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

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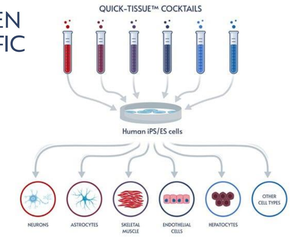
Introduction

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

ELIXIRGEN SCIENTIFIC



Source;
Company websites

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.

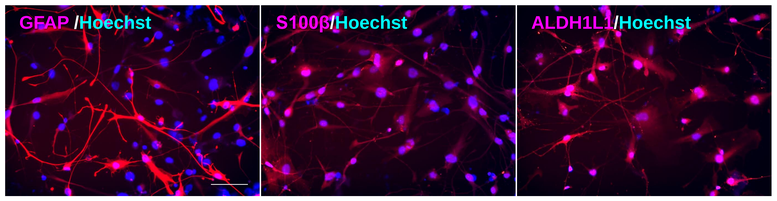
◆ Aim of this study

We evaluate whether the Quick-Glia™ Astrocytes (iPSC-derived astrocytes) provide supportive functions that are similar to that of human primary astrocytes and whether their co-culture system is suitable for HTS assays.

Materials and Methods

- **Cells :** Human iPSC-derived astrocytes (Quick-Glia™ Astrocyte) and iPSC-derived excitatory neurons (Quick-Neuron™ Excitatory) from Elixirgen Scientific, Primary Human astrocytes from ScienCell Research Laboratories (in 384-well plate assay) and Thermo Fisher Scientific (in MEA assay)
- **Plate:** 384-well plate (Thermo Fisher Scientific) or CytoView MEA 48 (Axion)
- **Medium conditions:** Neurobasal Plus Medium, B-27™ Plus Supplement, Neuron Culture Medium (FUJIFILM Wako Pure Chemical Corporation)
- **Culture period**: 6 or 7 weeks (post-differentiation)
- **Calcium imaging:**
  - Instrument: FDSS/µCell system
  - Ca^{2+} indicator: Cal-520, AM (f.c. 2 µM)
  - Measurement time: 20 min
  - Data acquisition interval: 0.1 sec
  - Coating methods: Ricoh's method
  - Neurons (10,000 cells /well)
  - Astrocytes (2,500 cells /well)
- **Monitoring electrical activity (MEA):**
  - Instrument: Maestro Pro (Axion)
  - Measurement time: 10min
  - Coating materials: PEI / Matrigel
  - Neurons (80,000 cells /well)
  - Astrocytes (20,000 cells /well)

Result 1: The expression of astrocytic markers



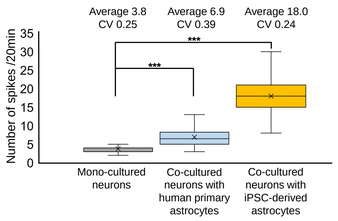
Immunofluorescence of Quick-Glia™ Astrocyte shows the expression of the astrocytic markers GFAP, S100β and ALDH1L1 on days 7 post-differentiation (scale bar = 100 µm).

More than 80% of cells with high expression of astrocytic markers GFAP, S100β and ALDH1L1.

iPSC-derived astrocyte is a pure cell population of astrocytes expressing astrocytic markers.

Result 2: Ca^{2+} transient assay in 384-well plates

Evaluation of neuronal-supportive functions



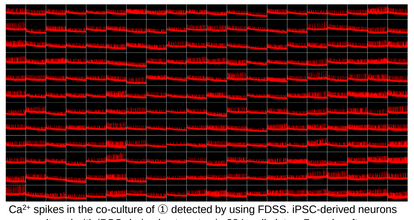
- Calcium spikes of the co-cultured neurons exhibited stronger synchrony and higher frequency.
- Co-culture with iPSC-derived astrocytes shows the same increase in spike number as when co-cultured with human astrocytes.

n ≧60, ***p<0.001. The significant difference for mono-cultured neurons was analyzed by unpaired t-test.

iPSC-derived astrocytes provide neuron-supportive functions.

Evaluation of variability in the co-culture

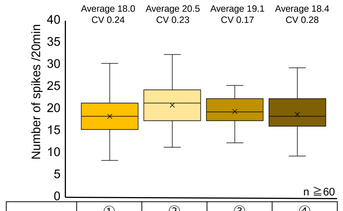
Difference between wells on one plate



Ca^{2+} spikes in the co-culture of ① detected by using FDSS. iPSC-derived neurons were cultured with iPSC-derived astrocytes in 384-well plates. 7 weeks after differentiation. Single squares represent single wells in the 384-well plate.

- Cells in all well exhibited spontaneous Ca^{2+} spikes.
- No difference in number of Ca^{2+} spikes between wells in all conditions (CV < 0.3), and median value is in the range 18-21.

