

高速分化技術によりヒトiPS細胞から作成した アストロサイトの神経細胞サポート機能の評価



Functional evaluation of iPSC-derived astrocytes generated by a rapid differentiation method

〇和田唯奈^{1*}, 矢本梨恵¹, 瀬尾学², 細谷俊彦¹ ¹株式会社リコー, ²Elixirgen Scientific, Inc.

○Yuina Wada^{1*}, Rie Yamoto¹, Manabu Seo², Toshihiko Hosoya¹ ¹Ricoh Company, Ltd., ²Elixirgen Scientific, Inc.

*e-mail; yuina.wada@jp.ricoh.com

Abstract

The Quick-Tissue[™] technology (Elixirgen Scientific, Inc.) is a transcription factor-based method for rapid differentiation of human induced pluripotent stem cells (iPSCs) into desired cell types. The method generates a pure population of neurons (Quick-Neuron[™] Excitatory) within 10 days and astrocytes (Quick-Glia[™] Astrocyte) within 28 days, respectively. Recent studies have revealed that astrocytes play important roles in the brain function and nervous system diseases. The neuron-astrocyte co-culture system is an in vitro tool to evaluate the supportive functions of astrocytes and therefore is an attractive test bed for drug screenings, toxicological assays, and disease research. In the present study, we examined whether the iPSC-derived astrocytes provide supportive functions. We co-cultured the iPSC-derived astrocytes and characterized their responses to various drugs using calcium imaging. The co-cultured neurons responded to antagonists and agonists for receptors of various neurotransmitters as expected. Calcium oscillations of the co-cultured neurons exhibited higher frequency and stronger synchrony compared to that of neuronal cultures without astrocytes, suggesting supportive functions of the astrocytes. These results suggest that the iPSC-derived astrocytes provide neuron-supportive functions that are similar to those of *in vivo* astrocytes and that they are useful for pharmacological assays of glial functions.

Introduction

◆ iPSC-derived neural and glial cells generated by the Quick-Tissue[™] technology



Quick-Glia[™] Astrocyte

Quick-Neuron[™] Excitatory

The Quick-Tissue[™] technology is a transcription factor-based method for rapid differentiation of stem cells (iPSCs or ESCs) into desired cell types. With this method, a pure population of differentiated cells can be produced typically within 10 days.

Aim of this study

- Differentiate within 28 days
- Pure populations



Immunofluorescence of Quick-Glia[™] Astrocyte shows the expression of the astrocytic markers ALDH1L1 and s100 β (scale bar = 100 μ m).

- Differentiate within 10 days
- Positive for neuronal markers



Source: Elixirgen Scientific website

Immunofluorescence staining of Quick-Neuron[™] Excitatory shows the expression of the pan-neuronal marker TUBB3 and the glutamatergic neuron marker vGLUT1 (scale bar = $100 \ \mu m$).

To assess the neuronal-supportive functions of the iPSC-derived astrocytes (Quick-Glia[™] Astrocyte), we examined the calcium spikes in excitatory neurons cultured with these iPSC-derived astrocytes.

Materials and Methods

- Cells : Human iPSC-derived astrocytes (Quick-Glia[™] Astrocyte) and iPSC-derived excitatory neurons (Quick-Neuron[™] Excitatory) from Elixirgen Scientific
- **Plate:** 96-well plate (Corning) or 384-well plate (Thermo Fisher Scientific)
- **Medium conditions:** Neurobasal Plus Medium, B-27[™] Plus Supplement, Neuron Culture Medium (FUJIFILM Wako Pure Chemical Corporation)
- Cultured period: 7 weeks
- Calcium imaging:
 - Instrument: FDSS/µCell system
 - Ca²⁺ indicator: Cal-520AM (f.c. 2μ M)
 - Measurement time: 20min
 - Data acquisition interval: 0.1 sec

Results

Evaluation of neuronal-supportive functions in 96-well plates



Calcium spikes of the co-cultured neurons:

- exhibited stronger synchrony and higher frequency
- supported robustly under a wide range of mixing ratios

iPSC-derived astrocytes provide neuron-supportive functions

20 min

Evaluation of drug responses in the co-culture in 384-well plates Neurons : astrocytes = 4:1× means 0.

Baseline amplitude [10 min after drug administration]

CNQX amplitude) D-AP5 Glutamate Kainic acid Chlorpromazine Amoxapine PTZ Linopirdine **4-AP** Carbamazepine DR, 5HTR NMDA receptor AMPA/NMDA Potassium channel AMPA/Kainate Kainate receptor Selective KCNQ GABA antagonist SSRI Sodium channel blocker channel blocker receptor antagonist antagonist receptor adonist selective agonist blocker antagonist ** baseline 0.1 ** 0.05 **DMSO** of (Ratio -0.05go **Spike number** [10 min after drug administration] **CNQX** Kainic acid **4-AP** D-AP5 Glutamate Linopirdine Chlorpromazine PTZ Carbamazepine er Amoxapine



iPSC-derived astrocytes are useful for HTS pharmacological assays

Summary

- iPSC-derived astrocytes (Quick-Glia[™] Astrocyte) provide neuron-supportive functions.
- These astrocytes are useful for pharmacological assays.
- The system is suited for HTS assays in 384-well plates.

NEURO2022

*p<0.05, **p<0.01, ***p<0.001. Statistical significance of the comparison

to 0.1 % DMSO was determined by the Wilcoxon rank sum test

Disclosure of Conflict of Interest Name of first author: Yuina Wada

> I have no COI with regard to the presentation.