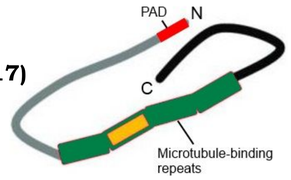
Introduction

Alzheimer's disease is the 6th leading cause of death in the United States yet has no current cure (Alzheimer's Association). It is a tauopathy: a neurodegenerative disease linked with abnormal neurofibrillary tangles (NFT) of aggregated tau protein in the central nervous system (UCSF Institute for Neurodegenerative Diseases).

The tau protein is an important microtubule-associated protein that typically attaches to and organizes neuronal microtubules, a function crucial to neurons which depend on microtubules to control cell shape and intracellular transport. Tau typically has 3 to 4 microtubule-binding repeat domains which code for parts of the tau protein where it binds to microtubules (Lee, G., Cowan, N. & Kirschner, M., 1988), which are part of the C-terminal of the gene, nicknamed the microtubule-assembly domain.

Figure 1: Tau protein structure (Lee, 2017)



However, tau's pathological roles of detachment from microtubules and aggregation have unclear mechanisms. It is clear, though, that the function and thus structure of the pathological tau protein is different from that of normal tau. Because of the role of mutations of altering protein structure, mutations, especially those in the C-terminal area, were investigated in this study as a possible link to tauopathy and neurodegeneration.

Objective

The objective is to explore the effect of C-terminal mutations on tau protein function, so as to understand the mechanisms underlying tau-related pathology as well as to investigate the possible role of mutations in neurodegeneration.

What is the effect of C-terminal mutations on tau protein function and cell viability?

- M0: 0N3R wild-type tau sequence (control)
- M1: 0N3R tau with deleted 621st base pair (experimental)
- M2: 0N3R tau with deleted 601st-660th base pairs (experimental)

Hypothesis: C-terminal mutations have negative effects on tau protein function and lead to decreased cell viabilities.

Materials and Methods

Two-pronged true experimental method:

(I): Bioinformatic/Database inquiry

- The effects of each sequence on protein function were inferred using comparisons of amino acid sequences (ApE software) and the number of tubulin binding repeat domains (NCBI Conserved Domain Search).

(II): Laboratory observation

- The effects on tau function were predicted through observation of the locations of the tau proteins of transfected COS-7 (monkey kidney tissue) cells expressing the sequences, done using GFP, DAPI, and tubulin staining. A cell viability assay using trypan blue dye was conducted to dye the dead cells.

Results and Interpretation (I)

Amino Acid Sequence Comparison:
M0 (352 a.a.)
M1 (205 a.a.): Frameshift mutation → Early stop → 147 a.a. Deletion
M2 (332 a.a.): 20 a.a. Deletion

Figure 2: Tubulin binding repeat domains from M0, M1, M2 amino acid sequences

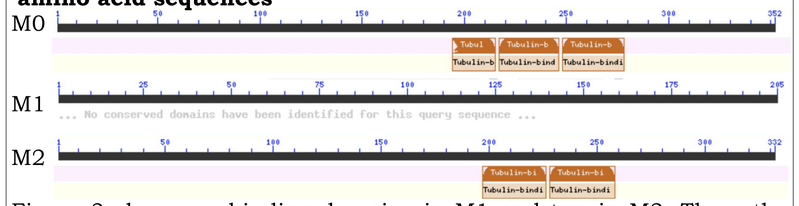
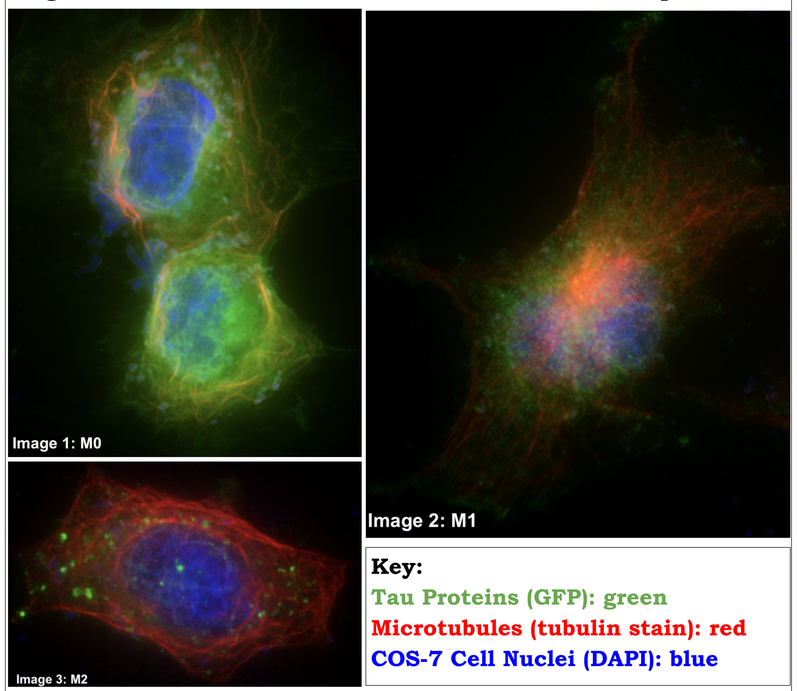


