HUMAN INDUCED PLURIPOTENT STEM CELL- DERIVED NEURONS AS A REPRODUCIBLE SYSTEM FOR DRUG DISCOVERY
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ABSTRACT
Neurotransmitter studies and compound screening for drug candidates for neurovascular and neurodegeneration disorders require models of developing and mature neural cells. Currently, primary rodent neurons are the widely accepted norm in drug screening and toxicity assays. New, human induced pluripotent stem (iPS) cells offer to fill this role of neurological disease models and improve the validity of drug screening data. One hurdle to the acceptance of iPS-derived neurons has been demonstrating that these neurons can mature and function in vitro in a manner similar to their primary rodent counterparts. At GlobalStem, we have developed a novel system for the differentiation, transfection, and maturation of human iPS-derived neural cells yielding reproducible cultures of functional neurons. Within this system, the neuronal progenitor cells can differentiate into mature neuronal cultures with functional properties such as calcium evoked responses and mature synaptic properties. We have compared the morphological and functional maturation of primary and iPS-derived neurons, and their potential to achieve the highly reproducible cultures required for pharmacological assays. Spontaneous activities in our iPS-derived neurons were measured and compared those of rat cortical neuronal cultures to estimate their degree of functional synaptic maturation and activity in response to various pharmacological agents. In summary, GlobalStem has developed a unique in vitro system for screening the viability of rodent and human neurons in supporting their morphological and functional maturation, making for reproducible, reliable cultures, and using our novel transfection reagents, allowing for easy regulation of gene expression in this system.

METHODS
Derived human neural progenitor neurons (GlobalStem, Gaithersburg, MD) were reseeded in Neurotrak medium with 24 10cm2 supranatant (GlobalStem Technologies) and 24 filters (GlobalStem, Gaithersburg, MD). For differentiation, the cells are grown in suspension for 2 to 3 weeks to generate hippocampal neuron cultures. Neuronal progenitor cultures were maintained in Neurotrak medium, following the induction of each neurite-outgrowing, the cells are plated onto multwell plates or multwell dishes with the accompanying mdr and neuron cultures (Minami et al., 2008). During neurotransfection, the cells were maintained in Neurotrak Medium (GlobalStem, Gaithersburg, MD) with 2% DMSO (supplement) (GlobalStem, Gaithersburg, MD). After five weeks maturation, further culture was fed in this supplemented media for continued immunofluorescence.

RESULTS
Hippocampal human iPS-derived neurons (GlobalStem, Gaithersburg, MD) were differentiated using a human cell-conditioned medium which rapidly cultured both neuronal morphologies and markers. Here we investigated the neuronal culture generated by this method from iPS cells in long term culture methods for improved neuronal health, and new quality HIP™ Neurons attain functional maturity. We also assess the reproducibility of HIP™ Neuron cultures is sufficient for multiwell pharmaceutical screening.

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