Pharmacological evaluation in human iPSC-derived cortical and sensory neurons using high-throughput MEA system

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Many neuronal phenotypes have been derived from human iPSCs

Application of these cells to toxicological assay and drug discovery
Human iPSC-derived neurons are expected as a new toxicological evaluation assay to replace animal experiments in preclinical studies.
Toxicological evaluation assay in human iPSC-derived neurons

Today’s topics

- MEA assay in CNS to predict the seizure risks
- MEA assay in PNS to predict the pain risks
Methods

Electrophysiological methods

High-throuput multi-electrode array system (Presto)

Features for MEA
- Non invasive
- Real time
- Multi-point measurement of activity of cultured neurons

24 wells (384 electrodes)
16 electrodes/well

Presto System

Extracellular signals of action potentials

Low impedance electrode

384 electrodes
Methods

human iPSC-derived cortical neurons

Human iPSC-Derived Neural Stem Cells + Human iPSC-Derived Mature Astrocyte

Co-culture neurons with astrocyte
Colocalization of pre- and postsynaptic components

Colocalization was confirmed, and synapses had mainly formed around thick dendrites and the soma.

Morphology (Pyramidal-like morphology)

Pyramidal-like morphology with thick apical and basal dendrites

These neurons were similar in appearance to cerebral cortical neurons in vivo

Pharmacological responses

hiPSC-derived neurons on the MEA

We confirmed electrophysiological responses of typical GABA and glutamate receptors

Plasticity in hiPSC-derived neurons

We reported that human iPSC-derived cortical neurons also have the plasticity.

Odawara A, et.al, BBRC 496, 856 (2016).
Induction of epileptiform activity

Major toxicity of new drugs in CNS is seizure-like firings

Convulsant drugs

■ 4-Aminopyridine (4-AP): $K^+$ channel antagonist
■ Pilocarpine: Muscarine receptor agonist
■ Chlorpromazine: $D_2$ receptor antagonist
■ Pentylenetetrazole (PTZ): GABA-A antagonist

Anti-convulsant drug

■ Phenytoin: $Na^+$ channel antagonist

Negative control

■ Acetaminophen: analgesic effect
The number of synchronized bursts were increased in a concentration-dependent manner.
Movie at before and 4-AP 10 μM administration

Before
Results: Raster plots in pilocarpine administration

The number of synchronized bursts were also increased in a concentration-dependent manner at pilocarpine administration.
The number of synchronized bursts were also increased up to 3 μM and disappeared at 10 μM administration.
Results: Raster plots in PTZ administration

The number of synchronized bursts were not changed. The duration of synchronized burst were increased in a concentration-dependent manner.
Results: Firing analysis in 4-AP, pilocarpine, chlorpromazine, and PTZ administration

Number of synchronized bursts were increased at 4-AP, pilocarpine and chlorpromazine administration.

On the other hand, number of synchronized bursts were decreased at PTZ administration.
Results: Firing analysis in 4-AP, pilocarpine, chlorpromazine, and PTZ administration

We detected the effects of four convulsant drugs in human iPSC-derived neurons and found that the responses of PTZ is different from 4-AP, pilocarpine and chlorpromazine.
The number of synchronized bursts disappeared at 100 μM phenytoin administration. We confirmed the responses of Na\(^+\) channel blocker.
The number of synchronized bursts and total spikes were not changed in a concentration-dependent manner at acetaminophen administration.
The induction of epileptiform activity by 4-AP, pilocarpine, chlorpromazine and PTZ and the suppressive effects by phenytoin were detected in hiPSC-derived cortical neurons made by Axol bioscience.
Peripheral nervous system

Sensory neuron


Human iPSC-derived sensory neurons are suitable to toxicological assay for pain
Methods

human iPSC-derived sensory neurons

Human iPSC-Derived sensory neuron progenitors

Human DRG tissue

Axol iPSC-Derived Sensory Neuron Progenitors

Dr Edward Emery (University College London).
Results: Sensory marker expression

We confirmed the typical human sensory marker.
Results: Capsaicin

Capsaicin

hiPSC-derived Sensory neurons on the MEA

We confirmed that TRPV1 channels was working electrophysiologically.
Results: Temperature change

The responses against temperature change

From 37 to 46°C

It was consistent with the activation temperature of TRPV1 channels

37°C  43°C
Results: Menthol and AITC (wasabi)

We also confirmed TRPM8 and TRPA1 channel was working electrophysiologically.
Results: Classification by electrophysiological responses

Human iPSC-derived sensory neurons were classified into 27 types depending on physiological responses against 3 compounds (Capsaicin, Menthol, and AITC).

- Percentage of positive responses against capsaicin were high both hiPSC-derived neurons and rat DRG neurons.
- hiPSC-derived sensory neurons have a variety of physiological properties and resemble to rat DRG neurons.
Results: Anti-cancer drug (Oxaliplatin and Vincristine)

MEA measurement in hiPSC-derived sensory neurons is useful to pain evaluation of anti-cancer drugs.
Results: Pain relief drug

We also confirmed no responses to pain relief aspirin in cultured hiPSC-derived sensory neurons.
Humen iPSC-derived sensory neurons (Axol Bioscience) show the expression of typical sensory neural marker Nav1.7, TRPV1, and TRPA1.

We detected the physiological responses to temperature change, capsaicin, menthol, and AITC.

Human iPSC-derived sensory neurons were classified into 27 types depending on physiological responses against 3 compounds.

We detected the responses to anti-cancer drugs oxaliplatin and vincristine in cultured hiPSC-derived sensory neurons. We will investigate the mechanism of action in the responses at anti-cancer drug administration.

MEA measurement in cultured hiPSC derived sensory neurons are suitable to toxicological assay for pain in peripheral nervous system.
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