In an AD brain beta-amyloid plaques and abnormal accumulations of neurofibrillary tangles largely consisting of the microtubular tau protein are the morphological hallmarks of this pathology. Mitochondrial dysfunction has been implicated in the development of Alzheimer’s Disease (AD) pathology and progression, but the mechanism is unknown. Changes in basic cellular processes such as oxidative phosphorylation (OXPHOS), the regulation of reactive oxygen species, and Ca\(^{2+}\)-sequestration and/or release have been observed. HEK cells were transfected with EGFp-Tau, MARCH5DSRed, mutated forms of MARCH5 H43WDSRed, mutated forms of Tau 6B and 8A, and wild types and mutated forms of MARCH5 and Tau. MARCH5 recognized the fission factor Drp1, leading to important role in morphological stability of mitochondria. MARCH5, also called MITOL, is a transmembrane protein and has 4 transmembrane regions and is a ubiquitin ligase. MITOL is another name for MARCH5.

**What is the role of MARCH5?**

**METHODS**

Clothing of MARCH5 and Tau into the fluorescent tag expression vectors was done by performing transformations, overnight bacterial culture, DNA isolation, and DNA quantification. Cell Culture was derived from the stock solution of HEK cells which were then placed on a plate by adding DMEM media which provides an appropriate environment for their growth. Transfection with a lipid-base transfection reagent, endolysin by aliquoting 1\( \mu \)L of DNA per well, bringing the volume to 50 \( \mu \)L with OptiMEM media, and then adding 50 \( \mu \)L of a mixed solution of endolysin and OptiMEM media. Cell Collection was performed by lysing the cells, centrifuging, and incubating them in TNEB with added protease inhibitor cocktail at -20\(^\circ\)C. The samples were quantitated using DC Protein Assay (BioRad).

**OxyBlot Analysis**

Immunoblot detection of carbonyl groups introduced into proteins caused by oxidative reactions using an OxyBlot analysis kit. The OxyBlot procedure was done using the OxyBlot detection kit for samples after electrophoresis and transfer to nitrocellulose membrane, blocking the non-specific sites, incubating with primary and secondary antibodies, and then adding a chemiluminescent reagent to visualize it by exposing it to U.V. light.

**RESULTS**

A hypothetical scheme of the formation of phosphorylated Tau and Tau mutant filaments. Phosphorylation of Tau and mutant Tau cause self-aggregation of the tau protein and the mutated forms. The mutated form of Tau is more prone to polymerize than the wild type tau based on the protein interaction but also, they self-aggregate at a lower level of phosphorylation.

**CONCLUSIONS AND FUTURE GOALS**

- MARCH5 overexpression causes a drastic increase in the production of reactive oxygen species (ROS) when analyzed with OxyBlot and dot OxyBlot.
- Wild-type Tau decreases the amount of ROS produced and rescues the cell when there is an overexpression of MARCH5 or when H43W is present in the cell.
- Confocal microscopy will be used to evaluate the colocalization of MARCH5 and Tau.

**REFERENCES**

1. Alonso AD, Cohen LS. Our Tau Tales from Normal to Pathological Behavior. J Alzheimer’s Dis 2018;64(s1):S507
3. Yonashiro Y, Inatome S, Roles of mitochondrial ubiquitin ligase MARCH5 overexpression further increases the number of ROS compared to the number of ROS in a normal cell. 4. For the double transfection samples with H43W and 8A also save the mitochondria from ROS increase in ROS compared to the ROS found in a normal cell. 5. Moreover, 6B and 8A proteins which are the mutated forms of MARCH5 seem to rescue the cells by drastically decreasing the number of ROS for MARCH5/6B and MARCH5/8A. For the MARCH5/Tau protein sample there is a smaller decrease in the ROS. 6. There is an increase in ROS for the MARCH5 protein sample and a decrease in ROS for the H43W sample compared to the amount of ROS in a normal cell. 7. There is a significant decrease in ROS for the Tau protein sample, a decrease in ROS is also observed in the 6B sample compared to the amount of ROS for normal cells, but the 8A protein sample does not seem to impact the amount of ROS based on the obtained data. 8. Moreover, 6B and 8A proteins which are the mutated forms of Tau seem to rescue the cells by drastically decreasing the number of ROS for MARCH5/6B and MARCH5/8A. For the MARCH5/ Tau protein sample there is a smaller decrease in the ROS. 9. For the double transfection samples with H43W seems that the mutated form of MARCH5 has a higher impact in the ROS species and Tau, 6B, and 8A do not seem to rescue the cells as compared to the double transduction with the wild-type MARCH5.

**Graph 1.** A hypothetical scheme of the formation of phosphorylated Tau and Tau mutant filaments.

**Graph 2.** Cartoon drawing of mitochondria. Mitochondria are opening or closed red-like structures surrounded by inner and outer membranes. Our protein of interest is located on the outer membrane and it has a transmembrane region and is ubiquinated on a “Y”-type for mitochondria.

**Graph 3.** Cartoon drawing of mitochondria. Mitochondria are opening or closed red-like structures surrounded by inner and outer membranes. Our protein of interest is located on the outer membrane and it has a transmembrane region and is ubiquinated on a “Y”-type for mitochondria.

**Graph 4.** Cartoon drawing of mitochondria. Mitochondria are opening or closed red-like structures surrounded by inner and outer membranes. Our protein of interest is located on the outer membrane and it has a transmembrane region and is ubiquinated on a “Y”-type for mitochondria.

**Graph 5.** Cartoon drawing of mitochondria. Mitochondria are opening or closed red-like structures surrounded by inner and outer membranes. Our protein of interest is located on the outer membrane and it has a transmembrane region and is ubiquinated on a “Y”-type for mitochondria.

**Graph 6.** Cartoon drawing of mitochondria. Mitochondria are opening or closed red-like structures surrounded by inner and outer membranes. Our protein of interest is located on the outer membrane and it has a transmembrane region and is ubiquinated on a “Y”-type for mitochondria.