Analysis of Alzheimer's Disease Therapeutics Effect on MitoNEET Expression

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disease that affects over 5 million Americans. The disease is characterized by the formation of senile plaques of the amyloid beta and neurofibrillary tangles within the brain that can impair the patient's memory and behavior. These symptoms of AD develop slowly and worsen over time. Currently there is no known cause or cure for AD, therefore treatment is restricted to alleviating symptoms. A new approach to AD focuses on mitochondrial dysfunction, which is when the mitochondria release reactive oxidative species that cause damage and changes to the expression of tissues, proteins, and genes. MitoNEET is a newly discovered mitochondrial protein that is thought to regulate bioenergetics in cells. The focus of our research is to help resolve the mechanism of AD by identifying potential targets for treatment. Fluorescence microscopy is used to evaluate changes in protein expression. This was used to assess changes in protein expression when exposed to current AD therapeutics. One treatment is isoproterenol, which is a bronchodilator that has been shown to upregulate mitoNEET. Our preliminary studies use fluorescence microscopy to verify that isoproterenol upregulated the expression of mitoNEET in N2a cells after a 24-hour exposure. The results showed a two-fold increase in the relative integrated density when exposed to 1, 10, 100 uM of isoproterenol. Further studies will investigate mitoNEET regulation in response AD therapeutics.

BACKGROUND OF ALZHEIMER'S DISEASE

AD is one of the most common neurodegenerative diseases and is the sixth leading cause of death in the United States. This disease is characterized by memory loss, language difficulties, and personality changes. This leads to neuron death, increased levels of reactive oxygen species, altered calcium signaling, and alternations in gene expression and transcription mechanisms.[4,8-11] There is currently no cure for AD.

Hallmarks of this disease include the development of the senile plaques of the amyloid beta peptide, and neurofibrillary tangles. These are present in the hippocampus, prefrontal cortex, cerebellum, and temporal lobe; regions of the brain that are involved with learning, memory, and behavior. The senile plaques are formed by the cleavage of the amyloid precursor protein (APP). Formation of these plaques cause connections between nerve cells to be destroyed, leading to decreased cell density, and ultimately neuronal cell death. The neurofibrillary tangles are affected by the tau protein, located on the microtubules of the neuron, which is hyperphosphorylated and then stick to each other.[12]

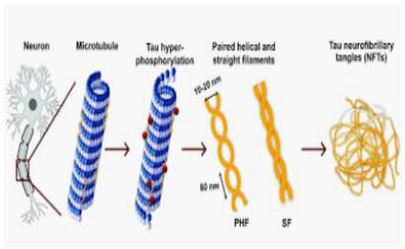


Figure 1. Process of hyperphosphorylation of tau protein.
Verwilst, Peter; Kim, Hyeong Seok; Kim, Soobin; Kang, Chulhun; Kim, Jong Seung. Sheffing light on the tau protein aggregation: the progress in developing high selective fluorophores. *Chemical Society Reviews.* 2018. 47(7). 2249-2265

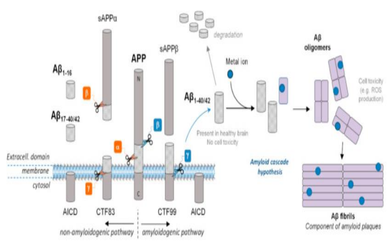


Figure 2. Cleavage of APP to form Aβ senile plaques.
Cheignon, C.; Tomas, M.; Bonnefont-Rousselot, D.; Faller, P.; Hureau, C.; Collin, F. Oxidative stress and the amyloid beta peptide in Alzheimer's disease. *Redox Biology.* 2018. 14. 450-464.

Research related to AD has been ongoing for several years. Past hypotheses have focused on the decreased activity of the acetylcholine esterase and the decreased expression of nicotinic and muscarinic receptors. Along with the accumulation of the amyloid beta formed by the cleavage of the APP, and the hyperphosphorylation of the tau protein. These hypotheses have only allowed for alleviation of the symptoms of AD rather than a cure.[13-14] By focusing on this more recently developed hypothesis, mitochondrial dysfunction is a new avenue provided to potentially find a cure.

