

Development of a High-throughput Assay for ALS Target Discovery using Human Stem Cell-derived Motor Neurons

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Introduction

Amyotrophic lateral sclerosis (ALS)

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that affects motor neurons in the brain and spinal cord. A key mechanism implicated in motor neuron death in ALS is glutamate excitotoxicity.

RNAi screening

BioFocus's target discovery platform is based on screening proprietary adenoviral knock-down libraries (SilenceSelect[®]) targeting the druggable human genome. These libraries contain over 11,000 shRNA vectors targeting over 4000 human genes. The combination of adenoviral knock-down technology with screening in human cells is designed to identify critical proteins in disease pathways.

Aim

As part of an ALS Association-sponsored project (TREAT-ALS), we aim to identify novel therapeutic targets for the treatment of ALS by screening our adenoviral shRNA library (SilenceSelect[®]) in human motor neurons (MN) exposed to disease-relevant stimuli such as glutamate excitotoxicity. Here we describe the development and characterization of high throughput screening-compatible cultures of motor neurons derived from human embryonic stem cells (hESCs).

Materials and Methods

Production of high purity Motor Neurons from hESCs at CSC

- Expansion of undifferentiated hES cells on appropriate substrate using feeder free/serum free Stemblast and Ectoblast media
- Differentiation and expansion of neural progenitors and neurospheres in non-adherent conditions using Neuroblast formulation.
- Continued differentiation of neurospheres into motor neuron progenitors in adherent conditions using Motorblast media.
- Final differentiation and maturation plating into 96-well plates, coverslips or flasks with CSCi substrate and Motorblast media designed for long term growth.
- Shipment of live cultures from CSCi to BioFocus.

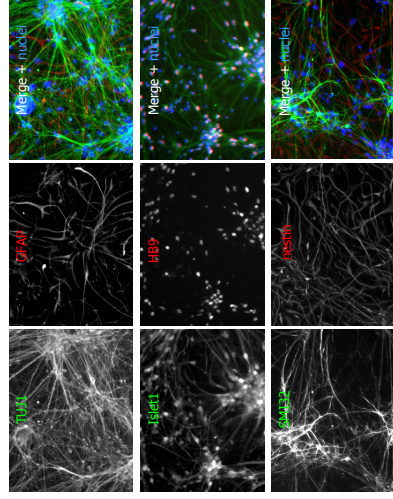
Electrophysiology

- Whole-cell patch-clamp recordings were performed at room temperature using an EPC10 amplifier and Pulse v8.76 acquisition software (HEKA). Cells were matured on glass coverslips for 4 weeks.
- Outward currents and ligand-gated currents - External solution (mM): NaCl 140, KCl 2.5, MgCl₂ 2, CaCl₂ 2, HEPES 10, D-Glucose 10, Sucrose 23.5 pH 7.4, NaOH. Pipette solution (mM): K-methane-sulphonate 140, NaCl 10, CaCl₂ 1, Mg-ATP 3, GTP 0.4, EGTA 0.2, HEPES 10, pH 7.25 KOH.
- Inward currents - External solution (mM): NaCl 115, KCl 3, MgCl₂ 1, CaCl₂ 2, HEPES 10, D-Glucose 10, TEA-Cl 30, 4-AP 4, pH 7.25 CsOH. Pipette solution (mM): Cs-methane-sulphonate 100, NaCl 10, CaCl₂ 1, Mg-ATP 3, GTP 0.4, EGTA 1, HEPES 10, pH 7.25 CsOH, which eliminate K⁺ currents.
- Inward and outward currents were evoked by a step voltage protocol. Cells were held at -80 mV and stepped for 1 sec in 10 mV increments from -80 to +60 mV.
- Ligand-induced currents: cells were held at -70 mV and current was measured for 30 secs. During this time 100 μM glutamate or 100 μM GABA was applied using a Burleigh rapid perfusion system.

Results

Motor neuron marker expression

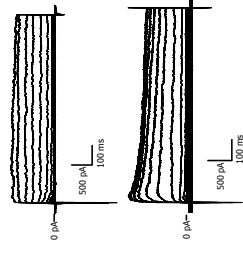
Cultures matured in 96-well plates for 5 weeks were positive for motor neuron markers SMI32, HB9, and Iset1. A minority of cells (<10%) stained positive for nestin (stem cells) and GFAP (astrocytes).



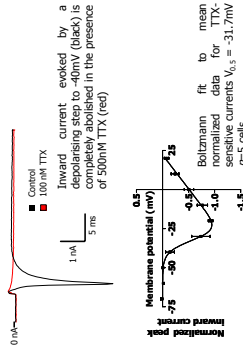
Images acquired using an ImageExpress micro high-content-imager (Molecular Devices)

Electrophysiology

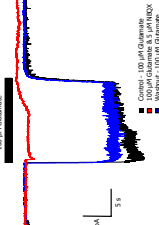
Outward K⁺ currents



TTX-sensitive Na⁺ current

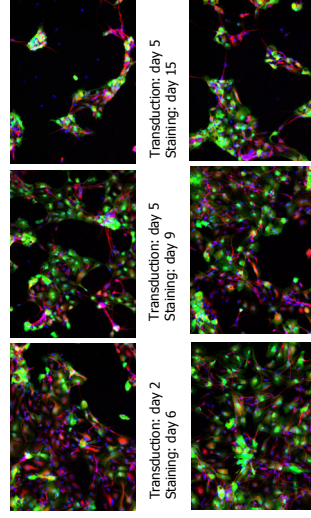


Ligand-gated ion channels



Human MN cultures grown in 96-well plates were efficiently transduced with adenovirus carrying cDNA for Reef Coral fluorescent reporter proteins AcGFP and ZsGreen. Reporter proteins were clearly detectable by fluorescence microscopy within 1 day. Expression continued for at least 10 days.

Transduction with adenovirus



Images acquired using a TN Cell 1000 Analyzer high-content-imager (GE Healthcare).

Conclusions

- Heterogeneous expression of outward K⁺ currents which could be inhibited by 10 mM TEA & 5 mM 4-AP (data not shown).
- Fast inactivating inward Na⁺ currents that were blocked by 500 nM TTX.
- GABA responses in 60% of cells tested with a mean inward current of -105 ± 55 pA (mean peak±sem, n=5 cells).
- Glutamate-induced responses in the majority (>85%) of cells with a mean inward current of -120 ± 34 pA (mean peak±sem, n=7 cells).
- Complete inhibition of glutamate-induced response by 5 μM NBQX indicating the presence of GluR's 1-4 (AMPA receptors).
- High-throughput-compatible cultures of human embryonic stem cell-derived motor neurons.
- Marker expression and electrophysiological characterization are consistent with human motor neurons.
- Cells can be transduced with adenoviral vectors enabling future screening of BioFocus Silence Select[®] library of shRNAs targeting the human druggable genome.

Acknowledgements

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