Use of pluripotent stem cells and stem cells-derived neurons in support of drug discovery and target validation

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1 INTRODUCTION

The advent of stem cells technologies has opened up new opportunities in drug discovery and development by providing new tools for drug screening, target identification and toxicity testing. Moreover the discovery of reprogramming patient derived somatic cells into induced pluripotent stem cells (iPSCs), which have the potential to differentiate into any cell or tissue type, has offered an attractive alternative to currently used cell models and primary cells to model disease phenotype.

Although stem cells offer huge potential in the drug development field many scientific and industrial challenges are yet to be overcome in order to increase their use in the different stages of drug discovery process.

Using both human embryonic stem cells and induced pluripotent stem cells derived from healthy controls and patients with neurodegenerative disorders (Huntington's disease, Amyotrophic Lateral Sclerosis, Alzheimer's disease), Charles River has developed a stem cell culture platform suitable for high-throughput screenings (HTS) and target validation. Moreover pluripotent stem cells have been routinely differentiated into striatal and cortical neurons using well validated protocols adapted from published papers, which employ the use of small molecules during neuronal induction.

Here we report examples of high and medium throughput assays using pluripotent stem cells and stem cells derived neurons which have been used for a Huntington's disease drug discovery program. Molecular biology (qPCR and branched DNA) and high content immunocytochemistry (ICC) methodologies have been developed for a thorough quality control during routine cell culture and differentiation. Electrophysiology approaches based on voltage clamp and current clamp protocols are also in place in order to measure neuronal activity during differentiation. Moreover using high content algorithms in fixed-cells and real time format we have developed low-throughput, high value assays to further support hit-to-lead and lead optimization programs.

2 METHODS

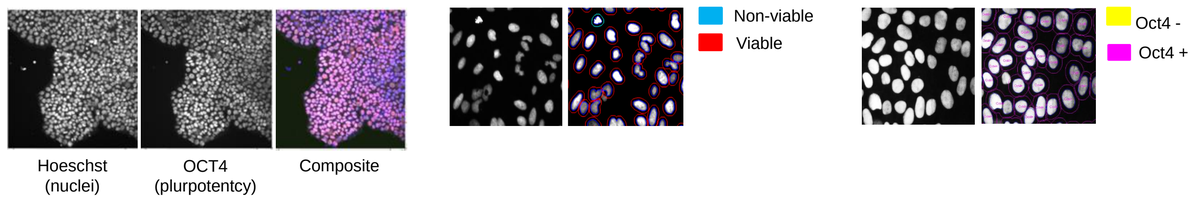

Figure 1. Pluripotent stem cell monolayer culture (left images) and quantitative viability and pluripotency assessment with high content algorithm.

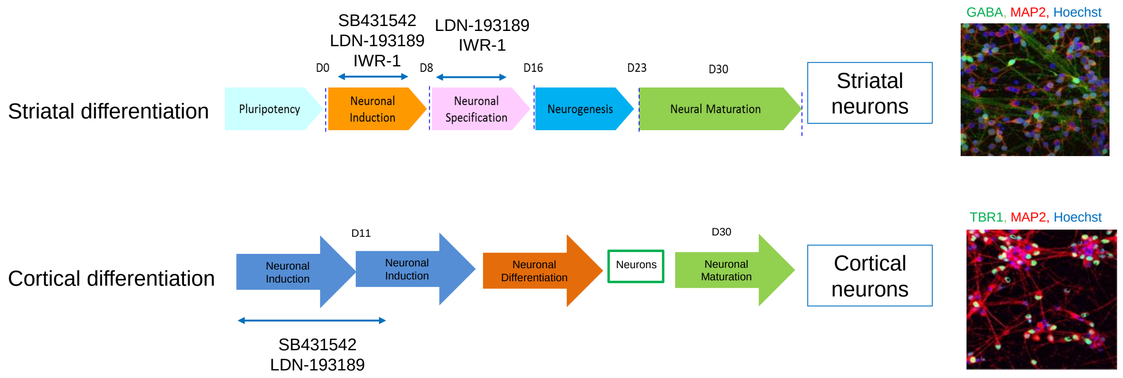

Figure 2. Schematic representation of pluripotent stem cells cultured in selection and maturation media over 40 days to obtain striatal and cortical neurons. Differentiation protocols were adapted from *Telezhkit et al., Am J Physiol Cell Physiol 2015, and Shi et al. Nat Protocols 2012.*

3 RESULTS

Pluripotent stem cells assays

HTRF assay

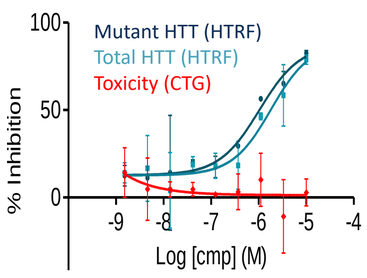

Figure 3. HTS using HTRF assay to measure HTT protein (mutant and total) in the context of Huntington's disease. Toxicity of compounds is measured with CellTiterGlo (CTG).

InCell Western

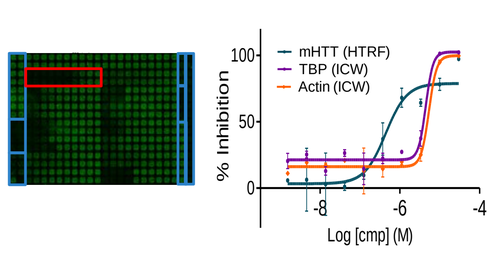

Figure 4. InCell Western in 384 well format for identification of compound and siRNA mediated effects on protein expression in pluripotent stem cells.

