Ready-To-Use hiPSC-derived Motor Neurons for Modeling Neurological Diseases and Toxicology Studies

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Abstract

In this study, we developed a robust method to produce highly pure, functional validated, and ready-to-use motor neurons from human induced pluripotent stem cells (iPSC), which can be used in neurological disease modeling and toxicology studies. Using xeno-free differentiation conditions, we were able to produce motor neurons from human iPSC, at greater than 85% purity as measured by immunostaining of ISL1, HB9, ChAT and Tuji. iXCells™ hiPSC-derived motor neurons are functionally verified by neuromuscular junction assay and electrophysiological assays. These cells can be cryopreserved, thawed, and cultured in defined maintenance media with or without glia cells for prolonged culture. Using iPSC lines from multiple donors, including Amyotrophic Lateral Sclerosis (ALS) patients, we have demonstrated the protocol is robust, and independent of the donor iPSC lines. We developed ALS isogenic models carrying multiple mutations in TDP-43 gene, which were used for identifying disease signature genes with transcriptome analysis. Taken together, these data show that our protocol is reproducible in human iPSCs, and is applicable in modeling MN-degenerative diseases, toxicology studies, and in proof-of-principle drug-screening assays.

Results

Derivation of highly pure, functional validated, and ready-to-use motor neurons from human iPSCs by a xeno-free differentiation protocol

Figure 1. Robust protocol for human motor neuron differentiation from human induced pluripotent stem cells (iPSCs) using xeno-free conditions. The ready-to-use iPSCs™ Motor Neurons are available for either cryopreserved or live culture formats.

Figure 2. On Day 2 post recovery of iPSCs™ Motor Neurons, more than 85% of the cells express HB9 (A and A') and ISL-1 (B and B').

Functional validation of iPSCs™ Motor Neurons

Figure 3. (A and B) Mature motor neurons in long term culture. Frozen iPSCs™ Motor Neurons were recovered and cultured in the Motor Neuron Maintenance Medium for 30 days, and the images of co-staining with ChAT & MAP2 (A) and TDP-43 & Neurofilament (B) are shown. (C) Immunostaining of mature motor neurons when co-cultured with mouse primary astrocytes for 10 days. (D) Mature motor neurons can be co-cultured with astrocytes more than 30 days. Mouse astrocytes were stained with GFAP (green), motor neurons were stained with Tuji (red).

Figure 4. Gene expression analysis during the motor neuron differentiation. (A) The RNA from iPSCs, neural stem cells (NPC) and motor neurons (MN) were analysed by qRT-PCR. (B) RNA-seq analysis of 9 lines of iPSC-motor neurons and their parental iPSC lines. The neuronal and synaptic markers are upregulated in the iPSC-motor neurons.

Figure 5. Neuronal network development of iPSCs™ Motor Neurons. Live culture images were recorded on DIV 2, DIV 3, DIV 6 and DIV 7 using IncuCyte. Neuronal body clusters are shown in yellow, and the neurites were in purple.

Figure 6. (A) Electric activities of iPSCs™ Motor Neurons were recorded using Maestro Multwell MEA System. iPSCs™ motor neurons derived from two different iPSC lines (Red and Green frames) were cultured on 48-well MEA plates for 30 days. (B) Electric activities increase with prolonged culture (about 2 months).

Figure 7. (A and A') 3-projection confocal image of iPSCs™ human iPSC-motor neurons co-cultured with C2C12 mouse myotubes for 6 days. Neuromuscular junction was stained with a-bungarotoxin (BTX). Motor neurons were stained with Neurofilament and MAP2. The framed area were enlarged in (A').

Figure 8. (A) iPSCs™ motor neurons co-cultured with C2C12 mouse myoblasts for 6 days. The well-bread synchronized bursting (red frames) were detected using MEA system. (B) Glutamate (100μM) treatment increased number of bursting electrodies. (C) Glutamate (100μM) treatment increased burst frequency.

Applications of iPSCs™ Motor Neurons in toxicology studies

Figure 9. Electrical activity of iPSCs™ iPSC-motor neurons measured in real-time using xCelligence RTCA CardioCR system (ACEA Biosciences) (A). (B) Zoomed in view of iX-Plate cardioCR 48 wells. (C) iPSCs™ motor neurons grew around the ECH probe. (D) Electrical activities of iPSCs™ iPSC-motor neurons and myoblast co-culture measured in real-time. 100μM glutamate increases the frequency and magnitude of electric spikes, while 100μM Curare, a neuromuscular blocker, completely inhibits the electric activity.

Figure 10. (A) The toxic effect of methylmercury (MeHg) on motor neurons. The frozen iPSCs™ Motor Neurons were recovered and cultured for 4 days. (B) A biphasic increase in intracellular Ca²⁺ was observed in hiPSC-MNs after MeHg exposure. The 1st phase indicates the increase in Ca²⁺ as a result of the release of Ca²⁺ from intracellular stores. The second phase is due to the influx of extracellular Ca²⁺. (C) The expression of the AMPA receptor GluA3 and 2 subunits. Distribution of neurofilament (H, Nfn), AMPA receptor subunits GluA3 and GluA2 in 7 day in vitro culture of hiPSC-MNs. (D) Time-to-offset of increase in fura-2 fluorescence during perfusion of hiPSC-MNs with MeHg (0.1 - 1.5 μM) in HEPES Buffered Saline (HBS). (E) Acute MeHg exposure induced increases of Ca²⁺, in hiPSC-MN are mediated in part by AMPA/kainate receptors. (F) Acute MeHg exposure induced increases of Ca²⁺, in hiPSC-MNs are mediated in part by Ca²⁺ permeable AMPA receptors.