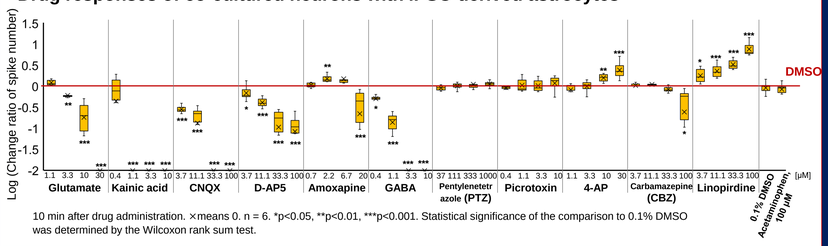
Variation of number of Ca^{2+} spikes



	①	②	③	④
Astrocyte lot	Lot A			
Neuron lot	Lot a			Lot b
Plate	Plate 1	Plate 2	Plate 3	
Date	X		Y	

Evaluation of drug responses

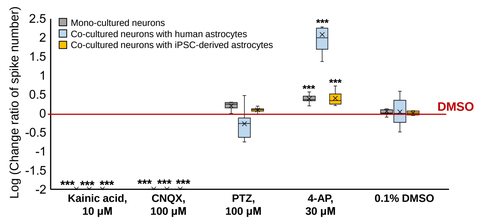
Drug responses of co-cultured neurons with iPSC-derived astrocytes



10 min after drug administration. ×means 0. n = 6. *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.

CNQX, D-AP5, GABA, 4-AP, CBZ and Linopirdine: Pharmacological effects detected dose-dependently
Amoxapine >6.7 µM: Pharmacological effects detected, <20 µM: Excitotoxicity detected
Glutamate and Kainic acid: Excitotoxicity detected
PTZ and Picrotoxin: No effects

Comparison of drug responses



Co-culture with iPSC-derived astrocytes shows the similar drug responses in spike number as when mono-cultured neurons and co-cultured with human astrocytes.

10 min after drug administration. ×means 0. n = 6. ***p<0.001. Statistical significance of the comparison to 0.1% DMSO was determined by the Wilcoxon rank sum test.

Summary of drug responses in 384-well plates

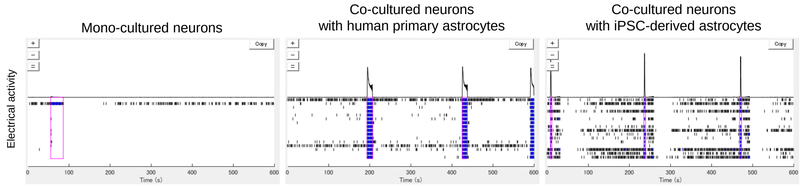
Receptor/ Ion channel	Mode	Compound	Expected changes in spike number		Spike number changes in cocultured neurons with astrocytes			
					Human primary		iPSC-derived	
AMPA/NMDA receptors	agonist	**Glutamate**	↑	Excitatory	↓	Excitotoxicity	↓	Excitotoxicity
Kainate receptors	agonist	**Kainic acid**	↑	Excitatory	↓	Excitotoxicity	↓	Excitotoxicity
AMPA/Kainate receptors	antagonist	**CNQX**	↓	Inhibitory	↓	○	↓	○
NMDA receptors	antagonist	**D-AP5**	↓	Inhibitory	↓	○	↓	○
Serotonergic receptors	Inhibitor	**Amoxapine**	↑	Excitatory	↑	○	↑	○
GABA receptor	agonist	**GABA**	↓	Inhibitory	↓	○	↓	○
GABA receptor	antagonist	**PTZ**	↑	Excitatory	→	×*	→	×*
GABA receptor	antagonist	**Picrotoxin**	↑	Excitatory	→	×*	→	×*
Potassium channel	blocker	**4-AP**	↑	Excitatory	↑	○	↑	○
Sodium channel	blocker	**CBZ**	↓	Inhibitory	↓	○	↓	○
KCNQ channel	blocker	**Linopirdine**	↑	Excitatory	↑	○	↑	○

*The excitatory neurons contains little GABAergic neurons.

iPSC-derived astrocytes are useful for HTS pharmacological assays.

Result 3: Multielectrode Array (MEA) assay

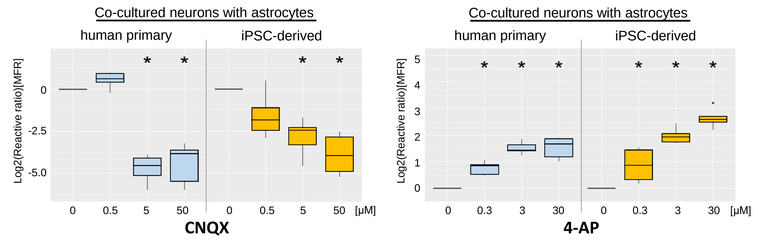
Evaluation of neuronal-supportive functions



- Firing of the co-cultures with astrocytes exhibited higher frequency than its of mono-culture of excitatory neurons.
- Synchronized network bursts were observed in co-cultures with astrocytes.

iPSC-derived astrocytes provide neuron-supportive functions.

Evaluation of drug responses



n = 5, *p<0.01, Statistical significance of the comparison to 0 µM (0.1 % DMSO) was determined by the Wilcoxon rank sum test.

Pharmacological effect was detected dose-dependently when co-cultures with astrocytes were exposed to drugs.

The cells co-cultured on MEA plates show similar drug responses in 384-well plate.

Conclusions

- **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.**
- **The cells co-cultured on MEA plates showed similar results in 384-well plate.**

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