Interaction of Different Tau Mutations In Vitro
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Abstract

The purpose of this research is to assist in the understanding of Alzheimer's disease mechanisms. Alzheimer's disease is a progressive disease that mainly destroys the memory of an individual and other important mental functions. Tau is a microtubule-associated protein whose purpose is to stabilize the microtubules in the neuronal axons in the Central Nervous System (CNS). Tau is a phosphoprotein and the degree of phosphorylation is very important for its normal functioning. Normally, tau contains 3 moles of phosphate per mole of protein. In Alzheimer's disease, tau protein gets hyperphosphorylated and contains 7.10 moles of phosphate per mole of protein. When hyperphosphorylated, tau cannot bind to tubulin and stabilize microtubules, instead, it binds to normal tau and sequesters it from microtubules resulting in microtubular disruption and ultimately death of the neuron. This research project was designed to examine the interactions between different tau constructs. In our lab, we have four tau constructs (wild type wt), tau hyperphosphorylated at Ser 199, Thr 212, Thr 231 and Ser 262 (P-Tau), tau with R406W (R406W) mutation and tau pseudo-phosphorylated at Ser 199, Thr 212, Thr 231, and Ser 262 with R406W mutation (P-H Tau) tagged with GFP. To achieve our objective, we want to tag our tau constructs with DsRed which will give us the ability to observe the interactions between two different tau mutants.

DNA cloning:
• Growing and purifying DNA expressing vector and insert
• Cutting DNA with restriction enzymes
• Agarose gel electrophoresis
• Gel cleaning of vector and insert
• Ligation
• Transformation
• Colony selection
• Restriction Enzyme re-digest

Methodology & Results

Transfections
• Grow HEK cells.
• Transfection using EndoFectin and OptiMem.
• Incubate for 48 hours.
• Mount microscope slides.

Fig.1 Results of restricted digest of the vector and insert. 1ug of DNA was digested using RE BOLLI and EcoRI. Top band (4.5kb) correspond to Vector containing GFP or DsRed. Lower band (1.2kb) correspond to Tau gene.

Fig.2. Results of transfection of HEK (human embryonic kidney) cells with DsRed-PH-Tau and GFP-PH-Tau plasmid. Cells were growing at 37°C and 5% CO2 until 90% of confluency and then transfected with plasmids. Cells were incubated for 48 hours post transfection and then fixed with 4%PFA in PBS and mounted on slides using mounting solution with DAPI. Cells were viewed using Leica confocal microscope.

Conclusion

Our project is not completed yet. Out of the 3 DNA plasmids coding for 3 tau mutants: P-Tau, PH-Tau and R406W. Only one, PH-Tau was successfully subcloned into DsRed vector, and as a result now we have PH-Tau in DsRed vector which would produce pathological tau hyperphosphorylated at Ser 199, Thr 212, Thr 231 and Ser 262 with inclusion R406W mutation conjugated to red fluorescent protein and PH-Tau in GFP vector which will produce a green color. We will use our constructs to observe the interactions between different tau mutants in the cells. This is important to understand these interactions because Alzheimer’s disease (AD) brain will have mutated and/or hyperphosphorylated tau present along with normal tau. Therefore, the experiment will aid in identifying how those mutations are interacting with the normal tau that is present in the brain and how they are interact with each other. In conclusion, the main purpose of this experiment is to assist in providing treatments for Alzheimer’s disease (AD).

References


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