PROPOSED PROJECT

The overarching goal of this research project is to understand the mitochondrial dysfunction occurring in Alzheimer's disease (AD) by evaluating changes in the mitochondrial protein, mitoNEET. This idea comes from the belief that mitochondrial dysfunction causes the formation of plaques and tangles in the brain of AD patients. Previous studies was completed on mitoNEET to identify it as a potential drug target for Type II diabetes (T2D) treatment. MitoNEET showed great potential as a target binding site in T2D trial. It is hoped that similar results will occur in our studies since T2D, and AD are conformationally similar.

There are two specific objectives that will be utilized to determine if mitoNEET is a potential cure target for AD. First, cell culture techniques will be used to examine proteins in an Alzheimer's disease model. The cell line being studied is a mouse neuroblastoma (N2A) cell line due to the cells being hearty, allowing reliable growth. Second, fluorescence microscopy will be used to study changes in the trafficking and expression of mitoNEET using $A\beta_{1\rightarrow42}$ and current therapeutics. MitoNEET can be labeled with GFP. The changes in the measured fluorescent signal correspond to the concentration of the protein within the cells.

MitoNEET

MitoNEET is a newly discovered mitochondrial protein. The mitoNEET is an integral protein localized in the outer mitochondrial membrane (OMM), its name is based on its subcellular localization and the presence of the amino acid sequence Asn-Glu-Glu-Thr (NEET). The first 32 residues present within the mitoNEET are an essential section of amino acids within the predicted transmembrane domain. This domain directs the mitoNEET to the outer membrane and is responsible for its colocalization. MitoNEET is also identified as part of the unique 39 amino acid sequence, CDGSH, a domain in residues 55-93 that act similarly to a zinc finger and is likely involved with iron binding. The protein also contains a N-terminal α-helix with a redox active iron-sulfur domain, [2Fe-2S]. These components have shown that mitoNEET is able to play roles in the regulation of energy metabolism in the mitochondria.[14]

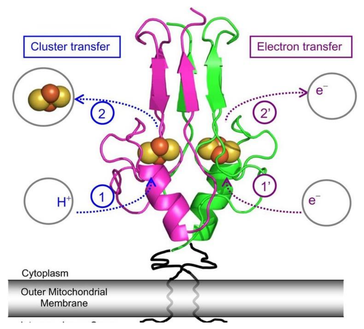


Figure 3. Structure and possible functional implications of mitoNEET. Paddock, Mark L.; Wiley, Sandra E.; Axelrod, Herbert L.; Cohen, Aina E.; Roy, Melinda; Abresch, Edward C.; Capraro, Dominique; Murphy, Anne N.; Nechushtai, Rachel; Dixon, Jack E.; Jennings, Patricia A. MitoNEET is a uniquely folded 2Fe-2S outer mitochondrial membrane protein stabilized by piog litazone. *PNAS.* 2007. 104(36). 14342-14347.

MitoNEET In The Presence of Isoproterenol

Preliminary studies were completed to verify that mitoNEET was upregulated when exposed to isoproterenol. In the study, N2a cells were exposed to 1 µM, 10 µM and 100 µM of isoproterenol and then compared to the control. The upregulation was found utilizing fluorescence microscopy tagged with Green Fluorescence Protein (GFP). A t-test was also completed to show that analysis completed was significant.

