Developmental delays in attention-deficit hyperactivity disorder: the point of view from induced pluripotent stem cells

Edna Grünblatt1,2, Asli Aybile Dogan1, Silvano Re1, Gregor Berger1, Anna Werling1, Susanne Walitza1,3,4,
1Department of Child and Adolescent Psychiatry and Psychotherapy, Psychiatric Hospital, University of Zurich, Zurich, Switzerland; 2Department of Neurology, Neurological Center Zurich, University of Zurich and ETH Zurich, Zurich, Switzerland; 3Zurich Center for Integrative Human Physiology, University of Zurich, Switzerland

INTRODUCTION
Attention-deficit hyperactivity disorder (ADHD) is one of the most common psychiatric disorders in children and adolescents with over 5% of the population affected worldwide [1]. Up to date no animal or cellular model exists which faithfully mirrors the disorder. To elucidate the pathomechanisms of ADHD a personalized human neuronal cell model is required to reflect ADHD (Gibco).

PROJECT WORKFLOW

- Keratinocytes isolation: Cells from the outer root sheet of 5 anagen hair follicles were dissociated by performing a two-round 0.05% Trypsin incubation. The cells were grown on Collagen I coated plates with serum free medium.
- Reprogramming: Low passage keratinocytes were transduced using “CytoTune™-iPSC 2.0 Sendai Reprogramming Kit” (Invitrogen) [3]. iPSC were manually isolated after 4 weeks and cultured on vitronectin coated plates with “Essential 8™ Flex” medium (Gibco).
- Differentiation: iPSC were differentiated using “PSC Neural Induction Medium” (Gibco) [4] on Cultrex® BME (Trevigen) coated plates and NSC were induced to differentiate into a mature neuronal culture using Neurobasal, 2% B27, 2 mM Glutamine, 1X CultureOne®.

PRELIMINARY RESULTS

- Transcriptomic: Gene expression profiles for MAP2, NES, PAX6, SeV, TERT, NANOG, OCT3/4 and Lin28A was conducted and normalized to reference genes HMBS, ACTB and GAPDH.

FUTURE DIRECTIONS

- Establishment of rapid and reproducible protocol for the isolation, culture and reprogramming of keratinocytes from plucked human hair follicles (Fig. 1).
- Expression of specific marker for reprogrammed keratinocytes, demonstrating pluripotency and similar phenotype as commercial iPSC (hiPSC control line) as well as hESC line (Fig. 2).
- Differentiation into post-mitotic neurons with measurable spontaneous electrical activity (Fig. 3), and gene expression profiles (Fig. 4).
- QC testing shows no gross genomic aberrations, no traces of mycoplasma contaminations and very low Sendai virus residue (Fig. 5).

REFERENCES


MATERIALS AND METHODS

Figure 1: From hair follicle to neurons. Keratinocytes were isolated from hair follicles plucked from a healthy control and expanded. Keratinocytes were reprogrammed using CytoTune 2.0 kit and iPSCs were expanded and differentiated into NSC. Finally, NSC were differentiated into a mature neuronal cell culture.

Figure 2: Heatmap showing the qRT-PCR result for the pluripotency markers LIN28A, NANOG, OCT3/4 and TERT and the keratinocyte marker KRT14. Upon reprogramming the keratinocyte marker KRT14 is highly downregulated, while markers for pluripotency are upregulated in all the generated iPSC lines. For the tested genes, the generated lines show similar expression pattern as the commercial iPSC line and the embryonic stem cell line (hES). Values were log2 transformed and the plot was generated with the R package heatSet. Levels of high expression are shown with red, while levels of low expression are upregulated in all the generated iPSC lines. For the tested genes, the generated lines show similar expression pattern as the commercial iPSC line.

Figure 3: Multi electrode array recording of iPSC derived neuronal culture. External cellular electrical activity was measured using the MEA2000-System (MCS) during terminal neuronal differentiation. The recordings suggest a rapid maturation of the neurons during the first weeks of differentiation.

Figure 4: Gene expression results of neuronal differentiation. Samples were assessed for the expression of PAR6, NES and MAP2 and normalized to ACTB and GAPDH as most stable reference genes. Neural progenitor makers PAR6 and NES are upregulated in passage 4 NSCs (N=4), while neuronal marker MAP2 is upregulated in neuronal cells at day 28 of neuronal differentiation (N=4). Values are given as mean ± SEM.

Figure 5: Quality control. Experiments of the generated iPSC line show no traces of contaminations and a normal karyotype. Mycoplasma contamination assay was performed using “LookOut® Mycoplasma PCR Detection Kit” (Sigma). G-bread staining of a P10 iPSC clone was kindly performed by the Institute of Medical Genetics, University of Zurich, Schleuen. Traces of Sendai virus was analyzed via qRT-PCR analysis.

Contact

Edna Grünblatt
Department of Child and Adolescent Psychiatry and Psychotherapy, Psychiatric Hospital, University of Zurich, Switzerland
E-mail: edna.gruenblatt@kjpd.uzh.ch