Alzheimer’s disease (AD) is a complex neurodegenerative disease that leads to a clinical decline in neurological function due to a loss of neuronal connectivity in the brain. The aberrant processing and accumulation of proteins is a hallmark of AD, particularly the extracellular deposition of β-amyloid (Aβ) peptides that leads to senile plaque formation and morphological abnormalities in developing neurites. An increase in the ratio of 42 amino acid Aβ species (Aβ42) that is longer and more insoluble, compared with the 40 amino acid long Aβ species (Aβ40), is a feature of the disease [1,2]. This study utilises iPSC-derived AD-model cell lines to study the process of Aβ-induced neurite inhibition and the molecular signalling pathways that drive the process. Aβ is known to bind to the Nogo receptor (NgR), which is involved in the inhibitory response that results in the inability of neurons to regenerate following spinal cord injury (SCI) [3,4]. NgR is known to activate Rho A and ROCK following SCIP, impacting actin dynamics and resulting in neurite retraction. Evidence also suggests that Aβ induction of neurite inhibition involves Rho A and ROCK activation.

The principle of this study was to further elucidate the molecular signalling events involved in Aβ-mediated neurite inhibition through adding inhibitors of Rho A (ibuprofen) and ROCK (Y-27632) signalling to two distinct models of Aβ-induced neurite inhibition. The first of which involves the exogenous addition of Aβ species to neuronal cultures, and the second involves the use of iPSC-derived patient and transfected AD-model cells.

Methodology

Induction of Neurite Outgrowth from Healthy and AD-Model Cells

Neurite Inhibition Induced by Exogenous Aβ

Fig. 2: Neurosphere formation and induction of neurite outgrowth in 2D and 3D culture from human pluripotent stem cells. Neurospheres were formed from both healthy and patient derived AD model cells. The 3D cell line, THK9患者, was first maintained in 20 neurosphere culture (A) before being seeded in suspension and differentiated for 21 days with the synthetic nerve growth factor. This generated the formation of mature neurones (B) for use in neurite outgrowth studies. Neurospheres were also formed from the iPSC-derived neuroepithelial line, NgRΔ, which was initially maintained as neurosphere cultures (C). Neurospheres from both cell lines were subsequently seeded onto 2D (E) or 3D (F) RGD coated growth substrates for a further 10 days during which time neurites are formed. In 2D culture neurite radiuses of the central neurones, whereas in 3D culture neurites possess the 200 μm depth of the scaffold and are visible from the outside of the scaffold. To study the effect of Aβ on neurite outgrowth, exogenous Aβ was added to cultures of SCID-derived neurospheres and NgRΔ-derived all cell types were used to further this mechanism.

Fig. 3: Exogenous Aβ species inhibit neurite outgrowth and are recovered through inhibition of Rho A and ROCK. Neurospheres derived from SCID cells were cultured in 2D (A-E) and 3D (F-I) conditions with the exogenous addition of Aβ40 to the culture medium (5 μM). In addition to this treatment, neurospheres were also treated with the selective ROCK inhibitor Y-27632 (G-I) and inhibitor of Rho A, C3(40 μM). ROCK inhibition (G) was used to determine if Aβ40 supplementation had any inhibiting effect on neurite growth and whether ROCK and Rho A inhibition is involved in downstream PLCG and ROCK signalling. This was also true when assessing the effect of ROCK inhibition. Feret's diameters were collected from 25 cultures for each Aβ concentration. Results show that ROCK and Rho A inhibition significantly reduce neurite outgrowth and neurite density at the highest concentration of Aβ40.

Fig. 4: Neurosphere outgrowth is inhibited from Alzheimer’s disease model cells in 2D culture and recovered by inhibition of Rho A and ROCK. Human AD iPSC-derived wild type (WT) and AD-model cells (35) were cultured in 2D with the selective ROCK inhibitor Y-27632 and inhibitor of Rho A, C3, to identify any differences in their ability to form neurites. Patient-derived neurospheres produce significantly less dense neurite scaffolds than any other cell type tested (J) and both patient and mutation AD-model cell lines produce significantly less dense neurite scaffolds than wild type cells. Reduction in neurite density with F-TβR2-1 enabled reversion to a level greater than the control, and reduced any differences between the cell types (K). Similarly, treatment also reduced any differences in neurite density between the cell types tested. Data points: 100 μm, *p < 0.005, **p < 0.001, ***p < 0.0001, ****p < 0.00001.

Conclusion

- Exogenous addition of Aβ40 to culture medium significantly inhibits neurite outgrowth in both 2D and 3D culture.
- Inhibition of Rho A and ROCK by ibuprofen and Y-27632 respectively can restore neurite outgrowth in the presence of inhibitory Aβ40.
- Alzheimer’s disease model cells with common AD-associated mutations provide a novel system to study the role of Aβ40 in neurite inhibition.
- Neurite density in 2D culture is reduced in AD-model cells compared with their wild type counterpart.
- Inhibition of Rho A by ibuprofen and ROCK by Y-27632 restores neurite density in AD-model cells.
- Rho A and ROCK signalling potentially involved in Aβ-mediated neurite inhibition.
- Neurite outgrowth assay utilizing AD-model cells can be used as a potential screening tool and has implications in the field of personalized medicine.

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Application of Patient-Derived Induced Pluripotent Stem Cells (iPSCs) to Study the Role of Neurite Inhibition and Mechanisms of Recovery in Alzheimer’s Disease

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Introduction

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