Neuroscience 301 (2015) 204–212

EVALUATION OF ESTABLISHED HUMAN IPSC-DERIVED NEURONS TO MODEL NEURODEGENERATIVE DISEASES

G. MENEGHELLO,[a†] A. VERHEYEN,[a†*] M. VAN INGEN,[a]
J. KUIJLAARS,[b] M. TUEFFERD,[a]
I. VAN DEN WYNGAERT[a] AND R. NUYDENS[a*]

[a] *Janssen Research & Development, A Division of Janssen Pharmaceutica N.V, B-2340 Beerse, Belgium*

[b] *Biomedical Research Institute, Hasselt University, B-3590 Diepenbeek, Belgium*

Abstract—Neurodegenerative diseases are difficult to study due to unavailability of human neurons. Cell culture systems and primary rodent cultures have shown to be indispensable to clarify disease mechanisms and provide insights into gene functions. Nevertheless, it is hard to translate new findings into new medicines. The discovery of human induced pluripotent stem cells (iPSC) might partially overcome this problem. Commercially available human iPSC-derived neurons, when thoroughly characterized and suitable for viral transduction, might represent a faster model for drugs screening than the time-consuming derivation and differentiation of iPSC from patient samples. In this study we show that iCell® neurons are primarily immature GABAergic neurons within the tested time frame. Addition of C6 glioma conditioned medium improved the bursting frequency of cells without further maturation or evidence for glutamatergic responses. Furthermore, cells were suitable for lentiviral transduction within the tested time frame. Altogether, iCell® neurons might be useful to model neurodegenerative diseases in which young GABAergic subtypes are affected. **© 2015 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: disease modeling, neurodegeneration, hiPSC, GABAergic neuron.

INTRODUCTION

An increased life expectancy brings enormous challenges for the healthcare systems, which are facing a fast pace increase in the number of people affected by neurodegenerative diseases. However, the existing treatments are limited at present, mainly due to inaccessibility of human neurons. This hampers the study of disease mechanisms and consequently also the development of novel therapies. Gradual decline in neurological function accompanied by neuronal loss are the common hallmarks of different neurodegenerative diseases. Cell culture systems, primary rodent cultures and transgenic animals have shown to be indispensable to clarify disease mechanisms and provide insights into gene functions (Agholme et al., 2010; Fishbein et al., 2014; Kumaraswamy et al., 2014; Tallaksen-Greene et al., 2014). Nevertheless, a reliable representation of human phenotypes still remains challenging at present, leading to the failure of many drugs in clinical trials (Tiscornia et al., 2011). Since the discovery that adult human somatic cells can be reprogrammed into induced pluripotent stem cells (iPSC) by overexpression of only four transcription factors (Takahashi et al., 2007), a new era of research has started. Recently, numerous studies using iPSC-derived neurons have been published, including iPSC-derived neurons to model neurodegenerative diseases (Israel et al., 2012; Mattis et al., 2012; Kaye and Finkbeiner, 2013; Ryan et al., 2013; Richard and Maragakis, 2014). These proof-of principle studies recapitulate the key aspects of the different pathologies, including aberrant synaptic abnormalities and functional deficits (Penzes et al., 2011; Hick et al., 2014; Wen et al., 2014).

Since derivation and subsequent differentiation of iPSC is rather time consuming and subjected to variability, commercially available sources of human neurons are more attractive to pharmaceutical companies, looking for fast-paced solutions to drugs screening. Commercially available iPSC-derived neurons or neural progenitors could potentially provide a faster *in vitro* system for preclinical drug discovery, neurotoxicity testing, and predictive disease modeling. iCell® neurons are human iPSC-derived neurons and have been used in the past by different groups to model various neurological disorders (Xu et al., 2013; Dage et al., 2014; Odawara et al., 2014). According to these previous studies, the cells behave like a highly pure population of functional forebrain neurons with both GABAergic and glutamatergic subtypes (Dage et al., 2014). Different subtypes of neurons are affected in different neurodegenerative models (Kriks et al., 2011; Shin et al., 2012; Vazin et al., 2014). Therefore, a thorough characterization of human iPSC-derived neurons to identify these neuronal subtypes is necessary before starting disease modeling.

*Corresponding authors. Address: Janssen R&D, Turnhoutseweg 30, 2340 Beerse, Belgium.
E-mail addresses: averhey1@its.jnj.com (A. Verheyen), rnuydens@its.jnj.com (R. Nuydens).
† Equal contribution.
Abbreviations: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; iPSC, induced pluripotent stem cells.

http://dx.doi.org/10.1016/j.neuroscience.2015.05.071
0306-4522/© 2015 IBRO. Published by Elsevier Ltd. All rights reserved.

In the present study we evaluate commercially available human iPSC-derived neurons as a model to study neurodegenerative diseases, at both morphological and functional levels. A thorough characterization by microarray, immunocytochemistry and functional calcium imaging is performed. As several studies have shown that conditioned medium can improve neuronal differentiation and maturation (Unsicker et al., 1984; Liu et al., 2013; Kaiser et al., 2014), cell culture medium was conditioned with rat glioma cell medium, in order to improve the functional and maturation properties of the cells. Last, the potential for gene overexpression is assessed using a viral approach.

