Scientific Literacy Background Essay

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The target of this experiment was to find the direct correlation to the neurodegenerative disease, Alzheimer's disease. Neurodegenerative diseases, such as Alzheimer's disease are due to abnormal aggregation of misfolded beta-sheet proteins (Hoshino et al., 2014). In proteins, shape determines function, so when a protein is misfolded, it is not performing its correct function. Abnormal protein aggregation is what leads to the misfolding of proteins, which directly caused the protein to not perform the proper function. The misfolded protein specifically discussed in the experiment is beta-amyloid. To prevent abnormal protein aggregation and the misfolding of proteins, there needs to be the import of proper chaperones. Proteins must fold into a three-dimensional shape to be functional, but newly synthesized proteins are at risk of misfolding and aggregation, which can lead to diseases. To prevent these issues, molecular chaperones are implemented and use ingenious mechanisms to prevent misfolding and aggregation (Hartl et al., 2011). Chaperones are extremely important in making sure that the proteins sustain homeostasis.

The experiment is an attempt to demonstrate how the anti-aggregate chaperone for the neuroendocrine protein proSAAS, which was associated with the neurogenerative disease, has a direct correlation to Alzheimer's disease (Hoshino et al., 2014). Alzheimer's disease is a brain disorder that will slowly destroy memory and the ability to think, which will lead to the inability to do simple things. Usually, it happens at an older age, and it happens to around 6 million people in America, typically over the age of 65 ("Alzheimer's Disease Fact Sheet", 2021). This disease, at the time, is ranked as the sixth leading cause of death in the United States, so it is clear why the experiment is being conducted to discover what proteins need attention to prevent

this disease ("Alzheimer's Disease Fact Sheet", 2021). In detail, Alzheimer's disease is described as neuronal loss in the hippocampus and the cortex (Hoshino et al., 2014). In this experiment, to see the correlation of the protein proSAAS in Alzheimer's disease pathology, 12-month-old APdE9 mice and donated human tissue was used to observe in fluorescent covered panels. It was observed that proSAAS immunoreactivity was highly colocalized with amyloid pathology. With this, both drugs and molecular chaperones will block the aggregation of the beta-amyloid 1-42, tua, or alpha-synuclein so that it slows down diseases such as Alzheimer's (Hoshino et al., 2014). If the researchers can demonstrate how there is a correlation of proSAAS and the aggregation of this protein to neurodegenerative diseases through the mice experiment, it can progress into importing the proper chaperones to correct the misfolding.

There are many methods of experimentation to determine the relations between proSAAS and beta-amyloid 1-42, which leads to neurodegenerative diseases such as Alzheimer's disease. One method of determining if proSAAS has a direct relation to Alzheimer's disease is immunofluorescent labeling of brain tissues for both proSAAS and AD (Alzheimer's disease) markers. The study was conducted on both human and mouse tissues. A hippocampal tissue sample was taken from a 73-year-old donor with AD, provided by NICHD Brain Tissue Bank for Developmental Disorders at the University of Maryland-Baltimore, where it went through multiple steps of being formalin-fixed, cryoembedded, and sectioned. (Hoshino et al., 2014) For immunohistochemistry, the tissue went through blocking in solution PBS, then incubated with rabbit anti-proSAAS and monoclonal mouse antibody. These sections were rinsed with Cy3-conjugated goat anti-rabbit and Cy2-conjugated donkey anti-mouse in a blocking solution.

(Hoshino et al., 2014) After the slides are rinsed again in PBS and covered with Fluoromount G, they were able to be visualized.

A similar experiment was conducted with the brain tissue with mice, where the sections were incubated in an avidin/biotin blocking kit and then incubated again with polyclonal rabbit anti-proSAAS for 1 hour. (Hoshino et al., 2014) Just like with the human tissues, the mouse tissues went through repetitive blocking and incubation with goat anti-rabbit antibodies, beta-amyloid mouse antibodies, and then covering with a form of fluorescent for observation. The results of these observations proved that proSAAS co-localizes with beta-amyloid deposits in both the human AD tissue and the mice AD tissues. In Figure 1, there are a set of images of H33342, proSAAS, 4G8 (beta-amyloid plaques), and the emergence of all of them. They are separated by a control group and also set from the two previous experiments with AD. In the control group, there is no 4G8 present, but there is proSAAS present since it is abundant in the brain tissues. In the group with AD, the sections with proSAAS are directly related to the sections where 4G8 are located, proving that proSAAS co-localizes with beta-amyloid in AD. There was a co-immunoprecipitation experiment also conducted from the mouse brain tissue that resulted in proSAAS being co-immunoprecipitations.

There were many preparations for all of the experiments conducted to observe the assay of proSAAS. From the mouse proSAAS plasmids already prepared, plasmids were encoding N-terminally truncated His-tagged proSAAS from cloning processes. The peptide beta-amyloid 1-42 was also prepared and was also in vitro fibrillation with the presence and absence of full-length and N-terminally truncated proSAAS. (Hoshino et al., 2014) Since insoluble beta-sheets

bind to ThT, through electron microscopy and dot blot analysis, it was tested and concluded that proSAAS prevents fibrillation of beta-amyloid 1-42 in vitro. ProSAAS was a highly potent inhibitor of fibrillation since it blocked 50% with a low presence. The TEM data supported this conclusion, with the addition of the dot-plot analysis showing that the beta-amyloid 1-42 is insoluble. To identify what region in proSAAS was responsible for the blocking of fibrillation, the three alpha-helices were observed. It resulted that the constructs #1-4 were able to prevent fibrillation, but since construct #5 (within 138-180) only had alpha-helix III, but with the observation of construct #6 (97-137) being inactive, it showed that both alpha-helices II and III are required. With the results, it was concluded that the region responsible for fibrillation and anti-aggregation of beta-amyloid resides within 97-180. With beta-amyloid oligomer preparation the cytotoxicity assay with Neuro2a cells was discovered that with the presence of proSAAS in Neuro2a cells with beta-amyloid oligomers, there was a blockage of cytotoxicity.

The basis of discovering that proSAAS is co-localized and co-immunoprecipitants with beta-amyloid, and also the blockage of fibrillation, is from observing the direct correlation between both the beta-amyloid and the proSAAS. 7B2 can also perform the same functions as the ProSAAS and this was prior knowledge to the experiment, so why was the experiment even conducted. ProSAAS had similar qualities to the 7B2, but it wasn't proven it has the same functions and same relationship with beta-amyloid. So, there was research conducted to see if it could prevent neurodegenerative issues like AD. After noticing how there were specific relations between ProSAAS and AD through tests like Immunofluorescent labeling and Co-immunoprecipitation, it was discussed that this methodology could be applied with other parts of the body outside just the brain tissues, but other degenerative issues across the body. These tests

can be used to see the relations between proteins and chaperones with amyloids that cause diseases like chronic disorders. If ProSAAS can help with AD, tests can be used to find other proteins that can help physical disorders and diseases across the body.

References

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