Metabolomics and neurite outgrowth as data rich developmental neurotoxicity assays in a pluripotent stem cell derived human neural model

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Introduction

A vast majority of chemical entities, ranging from pesticides to compounds of therapeutic interest, remain untested for potential toxic outcomes on human neural development. Current methods for evaluating developmental neurotoxicity (DNT) rely heavily on animal based testing and non-uniform cell lines, are often prohibitively expensive, and provide suboptimal predictive value. There is thus a need for rapid, cost effective, in vitro methods to identify such chemicals. To fill this critical gap we evaluated a robust, species representative and physiologically relevant cellular system, combined with high content imaging (HCI) and metabolomics, to address human DNT. Scalable and uniform populations of undifferentiated neural progenitor cells (hNP1TM), and differentiated neurons (hN2TM) and astrocytes (hAstroProTM), derived from human embryonic stem cells (hESC) were used to represent a neurodevelopmental continuum and evaluated for toxin induced changes in neurite outgrowth, metabolic signatures and viability. To address developmental stage specific neurotoxicity, Bis-1, a known neurotoxin, was applied to cultures pre or post neuronal differentiation, as well as in neuron-astrocyte co-cultures to represent complex brain tissue more accurately.

Methods

Cell culture: Cryopreserved neural progenitors (hNP1TM), neurons (hN2TM) and astrocytes (hAstroProTM) were plated by themselves or as co-cultures.

Viability: CellTiter96™ (Promega) was added to Bis1 treated cultures for last 2 hours and absorbance was read at 490nm. CellTiter96 contains a tetrazolium compound that is bioreduced into a colored formazan product by NADPH or NADH produced in metabolically active cells.

Metabolomics: Metabolomic profiling was done on an Agilent 6890/5973 GC-MS system using DB-SMS GC columns. MS data was collected monitoring mass ranging from 50 to 600 m/z and analyzed using PLS-DA with SIMCA-P+ after picking and aligning the peaks.

Neurite outgrowth: Neurites were labeled by immunocytochemistry for MAP2 & Nuclei stained with Hoechst 3342. Neurite outgrowth data was acquired on Cellomics ArrayScan VTITM with Neuprofiler™ software. Neurite tracing parameters were optimized manually.

Results

Metabolic profiling detects Bis-1 induced changes progenitors, neurons and astrocytes

Neurite HCl assays detect neuronal response at lower Bis-1 doses than viability assays

Neurite outgrowth: Neurites were labeled by immunocytochemistry for MAP2 & Nuclei stained with Hoechst 3342. Neurite outgrowth data was acquired on Cellomics ArrayScan VTITM with Neuprofiler™ software. Neurite tracing parameters were optimized manually.

Summary

• Level or stage of neural maturation differentially effected susceptibility to neurotoxins.
• Metabolomic profiling provides a sensitive pathway analysis approach to DNT for both mature neurons, astrocytes and pre-network neural progenitors.
• Significant alteration in neuronal cellular response in co-cultures with astrocytes suggests need for a more representative multi-cellular DNT assays.