Branched DNA

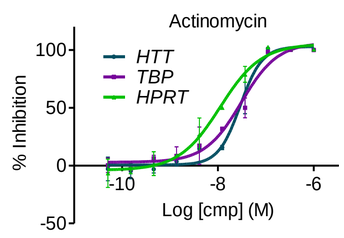

Figure 5. bDNA assay to determine RNA mechanisms of action e.g. transcription inhibitors

siRNA-mediated knockdown

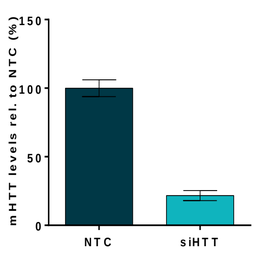

Figure 6. siRNA knockdown assay for HTT protein in pluripotent stem cells

Neuronal assays

Quantitative characterization of differentiation

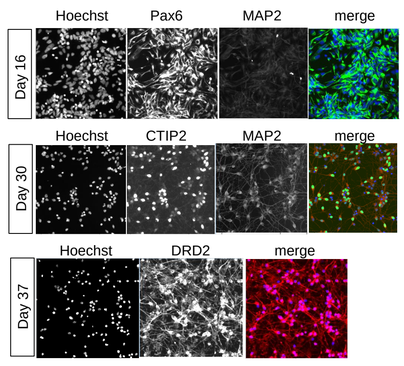

Figure 7. Example images of stem cells derived neurons stained with some of neuronal markers used to assess neuronal differentiation.

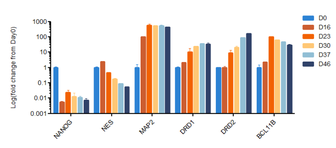

Figure 8. qPCR data for 6 markers used to assess neuronal differentiation and maturation.

Electrophysiology assessment of neuronal activity

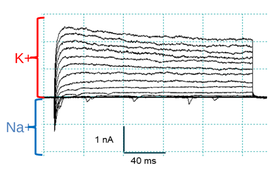

Figure 9. Example of voltage clamp method to assess the presence of outward (K+) and inward (Na+) currents in stem cells derived neurons at D24.

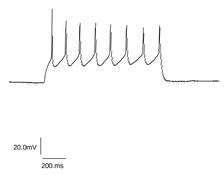

Figure 10. Current clamp assessment to measure action potential (AP) in stem cells derived neurons at D37.

High content assays

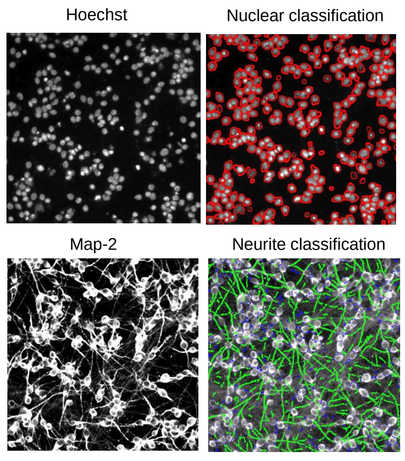

Figure 11. High content methodologies developed to asses neuronal cultures for neurites outgrowth and viability suitable for compound screening.

Stem cells derived neuron HTRF and CTG

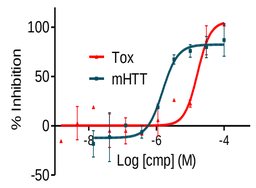

Figure 12. HTRF and toxicity assay (CTG) used for testing compounds that lower mHTT protein in stem cell derived neurons.

Real Time imaging for endpoint assays

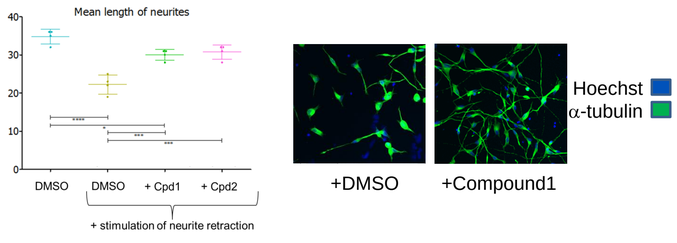

Figure 13. GFP- labelled neurites imaged real time +/- a modulator of neurite retraction to identify suitable endpoints to evaluate effects of small molecule modulators.

4 CONCLUSIONS

Charles River has developed a suite of assays using both pluripotent stem cells and stem cells derived neurons.

- HTS compatible assays using pluripotent stem cells with reproducible pharmacology
- Medium throughput assays with stem-cell derived neurons for hit compound deconvolution.
- Electrophysiology capabilities for measurement of neuronal activity.
- High content and molecular biology approaches for thorough quality control during culture and differentiation.
- Developed an array of high content algorithms in fixed-cell and real-time format to allow measurement of cell characteristics.

5 REFERENCES

1. Vsevolod Telezhkin et al. Forced cell-cycle exit and modulation of GABAA, CREB and GSK3β signalling promote functional maturation of induced pluripotent stem cell-derived neurons. Am J Physiol Cell Physiol (December 30, 2015). doi:10.1152/ajpcell.00166.2015.
2. Yichen Shi et al. Directed differentiation of human pluripotent stem cells to cerebral cortex neurons and neural networks. Nat Protocols 13 September 2012; doi:10.1038/nprot.2012.116