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Mitochondrial function in an in vitro model of Familial Alzheimer's disease

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Swiss Society of Toxicology
Annual meeting 2024

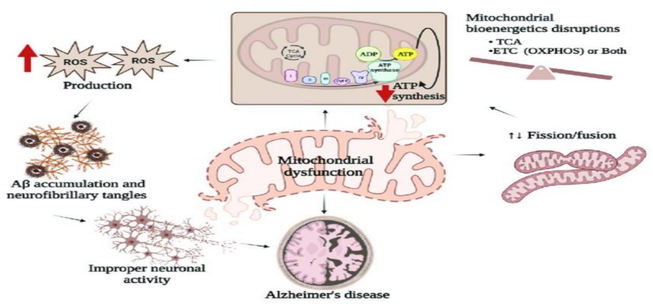
Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by

- accumulation of extracellular amyloid beta (Aβ) plaques
- formation of intracellular neurofibrillary tangles (NFT) and
- neuro-inflammation [1].

Mitochondrial toxicity is a crucial factor in Alzheimer's disease (AD) progression[2].

Understanding mitochondrial dysfunction is essential, as it directly contributes to AD pathology [3,4].



Mitochondrial toxicity and AD progression[5].

AIMS OF THIS STUDY:

1. Generate a thin layer-Matrigel based culture model using a Familial AD (FAD) cell line
2. Examine how FAD genotypes influence mitochondrial toxicity in neurons and glial cells after 2 and 6 weeks of differentiation.

Background

We generated FAD cell lines with mutations in human amyloid precursor protein (APP) alone (ReN-GFP-APP) or together with human presenilin 1 (PSEN1) (ReN-GFP-FAD) using neural progenitor ReN cells based on Choi *et al.* [6]. ReN-GFP and ReN VM were used as control cell lines.

Over time, FAD neurons cultured in Matrigel thin-layers displayed pathological features, including **increased Aβ deposits** at 6 weeks and **hyperphosphorylated Tau** at 9 weeks (Fig.1).

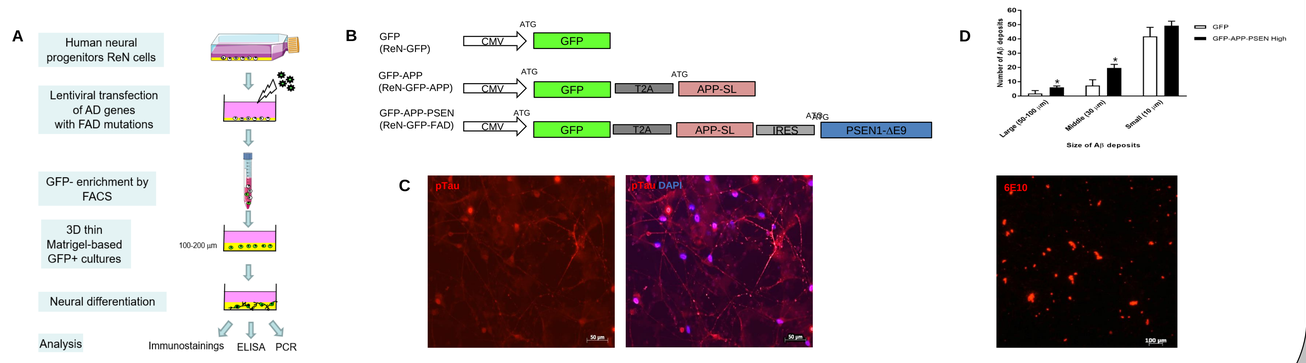


Fig.1. Aβ and Tau pathologies in GFP-FAD thin layer cultures A. Experimental design**. B.** Polycistronic lentiviral vectors used to engineer cell lines**. C.** hyperphosphorylation of Tau in FAD culture and **D.** Aβ deposition (C.Gaiser and L.Suter-Dick, unpublished data).