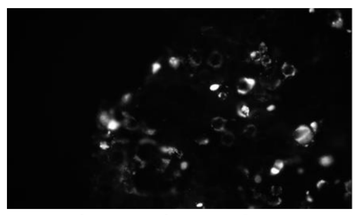


Figure 5. mitoNEET-GFP

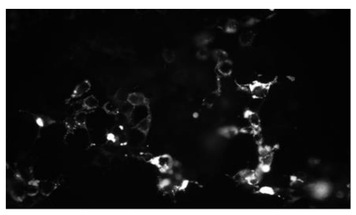
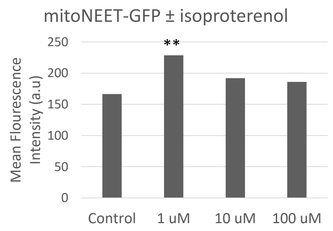
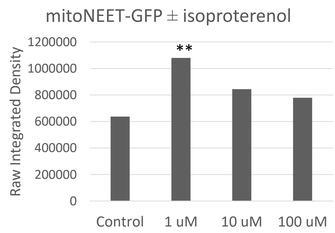


Figure 6. mitoNEET-GFP + 100 µM isoproterenol





MitoNEET IN THE PRESENCE OF DONEPEZIL

A new study was performed to determine if mitoNEET expression was changed when exposed to donepezil, a current treatment or AD. In the study, N2a cells were exposed to 5 µM, 50 µM, and 500 µM donepezil and then compared to the control. MitoNEET concentration showed no change when utilizing fluorescence microscopy tagged with Green Fluorescence Protein (GFP).

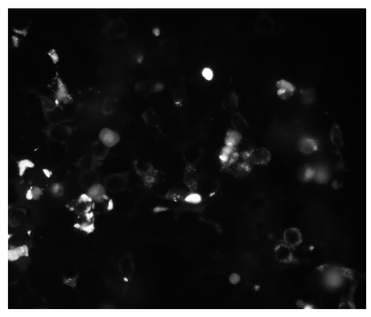


Figure 7. mitoNEET-GFP + 0 µM donepezil

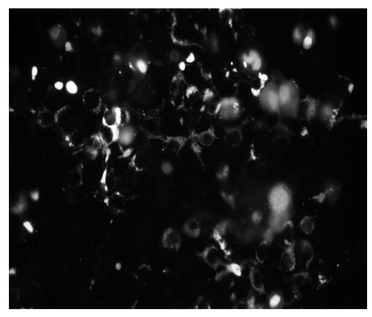


Figure 8. mitoNEET-GFP + 5 µM donepezil

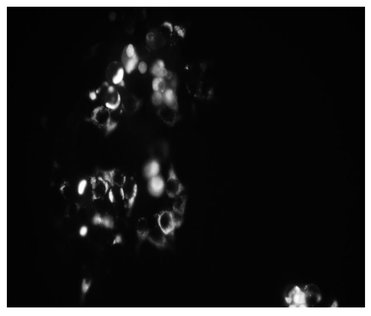


Figure 9. mitoNEET-GFP + 50 µM donepezil

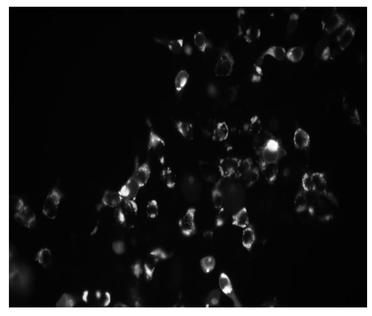
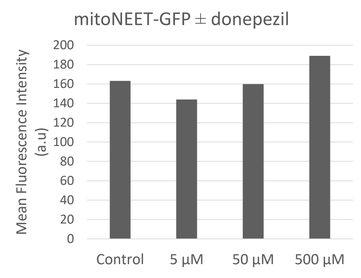
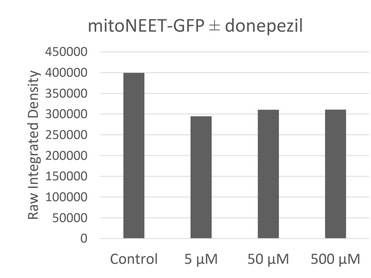


Figure 10. mitoNEET-GFP + 500 µM donepezil

Utilizing the processing program Image J, the average concentration of donepezil was found for the control, 5 µM, 50 µM, and 500 µM. The mean fluorescence intensities (a.u.) were determined to be 163, 144, 160, and 189, respectively. Also, the average raw integrated densities were determined to be 399000, 295000, 310000, and 311000, respectively.





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