

Introduction

Tau protein can form aggregates in the brain and cause Alzheimer's Disease. Oxidative stress can play a role in this aggregation.

It has been recently shown that the protein part of telomerase (TERT) can be expressed outside the nucleus in nerve cells independent from its function on telomeres in dividing cells. Studies have shown that TERT can reside in the mitochondria which also produce oxidative stress. MitoSOX is a reagent used to visualize the production of superoxide by mitochondria.

So we performed a MitoSOX analysis in order to compare the level of oxidative stress in SH-SY5Y cells transfected with pcDNA 3.1 (control vector), truncated tau and wildtype tau. In addition, differentiation of SH-SY5Y cells was performed.

Aim

- To investigate the protective role of mitochondrially overexpressed TERT protein in human neuroblastoma (SHSY5Y) cells against truncated tau and oxidative stress.
- To characterise neuronal differentiation marker, rate of division and telomerase activity in differentiated compared to undifferentiated cells.

Methods

- ❖ Transfection using Optimem, Lipofectamine 2000 (a transfection reagent) and pGFPmax plasmid at a concentration 0.5µg/µl in the proportions of 3:1 and 6:1.
- ❖ Differentiation of cells into neuron-like cells using Retinoic acid at a concentration of 10µM.
- ❖ Immuno-staining on differentiated and undifferentiated SH-SY5Y cells using mouse anti-TERT and anti-betaIII tubulin antibodies.
- ❖ Staining of live cells with MitoSOX and after fixation stained with a myc-tag mouse antibody in order to detect the TERT shooter vectors.

Results

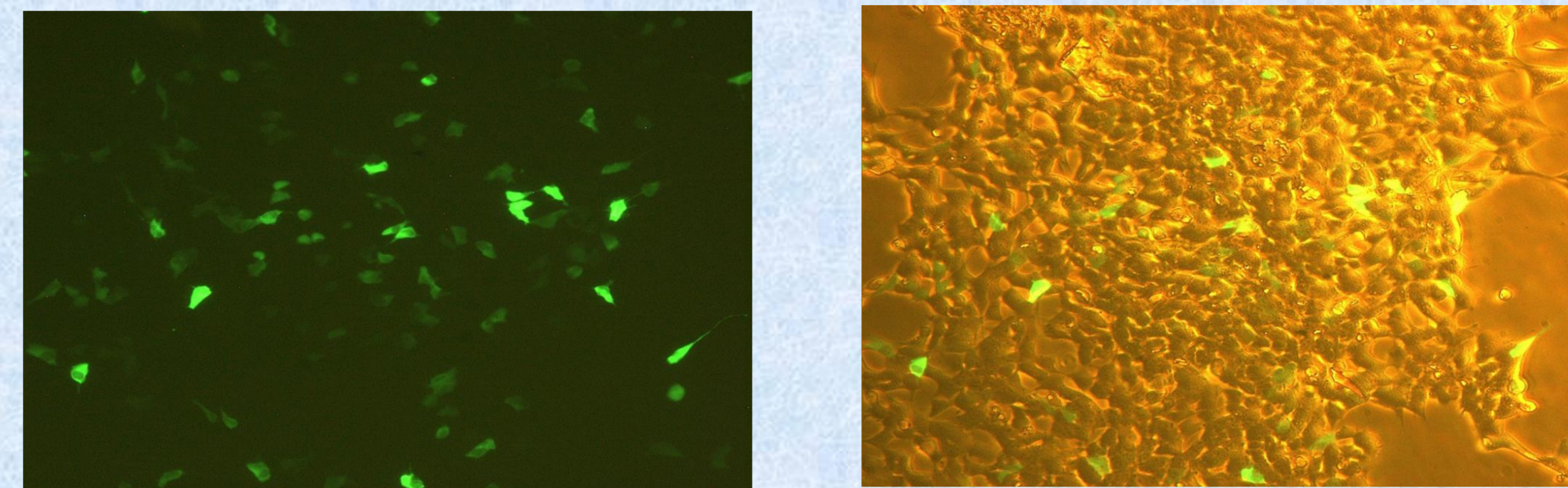


Figure 1: pGFPmax transfected SH-SY5Y cells 6:1. left: fluorescence image, phase contrast on the right