Experimental approach

Cells: Parental ReN VM cells (Millipore, SCC008): immortalized human neural progenitor cell line derived from the ventral mesencephalon (VM) region of a human fetal brain tissue; control ReN-GFP, diseased ReN-GFP-APP, ReN-GFP-FAD.

Method:

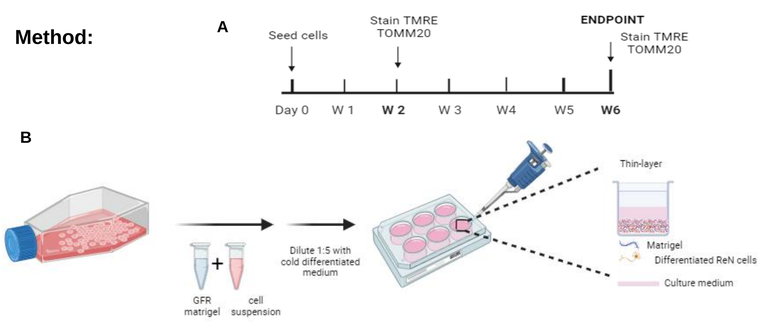


Fig.2. A. Timeline **B.** ReN parental, ReN-GFP,ReN-GFP-APP and ReN-GFP-FAD were mixed with growth factor reduced (GFR) matrigel 1:10 with DMEM-F12. Cells were differentiated in thin layer-based culture up to 6 weeks**. B.** Cells were evaluated at week 2 and 6 of differentiation as a matter of activity, distribution and quantification of superoxide production.

Mitochondrial activity

2 WEEKS OF DIFFERENTIATION

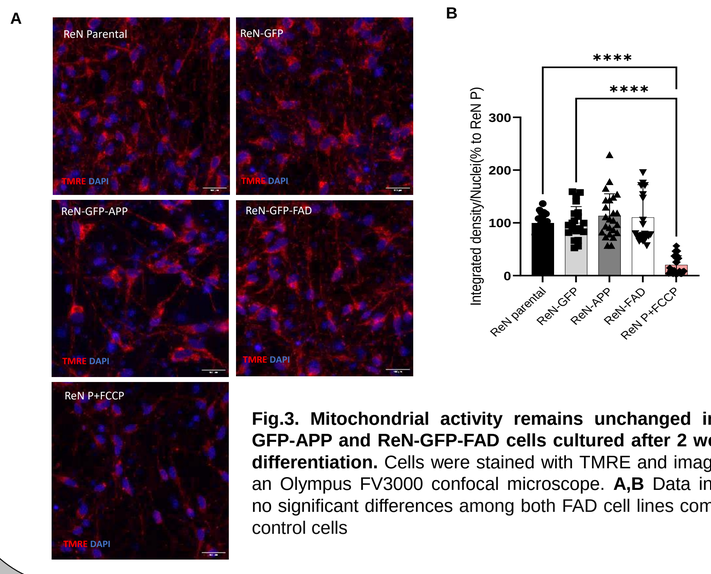


Fig.3. Mitochondrial activity remains unchanged in ReN-GFP-APP and ReN-GFP-FAD cells cultured after 2 weeks of differentiation. Cells were stained with TMRE and imaged with an Olympus FV3000 confocal microscope. **A,B** Data indicated no significant differences among both FAD cell lines compare to control cells

