Stem Cell Derived Human Neurons in CNS Drug Discovery

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INTRODUCTION

Central nervous system disorders affect many people worldwide. To increase the quality and number of drugs available for treatments of CNS disorders such as Alzheimer's, schizophrenia and anxiety, the success rate of CNS drug discovery programmes must be improved. The challenge in drug discovery is to develop more relevant assay systems to identify lead compounds for further development. Cell based screening assays currently used for CNS drug development involve primary cells or commercially available cell lines, both of which have many disadvantages. Here, we have overcome these limitations by generating unlimited supplies of physiologically relevant neurons from human stem cells. These neurons can be successfully maniplulated and used in CNS drug discovery programmes to identify lead candidate compounds and limit the need for *in vivo* experimentation.

MATERIALS AND METHODS

Differentiation Process

Undifferentiated stem cells were seeded by mechanical passaging of hESC colonies onto matrigel-coated IVF dishes. Cells were differentiated to human neurons according to Gerrard et al, 2005.

Immunocytochemistry

Images were taken with confocal microscope 63X oil lens.

Western Blot Analysis

Cell lysate was assayed using western blot analysis for expression of neuronal markers.

Electrophysiology

The whole-cell ruptured patch-clamp technique was used to record ionic currents and action potentials at room temperature, with a physiological extracellular solution containing (mM): NaCl (140), KCl (20), $CaCl_2$ (2), $MgCl_2$ (1), glucose (10), HEPES (10), pH 7.4. The intracellular pipette solution containing (mM): KCl (30), HEPES (5), $MgCl_2$ (1), K-aspartate (110), Na_2ATP (4), NaGTP (0.4), EGTA (0.015) was also used for these experiments. Pipette resistance between 2-6 MΩ was used. Capacitative transients were compensated electronically from the recordings and the voltage drop across the series resistance was also compensated (68–80%). Junction potentials were not compensated electronically from the recordings.

Screen

Responses were measured in Flexstation III using calcium3 and membrane polarization kits (Molecular Devices).

RESULTS

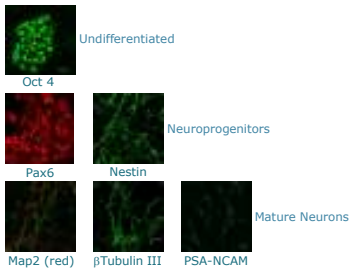

Figure 1: Differentiation process from undifferentiated stem cells to mature neurons. Upon neuronal differentiation cells lose expression of Oct 4 and express Pax6 and Nestin as the neuroprogenitor stage is reached. Cells then lose these markers upon further maturation and begin expression of Map2, BtubulinIII and PSA-CAM, markers of mature neurons.

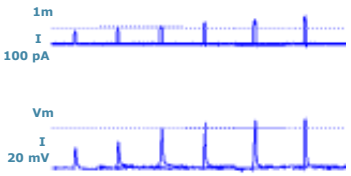

Figure 3: Evoked action potentials in mature neurons. Increasing depolarising current steps (50 pA steps) elicit overshooting action potentials (> 0 mV).

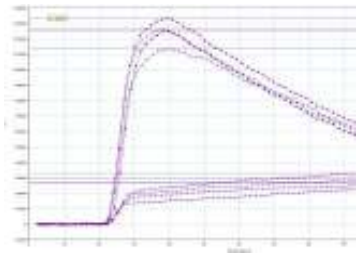

Figure 5: Flexstation calcium assay results of mature neurons stimulated with two concentrations of GPCR ligand.

Figure 2: Expression of Gad 65/67 in three different batches of mature neurons. Batches produced are reproducible in expression of markers and functionality.

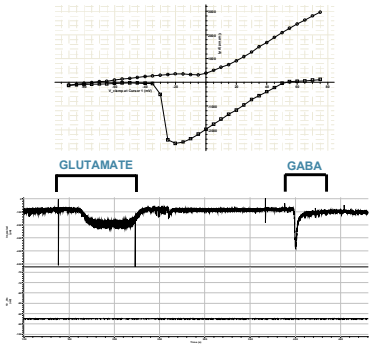

Figure 4: Top: Current-to-voltage plot of inward and outward currents peaks in the same cell reveal typical behaviour of voltage-gated Na^+ (square) and K^+ (circle) currents. Bottom: Transmitter-gated currents in neurons. Typical whole-cell currents evoked by glutamate (100 μM) and GABA (1 mM) in one mature neuron from a holding potential of –90 mV.

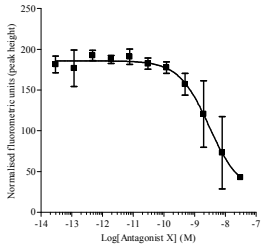

Figure 6: Dose response to identified hit compound from GPCR library screen.

DISCUSSION

We have generated reproducible batches of human neurons that will significantly impact and improve CNS drug discovery. The stem cell derived neurons express all known markers typically associated with mature neurons and are functionally active. They express typical sodium (TTX-sensitive) and potassium currents and respond to saturating concentrations of neurotransmitters GABA and glutamate. Furthermore, these cells have the ability to fire overshooting action potentials.

We are able to format the neurons for screening purposes and can measure calcium and membrane potential responses upon stimulation. We have ascertained an expression profile of transporters and receptors endogenously expressed in the neurons and are able to manipulate them to over-express proteins of interest. This gives us the ability to further enhance the superior screening systems that we can build which can based around multiple CNS related targets.

We have used these neurons in a recent screen to identify antagonists of a novel GPCR target implicated in schizophrenia. The neurons were shown to express this GPCR in high levels and we generated a stable cell line over-expressing the GPCR for a comparitive study. We identified hit compounds from a focused library screen by measuring calcium responses. We found that the neurons gave us increased data content over the stable cell lines, which was proven to be data necessary for the successful outcome of the project.

CONCLUSION

We have developed stem cell derived neurons which can be used as a tool for improved CNS drug discovery.