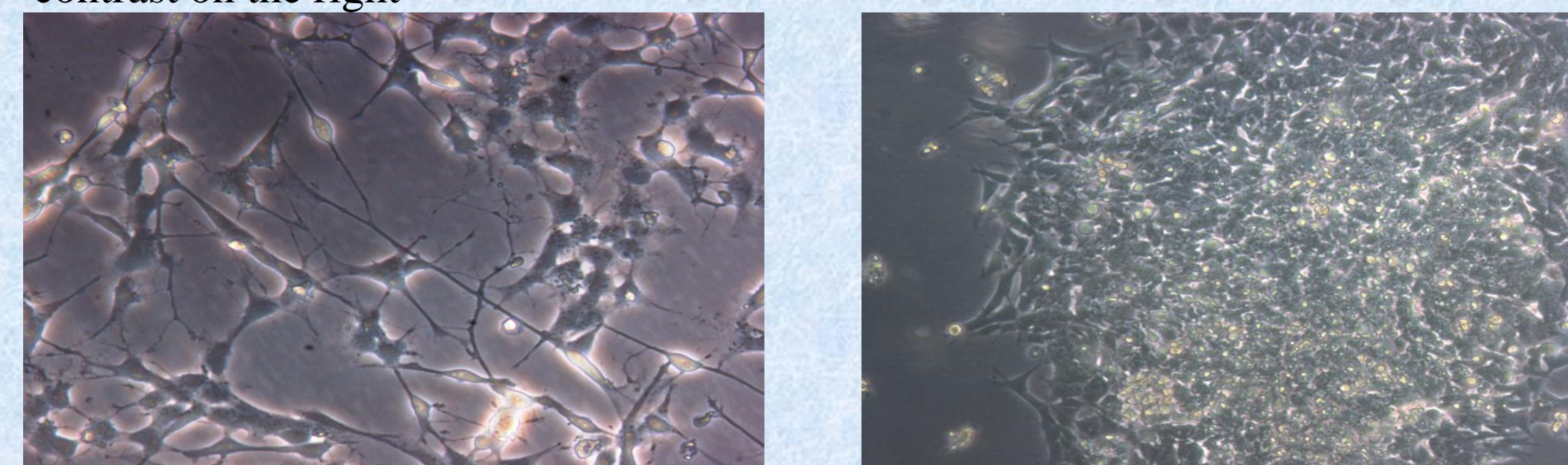


Figure 2: Differentiated SH-SY5Y cells. Phase contrast pictures show differentiated cells after nine days (left) and undifferentiated cells (right)

