Alzheimer’s disease model cells derived from human iPS cells

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Introduction

Alzheimer’s disease (AD) is the most common cause of dementia characterized by impaired memory and cognitive function due to neurodegeneration. The increased risk in AD mortality coupled with the socioeconomic impact of the disease has necessitated the urgent development of an effective therapy. However, the currently available models of AD are challenging and a more humanized, scalable assay system is required to better understand the disease and identify novel therapies.

There are two methods to develop AD model cells based on human iPS cells. The first method uses targeted genetic modification of human iPS cells, which introduces mutations that are AD-specific. In this method, human iPS cells with mutations in the α-secretase (ADAM10 and ADAM17) and γ-secretase (PSEN1 and PSEN2) genes, which are responsible for familial AD, are differentiated into neurons and evaluated for their effect on Aβ production. The second method uses AD patient-derived human iPS cells and AD model cells. In this paper, we mainly describe how we developed the AD model cells (ReproNeuro Ach-AD™ and ReproNeuro Glu-AD™) by generating human iPS cells containing a mutant PS1 gene (P117L), which is responsible for familial AD, and differentiating these cells into cholinergic or glutamatergic neurons. In the brains of patients with AD, cholinergic neurons are damaged and the glutamate system is disrupted. To confirm whether these neurons can be used for drug screening, we performed characterization of these neurons as well as the Aβ assay. The effect of these neurons on the production of Aβ40 and Aβ42 was measured using the AlphaLISA Human Amyloid (1-40/42) Immunoassay kit (PerkinElmer) for high-throughput screening (HTS).

Generating the AD model cells by using human iPS cells

Induction of human iPS cells into cholinergic or glutamatergic neurons

Upper panels: Neurons derived from human iPS cells containing the mutated PS1 (P117L) gene are stained by monoclonal anti-ChAT (cholinergic neurons), anti-GFAP (glial cells), and anti-VGLUT1 (glutamatergic neurons) antibodies. Lower panels: Neurons derived from human iPS cells are stained by anti-NFT (neurofibrillary tangle; cholinergic neurons) and anti-βIII-tubulin (pan-neuron) antibodies. Inset: Two representative images of differentiated human iPS cells stained for PS1 (green) and DAPI (red). Scale bar = 250 μm.

Expression of markers for the undifferentiated state of human iPS cells

Induction of human iPS cells into cholinergic or glutamatergic neurons

Upper panels: Neurons derived from human iPS cells containing the mutated PS1 (P117L) gene are stained by monoclonal anti-ChAT (cholinergic neurons), anti-GFAP (glial cells), and anti-VGLUT1 (glutamatergic neurons) antibodies. Lower panels: Neurons derived from human iPS cells are stained by anti-NFT (neurofibrillary tangle; cholinergic neurons) and anti-βIII-tubulin (pan-neuron) antibodies. Inset: Two representative images of differentiated human iPS cells stained for PS1 (green) and DAPI (red). Scale bar = 250 μm.

Increase of the secreted Aβ42/40 ratio caused by the mutated PS1 gene

Aβ secretion was modulated by the γ-secretase inhibitor (DAPT)

Aβ level in the culture media of the neurons containing the mutated PS1 gene is decreased by the γ-secretase inhibitor (DAPT) in a dose-dependent manner. DAPT was added to the culture media at 5 μM, and the effect was measured using the Meso Scale Discovery Human Aβ assay kit. The calculated IC50 was close to the IC50 of DAPT in the reported study (5 μM, 2017).

Summary

We constructed AD model cells (ReproNeuro Ach-AD™ and ReproNeuro Glu-AD™) via genetic modification of human iPS cells and their induction into neurons.

The Aβ model cells containing the mutated presenilin-1 (PS1) gene showed increased secreted Aβ42/40 ratio, which is one of AD-specific phenotypes.

The measurement of Aβ concentration should be used for high-throughput screening.

AD patient-derived human iPS cells were established originally by ReproCELL.

Materials

Human iPS cell culture

- Human iPS cell line: RCHEP006 (ReproCELL)
- Reprogramming: RCHEP103 (ReproCELL), RCHEP104 (ReproCELL)
- Differentiation: RCHEP006 (ReproCELL), RCHEP103 (ReproCELL), RCHEP104 (ReproCELL)

AD model cell generation

- Recombinant human fibroblast growth factor-2 (rhFGF-2), RCHEOT003 (ReproCELL)
- ReproNeuro Ach-AD™ and ReproNeuro Glu-AD™

Aβ assay

- AlphaLISA® human amyloid beta 1-40 (Aβ1-40) (high specificity) kit, AL275 C/F (Perkin-Elmer, MA, USA)
- AlphaLISA® human amyloid beta 1-42 (Aβ1-42) (high specificity) kit, AL276 C/F (Perkin-Elmer, MA, USA)
- Enzyme-linked immunosorbent assay (ELISA) kit, 354277 (BD Biosciences, Franklin Lakes, USA)
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Screening

- Enzyme-linked immunosorbent assay (ELISA) kit, 354277 (BD Biosciences, Franklin Lakes, USA)
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