6 WEEKS OF DIFFERENTIATION

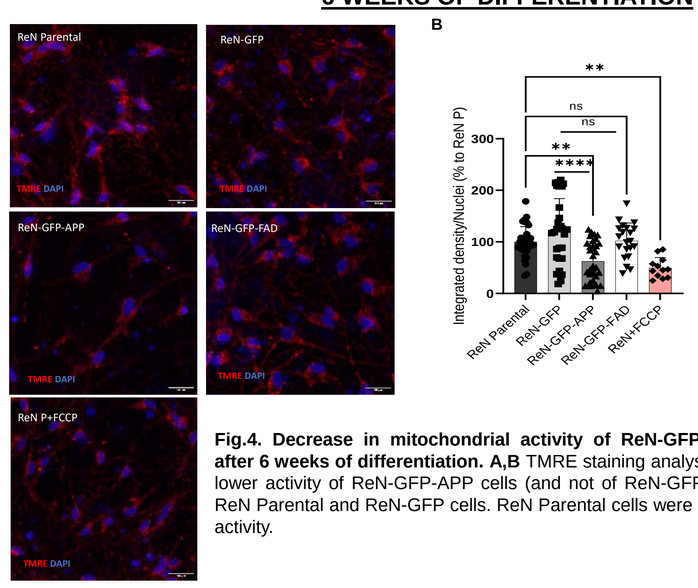


Fig.4. Decrease in mitochondrial activity of ReN-GFP-APP cells after 6 weeks of differentiation. A,B TMRE staining analysis indicated lower activity of ReN-GFP-APP cells (and not of ReN-GFP-FAD) than ReN Parental and ReN-GFP cells. ReN Parental cells were set at 100% activity.

MitosegNet mitochondrial analysis

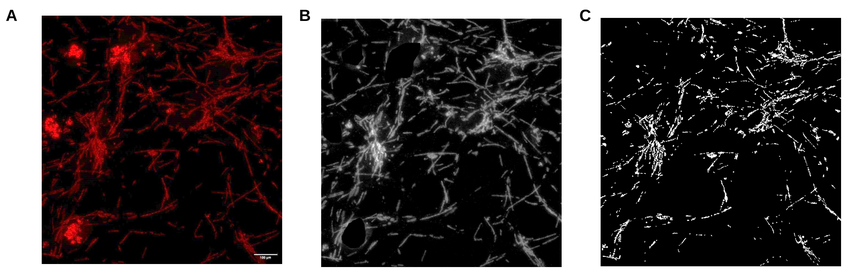


Fig.5. Deep Learning segmentation with MitoS and analysis of Mitochondrial Morphology using MitoA. A. Cells stained with TOMM20 and imaged with confocal microscopy **B.C** Contrast-enhanced raw images were stored as 8-bit images. The signal was segmented into individual mitochondria by MitoS for clear boundary definition. Segmented images were then analyzed by MitoA to determine branch length and mitochondrial count.