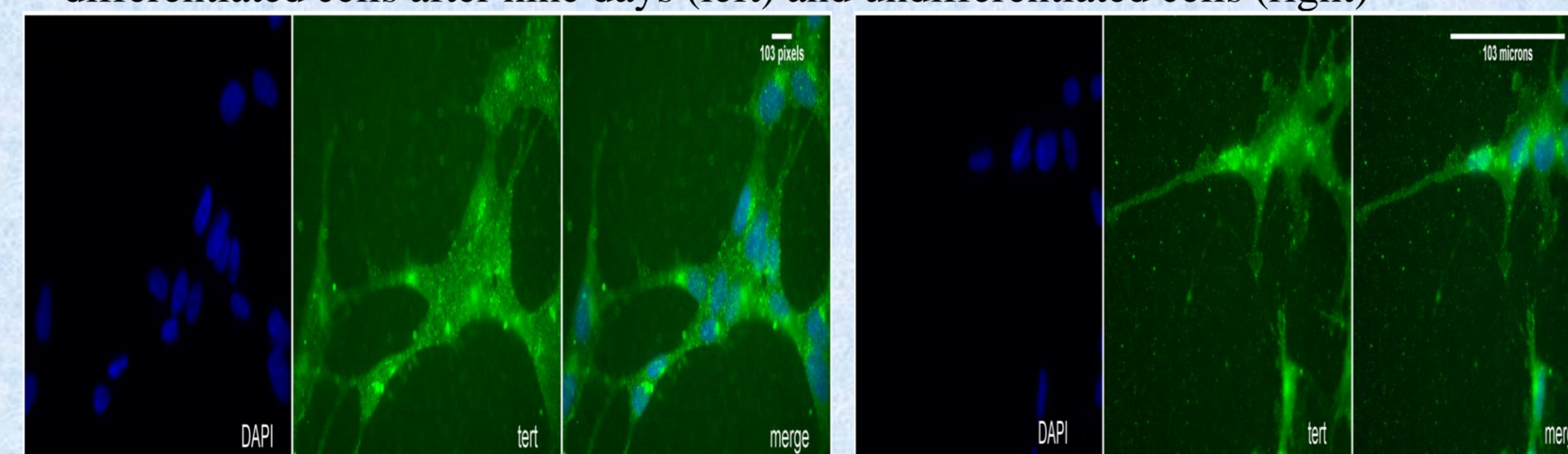


Figure 3: Tert staining (green) on undifferentiated cells (left) and differentiated cells (right).

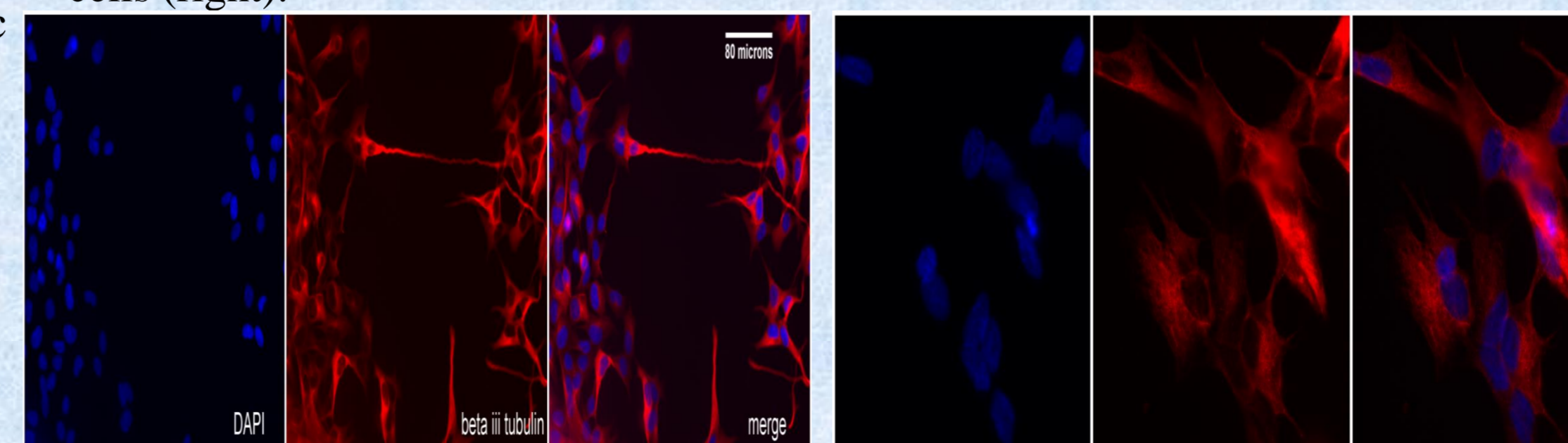


Figure 4: Beta iii tubulin staining (red) on differentiated (left) and undifferentiated (right) SH-SY5Y cells. Dapi = blue signal