Figure 3 shows no binding domains in M1 and two in M2. Thus, the binding function would be negatively impacted in M1 and M2 tau, with M1 tau likely experiencing the least binding and greatest aggregation.

Results and Interpretation (II)

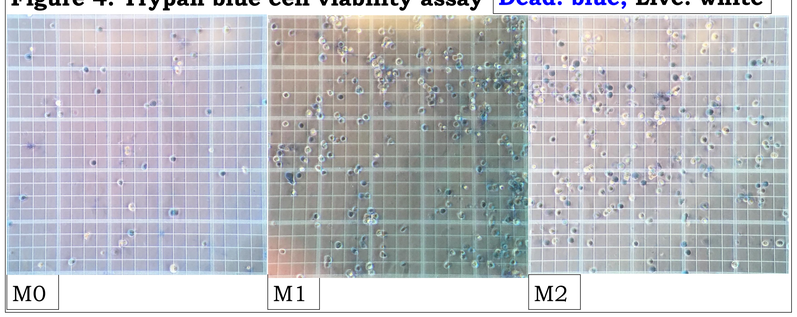
Figure 3: COS-7 cells transfected with M0, M1 and M2 plasmids



Key:
Tau Proteins (GFP): green
Microtubules (tubulin stain): red
COS-7 Cell Nuclei (DAPI): blue

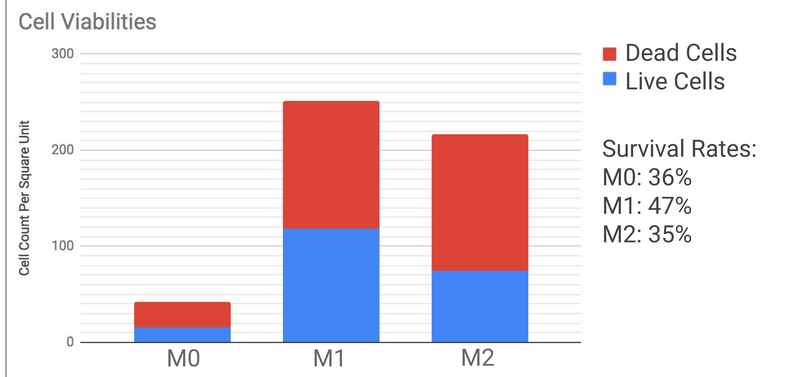
Aggregated, detached tau and unorganized microtubule shape in M1 and M2 cells (Figure 4) confirm the hypothesized negative mutation effect on the tau functions of binding and organizing.

Figure 4: Trypan blue cell viability assay **Dead: blue, Live: white**



Results and Interpretation (II) (cont.)

Figure 5: Cell Viability Comparison Graphics



The similar cell survival rates do not support the hypothesized decreased cell viabilities for mutated M1 and M2. The observed cell viabilities could be affected by other factors unrelated to the mutation, such as cell death through poor environmental conditions and the detachment and washing off of dead cells from the dish during buffer washes done before viewing.

Conclusion

Tau mutations lead to a negative effect on the functions of the tau protein and the decreased microtubule binding and tau aggregation apparent in neurodegeneration.

Implications and Next Steps

Utilizing neurons instead of COS-7 cells in the laboratory experiment, as well as more trials, could lead to more accurate modeling of the relationship between tau mutations and cell viability. However, the correlation between the functions of mutated tau and pathological tau illustrates the need to further investigate the possible role that mutated tau may play in neurodegeneration. A clearer understanding of the causes of pathological tau will greatly enable the discovery of drugs or treatments that can most effectively battle neurodegenerative diseases such as Alzheimer's disease.

Acknowledgements and References

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