EXPERIMENTAL PROCEDURES

Cell culture conditions

iCell® neurons (Cellular Dynamics International, NRC-100-010-001, lot numbers 1388825 and 1360101) were cultured at 40,000 cells/well in MW96 µclear plates (Greiner) according to the manufacturer's instructions. Cells were kept in culture for maximum 35 days, as they detached in our hands after 4–5 weeks in culture Half of the medium was changed twice per week, either with iCell® medium (CDI) or C6 glioma conditioned medium. Laminin (2 µg/ml, Sigma, St. Louis, MO, USA) was added to the culture medium to improve cells attachment.

The rat C6 glioma cell line (Benda et al., 1968) was cultured in DMEM high glucose supplemented with 1% L-Glutamate 200 mM, 1% sodium pyruvate 100 mM, 1% penicillin–streptomycin (all Gibco, Life Technologies, Carlsbad, CA, USA) and 10% FBS (Hyclone). At confluence, medium was replaced with iCell® neuron maintenance medium (CDI, 30 ml in T175). Two days later, conditioned medium was collected, centrifuged (5 min at 2000 rpm), filtered and stored at −20 °C until use.

Primary cortical neurons: cortices were dissected from wild-type E18/19 Wistar Crl:WI rats (Charles River) in HEPES (7 mM) buffered Hanks balanced salt solution (HBSS) (all Gibco), and dissociated enzymatically and mechanically. After centrifugation, the cell pellet was resuspended in minimal essential medium (MEM, Gibco) supplemented with 10% heat-inactivated normal horse serum (Innovative Research, MI, USA) and 30 mM glucose (Merck). Cells were plated in MW96 µclear plates (Greiner). After 4 h, medium was replaced with B27 supplemented Neurobasal medium, containing L-glutamine (2 mM) (all Gibco), and cultures were kept in a humidified CO_2 incubator (37 °C; 5% CO_2) for 12 days.

Immunocytochemistry

Cells were fixed for 15 min using 4% paraformaldehyde in PBS, washed and permeabilized with Triton-X100 (0.25%) in PBS. After 30-min blocking with normal goat serum in 0.1% BSA in PBS, cells were incubated overnight at RT with the following antibodies: rabbit anti-β3 tubulin TUBB3 (Covance), rabbit anti-MAPT (Dako), rabbit or chicken anti-MAP2 (Millipore, Billerica, MA, USA and Aves Labs, Tigard, Oregon, USA), rabbit anti-nestin, mouse anti-PSD95 (both Abcam, Cambridge, UK), mouse anti-synapsin, mouse anti-synaptophysin, rabbit anti-VGLUT1, rabbit anti-VGLUT2, rabbit anti-VGAT, mouse anti-GAD65 (all Synaptic Systems). Subsequently, cells were washed with PBS/BSA (0.1%) and incubated for 1 h at RT with Alexa 488, Alexa 555 or Alexa 647 secondary antibodies (Life Technologies, Carlsbad, CA, USA). DAPI was used to stain the nucleus. Confocal images were taken either manually with a Zeiss LSM 510 or automated with the C7000 high content imaging system (Yokogawa). Different neuronal subpopulations were quantified using Image J software. At least 200 cells from different images were counted for each condition. Quantifications are shown as the % average ± standard error (SE).

RNA in situ hybridization

RNAscope® or RNA-ish was performed according to the manufacturer's instructions (RNA fluorescent multiplex kit, Advanced Cell Diagnostics, Hayward, CA, USA). Cells were fixed with PFA (4%) in PBS and dehydrated in ethanol. Cells were then rehydrated followed by protease treatment and PBS washes. Cells were subsequently incubated for 2 h at 40 °C with positive control probe (ubiquitin C), negative control probe, target probes (*MAP2, GAD2, SLC17A7, SCL17A6*) or blanks. Afterward Amp 1-FL (30 min at 40 °C), Amp 2-FL (15 min at 40 °C), Amp 3-FL (30 min at 40 °C) and Amp 4-FL (15 min at 40 °C) were subsequently added with washing steps in between. Finally, RTU DAPI solution was added for 30 s at room temperature followed by final washes in PBS. Imaging was performed using the high content imaging system CV7000 (Yokogawa).

RNA extraction and microarray analyses

RNA extraction was performed using the RNeasy mini kit (Qiagen, Hilden, Germany). cDNA targets were prepared and labeled using the IVT express kit and then hybridized on Affymetrix® Human Genome U219 array plate in the GeneTitan® instrument (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's protocol.

Microarray analysis was performed using the Bioconductor package version 2.12 (working with R version 3.0.1) (Gentleman et al., 2004). Target transcripts of probes were annotated using Entrez Gene based alternative cdf version 15.1.0 (Dai et al., 2005), assigning probes to 18,567 unique transcripts. RMA algorithm was used for pre-processing (Irizarry et al., 2003).

Viral transduction

DIV7 iCell® neurons were transduced with either AAV (vector AAV-6-EGFP-WB(X), 2.4 × 10e8 transduction units/µl), produced by Dr. Sebastian Kügler (Dept. of Neurology, University Medicine Goettingen, Germany) or Lentivirus (Vector pLX_TRC315, Janssen Pharmaceutica, with a relative titer in the range of 10e7 transduction units/ml), both carrying the GFP gene

Download English Version:

https://daneshyari.com/en/article/6272143

Download Persian Version:

https://daneshyari.com/article/6272143

Daneshyari.com