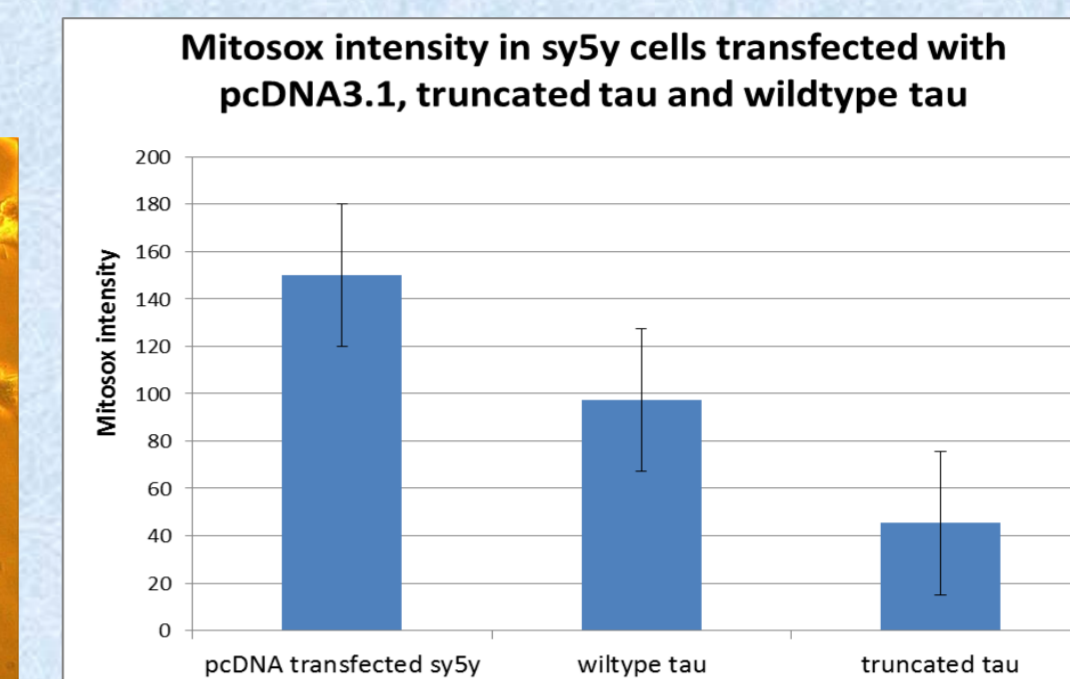


Fig 5: Graph showing mitosox intensity in pcDNA transfected, wildtype and truncated SH-SY5Y cells. Mitosox intensity shows the amount of oxidative stress in cells. Cells transfected with truncated tau show low oxidative stress levels This means that truncated tau reduced oxidative stress in the SY5Y cells.

Discussion

This project aimed to transfect tau and mitochondrial TERT protein into a neuroblastoma cells as a model. Although the planned double transfection was not successful we studied the induction of oxidative stress after transfection of normal and pathological (truncated) tau protein compared to an empty vector. Surprisingly we found that tau seemed to decrease the oxidative stress in our cell system. We also successfully differentiated the neuroblastoma cells into more neuron-like cells that produce more nerve cell marker such as a brain specific tubulin – beta III tubulin (fig 4). Anti-tert staining showed that there was tert migration from the nucleus into the cytoplasm (fig 3). A TRAP assay for measuring telomerase activity was performed but we did not find a decrease in telomerase activity after differentiation (data not shown). Thus, we gained important insights into various parameters of our model system.

Conclusion

- ❖ Since tau decreased oxidative stress in SY5Y cells, it was concluded that an independent experiment will be essential to verify the observation since it was the opposite from what we expected.
- ❖ SH-SY5Y cells are not a perfect model for normal human nerve cells that do not express telomerase activity.

References

1. Gabriele Saretzki, (2009). 'Telomerase, mitochondria and oxidative stress,' Elsevier, pp.485-492.
2. Singhapol C, Pal D, Czapiewski R, Porika M, Nelson G, et al. (2013) Mitochondrial Telomerase Protects Cancer Cells from Nuclear DNA Damage and Apoptosis. PLoS ONE 8(1): e52989. doi:10.1371/journal.pone.0052989.