**BICD110 SP21**

**Cell Biology Assignment 2**

1. What do you think is a **public misconception** about dementia and what could be the consequences (implications)?

A common misconception is thinking that dementia and Alzheimer’s disease are synonymous. Alzheimer’s disease is only a type of dementia, and accounts for majority of dementia cases. There are other types of dementia such as frontotemporal dementia (FTD), Lewy Body Disease and vascular dementia to name a few. Different types of dementia exist due to the difference in underlying pathology, but all of them involves loss of cognitive function. Being knowledgeable on the specific type of dementia a person has will help him/her or his/her caretaker to understand his/her symptoms, monitor the progression, and know how to manage his/her symptoms.

1. From the **Figure 2 A (and video)** what difference do you observe in the microtubule extensions when comparing non-demented control neurons with demented cases (FTD-MAPT)? Is microtubule dynamics different in the control vs. FTD-MAPT neurons?

The microtubule dynamics between the control neurons and FDT-MAPT neurons were found to have qualitative differences. In comparison to the non-demented control neurons which had actively growing microtubule extensions around an oval nucleus showing smooth surface, multiple microtubule extensions can be seen projecting into the nuclei of FTD-MAPT neurons. These microtubule extensions observed on the FDT-MAPT neurons were also found to have a focused origin.

1. What could be the scientific causes of this phenomena?

Tau is known to be distributed predominantly on axons where it regulates and provides microtubule stabilization. It is assumed that the mislocalization of tau from axons to the cell bodies and dendrites due to MAPT mutations which can be observed in the early stages of FTD in vivo may have contributed to the marked changes in microtubule dynamics of FTD-MAPT neurons.

1. What could be the consequences of this phenomena?

Due to the abnormal extension of microtubules into the nuclei of FDT-MAPT neurons, mechanical forces are transmitted due to the coupling of microtubules to the nuclear membrane through the LINC complex. These events affect the shape and integrity of the nucleus leading to pronounced deformation of the nuclear lamina as shown in the confocal images of FTD-MAPT neurons and schematic image analysis method measuring the distortion of the nuclear membrane by quantifying nuclear invaginations.

1. In Figure 4A, scientists used Lamin B to stain nuclear lamina – a fibrillary network inside of the nucleus. What differences do you notice in the nuclear lamina when comparing non-demented control neurons with demented neurons?

The demented neurons, compared to the non-demented control neurons, show distinct nuclear invaginations as shown on three-dimensional stimulated emission depletion (STED) super-resolution imaging. Moreover, the nuclear invaginations seen in the demented neurons were observed to have deep extensions, with some extending from one end of the nucleus to the opposite end, giving the appearance of large folds.

1. At this point, what conclusion could you elaborate? Begin to draw a graphic model illustrating the disease-like phenotype you have identified. Your model should compare the normal (non-mutated) vs the MAPT (mutated) neurons.

Diagram

Description automatically generated

**LEGEND**

Dependency

The presence of MAPT mutation causes the tau protein to be predominantly localized in the cell bodies and dendrites instead of the axons as seen on healthy neurons without MAPT mutation. Localization of tau in the cell bodies alters the microtubule dynamics causing multiple microtubule extensions into the nuclei compromising the integrity of the nuclear membrane as opposed to healthy neurons which show growing microtubules within the cell body that does not project into the nuclei. These are depicted in the graphic model which shows that tau localization is affected by the presence of MAPT mutation, which consequently affects microtubule dynamics and nuclear membrane integrity.

1. Using immunofluorescence and confocal microscopy, scientists investigated the expression and localization of the Tau protein in non-demented neurons and demented neurons (FTD-MAPT). They used MAP2 staining as a neuronal somatodendritic marker, beta 3-Tubulin to identify microtubules in the neuronal cells and DAPI to visualize the nucleus. From **Figure 1 D, E and Fig 4 B,** identify the role of Tau protein in the nuclear invagination induced by the MT polymerization into the nucleus. What do you observe about the Tau neuronal localization in non-demented control neurons vs. demented neurons (FTD-MAPT)?

On confocal imaging, tau protein was found to be extensively found on the axons of non-demented control neurons. On the other hand, tau was observed in MAP2-positive cell bodies and dendrites of FTD-MAPT neurons. In FTD-MAPT neurons visualized through STED imaging, tau protein was observed to be within the nuclear invaginations lining the outer membrane of the nucleus and is within hundreds of nanometers of the nuclear lamina. Since tau is a microtubule-binding protein which stabilizes microtubules, the presence of tau in close proximity to the nuclear lamina causes microtubules to protrude into the nuclear membrane due to mechanical pressure distorting the shape and integrity of the nucleus causing invaginations.

1. Proteins that shuttle in and out of the nuclei have NLS (nuclear localization signals) and/or NES (nuclear export signals). In the experiment presented in **Figure 6 A, C,** scientists have tagged these signals with fluorescent tags such as RFP (red color) and GFP (green color). By measuring the fluorescent intensity (confocal microscopy) of RFP and GFP signal they can localize the proteins exported from or imported from the nuclei. From **Figure 6C**, analyze the graph bar representing the ratio NLS/NES. Elaborate a conclusion.

Non-demented control neurons showed predominant NLS:RFP concentration in the nucleus and NES:GFP concentration in the cytosol. On the other hand, upon quantification of the nuclear/cytoplasmic ratio of both signals on the demented FDT-MAPT neurons, there was evidence of altered nuclear transport as the NES:GFP was more localized in the nucleus while the NLS:RFP is decreased. The bar graph illustrated on Figure 6C demonstrating the increased nuclear/cytoplasmic ratio of NES:GFP and decreased nuclear/cytoplasmic ratio of NLS:RFP in the demented FTD-MAPT neurons supports this finding.

Since NLS and NES are localization and export signals, respectively, we can conclude that the altered nuclear membrane of FDT-MAPT neurons affects the selective permeability of the nuclear membrane causing leaks which leads to defects in nucleocytoplasmic transport within the demented neurons.

1. Complete your graphic model including the conclusions/information obtained from **Figure 1, 4 and 6**. Write a legend associated to your graphical model.

Diagram

Description automatically generated

**LEGEND**

Dependency

In addition to the previous graphic model, it has been established that the presence of tau in close proximity to the nuclear membrane leads to abnormal nuclear shape. The defective nucleocytoplasmic transport are assumed to be caused by microtubule-mediated deformation of the nuclear membrane or protracted damage to the nuclear membrane.

1. How disruption of the microtubule polymerization and nucleocytoplasmic transports could affect the overall neuronal function?

The presence of tau in the cell body causes defects in the microtubule dynamics which compromises the integrity of the nuclear membrane and leads to dysfunctional nucleocytoplasmic transport evidenced by NLS:RFP on the cytoplasm and NES:GFP on the nuclei of FDT-MAPT neurons, suggesting leaky nuclear pores. Integration of DNA replication and protein synthesis which are vital neuronal functions depend on selective transport of proteins and other substances between the nuclear and cytoplasmic compartments, which means that defect in the nucleocytoplasmic transport may negatively affect these processes.