Alzheimer's disease model cells derived from human iPS cells

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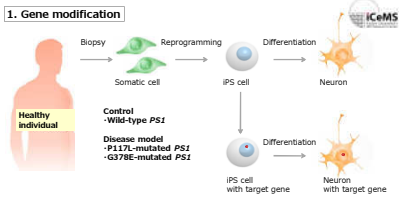
Introduction

Alzheimer's disease (AD) is the most common cause of dementia characterized by impaired memory and cognitive dysfunction due to neurodegeneration. The predicted increase in AD morbidity 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.

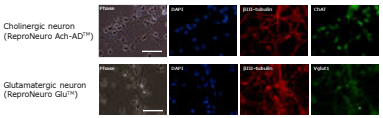
There are two methods to develop AD model cells based on human iPS cells. The first method uses targeted genetic modification of human iPS cells, and the other involves the production of human iPS cells from patients with AD. We supply both types of AD model cells depending on request.

In this poster, 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 *Presenilin1*(*PS1*)gene, which is responsible for familial AD, and differentiating these cells into cholinergic neurons 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 β1-40/1-42 immunoassay kit (Perkin-Elmer) for high-throughput screening (HTS).

Generating the AD model cells by using human iPS cells



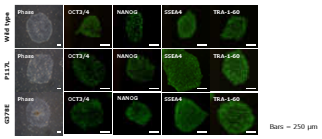
Induction of human iPS cells into cholinergic or glutamatergic neurons



Upper panels: Neurons derived from human iPS cells containing the mutated *PS1* (P117L) gene are stained by anti-choline acetyltransferase (ChAT; cholinergic neurons) and anti-βIII tubulin (pan-neuron) at day 14. Scale bar = 100 μm.
Lower panels: Neurons derived from human iPS cells are stained by anti-vesicular glutamate transporter 1 (Vglut1; glutamatergic neurons) and anti-βIII tubulin (pan-neuron) at day 14. Scale bar = 100 μm.

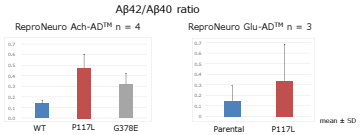
Irrespective of the source and type of cells, there were no differences in their degree of differentiation into neurons (data not shown).

Expression of markers for the undifferentiated state of human iPS cells



Bars = 250 μm

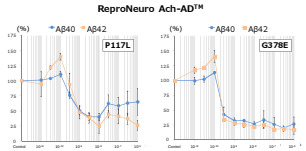
Increase of the secreted Aβ42/40 ratio caused by the mutated *PS1* gene



Aβ40 and Aβ42 secreted into the media were measured using the AlphaLISA® human Aβ kit after the neurons differentiated from the two types of human iPS cells (containing the wild-type and mutated *PS1*) cultured for 14–28 days. Culture media were directly applied to each AlphaLISA® human Aβ kit. The Aβ42/Aβ40 ratio was increased in neurons derived from human iPS cells containing the *PS1* gene with the P117L and G378E mutations. In patients with AD, the higher Aβ42/Aβ40 ratio induced by the elevation of Aβ42 production is associated with Aβ aggregation and deposition, which cause neuron loss. Neurons containing the mutated *PS1* showed a similar phenotype to neurons from patients with AD because their Aβ42/Aβ40 ratio was higher than those of neurons containing the wild-type *PS1*. Thus, neurons containing the mutated *PS1* can be used for AD drug development as a disease model cell reproducing an AD phenotype.

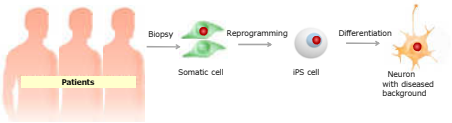
WT; cells with transfected normal PS1 gene Parental;cells without transfection

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

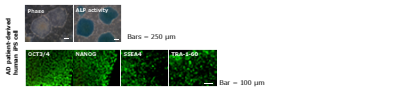


Aβ level in the culture media of the neurons containing the mutated *PS1* gene is decreased by the γ-secretase inhibitor XXI/compound E (DAPT) in a dose-dependent manner. DAPT was added into the culture media at day 12, and the culture was continued for 2 more days. Amounts of Aβ40 and Aβ42 secreted into the media were measured using the AlphaLISA® human Aβ assay kit. The calculated IC_{50} was close to the IC_{50} of DAPT against γ-secretase (i.e., 300 pM)[※].
Data represent the mean ± SD of 3 assays.
※Reference: Data sheet of γ-secretase inhibitor XXI, compound E, 565790, Millipore

2. Patient-derived cells



Expression of markers for the undifferentiated state of human iPS cells



Bars = 250 μm
Bar = 100 μm

Phase contrast, ALP activity stain and immunocytochemistry images of the human iPS cells.
Both types of human iPS cells maintained the undifferentiated state, because these cells formed colonies with smooth edges and expressed some typical markers for the undifferentiated state (OCT3/4, NANOG, SSEA4, and TRA-1-60). Scale bar = 250 μm.

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 AD model cells containing the mutated presenilin1 (*PS1*) gene showed an 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 cells, RCHIPC0001
·ReproFF2™, RCHEMD006 (ReproCELL, Kanagawa, Japan)
·Matrigel™ , 354277 (BD Biosciences, Franklin Lakes, USA)
·Recombinant human fibroblast growth factor-2 (rhFGF-2), RCHEOT003 (ReproCELL)

AD model cells
· ReproNeuro Ach-AD™, RCESDA103 RCESDA 105 (ReproCELL)
· ReproNeuro Glu-AD™, RCESDG103 RCESDG 105 (ReproCELL)

Amyloid β 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)
·White ½ AreaPlate-96, 6005560 (Perkin-Elmer)
·γ-secretase inhibitor XXI, Compound E (DAPT), 565790 (Millipore)
·Enspire® 2300 Multilabel Reader (Perkin-Elmer)

ReproNeuro series

Immunocytochemistry
·4% Paraformaldehyde Phosphate Buffer Solution (PFA), 163-20145 (Wako Pure Chemical Industries, Tokyo, Japan)
·Triton X-100, T8787 (Sigma, MO, USA)
·Bovine serum albumin (BSA), A9418 (Sigma)
·DAPI, D1306 (Life Technologies, CA, USA)

Primary antibodies
·Anti-Oct3/4, sc-5279 (Santa Cruz, CA, USA)
·Anti-Nanog, RCAB004P-P (ReproCELL)
·Anti-SSEA4, MAB4304 (Millipore, MA, USA)
·Anti-TRA-1-60, MAB4360 (Millipore)
·Anti-Choline acetyltransferase (ChAT) , ab137349 (Abcam, Cambridge, UK)
·Anti-Vglut1, V0389 (Sigma)
·Anti-β tubulin isotype III, T8660 (Sigma)

Secondary antibodies (all purchased from Life Technologies)
·Alexa® 488 goat anti-mouse IgG, A-11029
·Alexa® 488 goat anti-mouse IgM, A-21042
·Alexa® 488 goat anti-rabbit IgG, A-11008
·Alexa® 546 goat anti-mouse IgG, A-11003