The Fate of the PH-TAU, Exocytosis or Degradation?

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Abstract

Tau is a microtubule-associated protein (MAP). Tau is essential because it aids in the maintenance of microtubules in the central nervous system’s (CNS) neuronal axons. Tau is a phosphoprotein, and its level of phosphorylation is critical for its proper functioning. Normally, each mole of tau contains three moles of phosphate. However, tau protein becomes hyperphosphorylated in Alzheimer’s disease patients, resulting in 7-10 moles of phosphate per mole of protein. When Tau gets hyperphosphorylated it can’t bind to tubulin and stabilize microtubules. Additionally, aberrant and hyperphosphorylated tau attaches to normal tau, separating it from the microtubules. As a result, the microtubules are disrupted, and the neurons die.

When pathogenic TAU (PH-TAU) is taken up by cells, a portion of the protein is transferred by the endo-lysosomal route to lysosomes for degradation or can be exported in the form of exosomes, resulting in the disease spreading further. The purpose of this research is to figure out what happens to PH-TAU after it has been taken up by cells. To do so, we looked for tau protein colocalization with RAB5 (early endosome marker), RAB7 (late endosome marker), and RAB11 (endosomal marker). Moreover, the Direct Current (DC) stimulation is the technique that was shown to promote the upregulation of heat shock proteins, which are responsible for the identification and degradation of misfolded proteins. In this project, we also want to see if DC stimulation will lead to increased PH-Tau degradation (colocalization with RAB11) compared to unstimulated cultures.

Introduction

Tau is a microtubule-associated protein (MAP) that’s important in aiding the maintenance of microtubules of the neural axons in the central nervous system (CNS). Tau is a phosphoprotein and degree of phosphorylation is very important for the proper biological activity. During the normal conditions tau has three moles of phosphate, and it assists with stabilizing the internal skeleton of the neurons in the brain. Unfortunately, when it comes to patients that have Alzheimer’s disease (AD), tau protein becomes hyperphosphorylated, which concludes in about 7 to 10 moles of phosphate per mole of protein. Due to the hyperphosphorylated tau, it cannot bind to tubulin and stabilize microtubules. In addition, it will automatically bind to normal functioning tau which causes the tau and the microtubules to separate. Thus, it will result in the death of the neurons. It was shown that phosphorylation at Ser199, Thr212, and Ser262 (PH-Tau) results on tau conformation similar to one found in AD patients.

The purpose of this research project is to observe what process does PH-TAU goes through after it has been taken up by cells. Originally, when cells take pathogenic tau which is transferred by the endo-lysosomal route to lysosomes for degradation. Therefore, in this research, we have observed PH-Tau colocalization with RAB5 (early endosome marker), RAB7 (late endosome marker), and RAB11 (endosomal marker).

RABs are small GTPases triphosphatases. They regulate various steps of membrane trafficking, which include vesicle formation, vesicle movement. RAB 5 is important in the regulation of early endocytosis, and membrane transport which contributes to gene transcription and other types of processes. While RAB 7 contributes to the control of the transport system to the endocytic compartments, which involves lysosomes and late endosomes. RAB 11’s key function is the recycling of early endosomes and endosomal compartments to the TGN (trans-Golgi network) and plasma membrane. Direct Current (DC) stimulation is the technique that was used for this project. It assists in the upregulation of heat shock proteins that are responsible for identifying and degrading misfolded proteins. We want to observe if DC will speed up the transportation of TAU through the endo-lysosomal system. As a final step, we observed and compared between unstimulated cultures and increased pathological human tau localization.

Methodology

To achieve our goal, we performed transfection of HEK293 cells with RAB5-GFP, RAB7-GFP, and RAB11-GFP plasmids following its exposure to Tau or PH-TAU protein. Since our focus was to see them over different time periods, we incubated them for 24 or 48-hour. Following the transfection cells were fixed and processed for immunocytochemistry and labeled with DAKO Tau antibody (Human tau, rol). Cells were mounted on the slides using prolang with DAPI and viewed under the confocal microscope. Finally, we used ImageJ software to analyze tau PH-TAU colocalization with RAB5, RAB7, and RAB11.

Results

In this project, we are attempting to determine the fate of tau and PH-Tau proteins after they were taken up by the HEK cell. We want to see the localization of these proteins as they go through the endo-lysosomal pathway. According to the data obtained, there was higher colocalization of proteins with RAB11. Which being said they went through the early and late endo-lysosomal phase to the recycling phase. According to our knowledge, proteins were being extracted out before they reached the recycling step. However, in this situation, they were not removed and instead went through the recycling phase. In addition, DC stimulation was used to see how it affects the protein colocalization and it shows that there were less proteins in each compartment compared to Tau 48h non-stimulated. However, we do not know if they are being removed through exocytosis or being destroyed; for which further studies need to be done.

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