Mitochondrial distribution

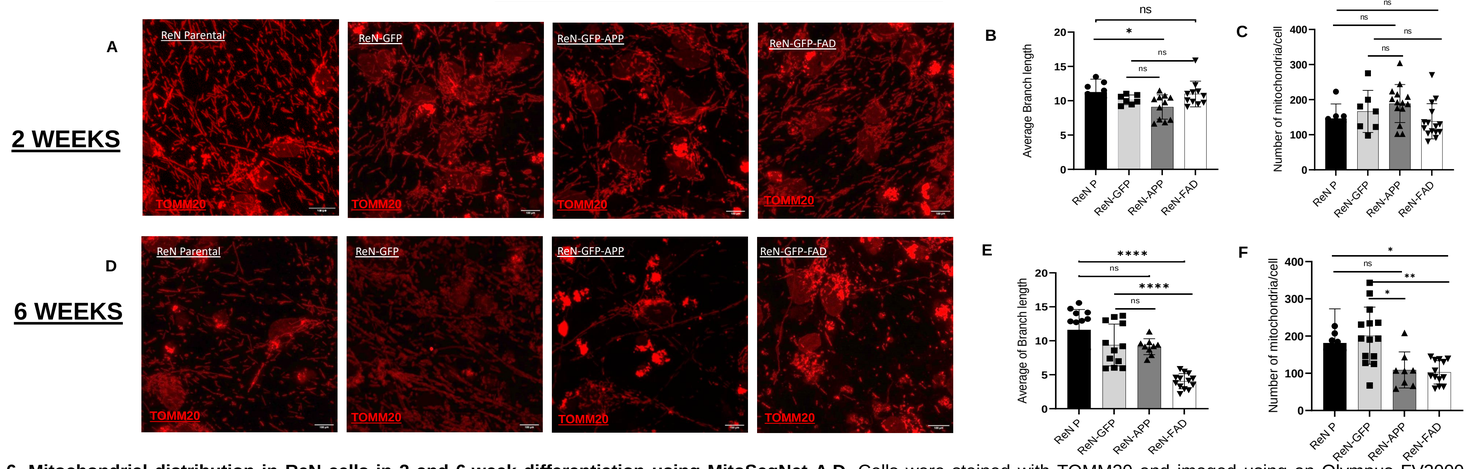


Fig.6. Mitochondrial distribution in ReN cells in 2 and 6-week differentiation using MitoSegNet A,D. Cells were stained with TOMM20 and imaged using an Olympus FV3000 confocal microscopy. **B,C** ReN-GFP-APP cells indicated shorter average branch lengths than ReN parental controls after 2 weeks of differentiation, with no significant change in mitochondrial numbers **E,F** ReN-GFP-FAD cells at 6 weeks showed shorter branches than ReN-GFP and parental controls, with both ReN-GFP-FAD and ReN-APP cells displaying reduced mitochondrial numbers compared to ReN-GFP cells.

Neuronal morphology

2 WEEKS OF DIFFERENTIATION

6 WEEKS OF DIFFERENTIATION

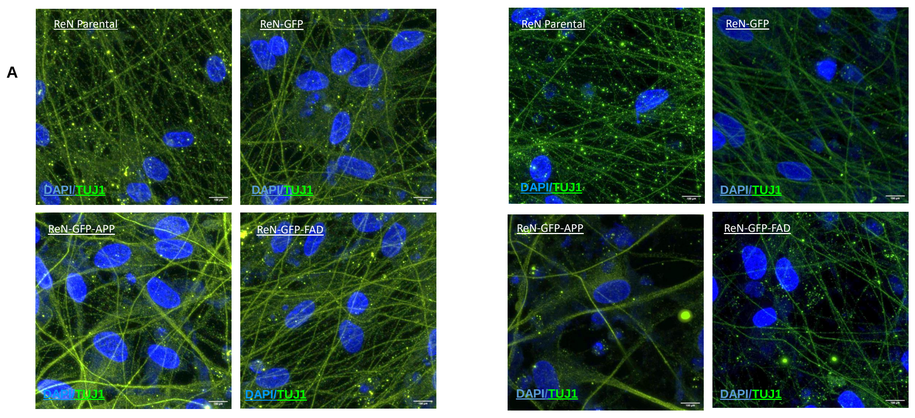


Fig.7. Effects of FAD mutations on the Neuronal morphology. A.B Immunofluorescence staining of 2 and 6 weeks old neuorns with Tuj-1 and imaged using Olympus confocal microscopy. There was no significant difference in neuronal morphology observed at 2 weeks; however, neuronal loss was noted in both FAD cell lines at 6 weeks compared to the 2-week-old cells.

Conclusions and outlook

We successfully developed an in vitro model to assess mitochondrial impairment in FAD cell line:

1. After 2 weeks of differentiation, there is no difference in cell activity between both control and ReN-GFP-APP and ReN-FAD cell line. However, at the 6th week of differentiation, the activity of ReN-GFP-APP cells decreased significantly in comparison to ReN parental cells.
2. The average branch length of ReN-GFP-FAD cells was markedly reduced after 6 weeks of differentiation in comparison to control cells ,while no difference was observed between parental and ReN-GFP-APP cells .Furthermore, there was a notable decrease in the number of mitochondria in ReN-GFP-APP cells compared to control ReN-GFP cells, as well as a decrease in ReN-GFP-FAD cells compared to both parental ReN and ReN-GFP cells.
3. Additionally, Tuj1-positive FAD neurons showed significant axonal degeneration, indicating neurotoxic effects.

Outlook:

1. Mitochondrial respiration, oxygen consumption rate (OCR), and oxidative phosphorylation (OXPHOS) will be assessed in ReN cell lines at 2 and 6 weeks using Seahorse assays and western blotting.
2. Mitochondrial morphology will be evaluated using transmission electron microscopy (TEM) at 2 and 6 weeks of differentiation.

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