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14. ABSTRACT

As a more biologically relevant model of human physiology, human neural progenitor cells have a strong potential as in vitro, cell-based biosensors for environmental toxins. The overall goal of this project is to develop a human neural cell based biosensor using ArunA's neural cell lines. ArunA's neural cell lines, hNP1™ Human Neural Progenitor Cells and hN2™ Differentiated Neuronal Cells, are derived from human embryonic stem cells (hESC), and more recently ArunA's neural cells lines are being derived from human induced pluripotent stem cells (hiPSC). This report describes progress in these major areas: (1) production of induced pluripotent stem cell derived neural progenitor cells, (2) directed differentiation of progenitors into dopaminergic neurons and astrocytes and (3) assay development of ArunA's neural cell lines as sensor elements for neurotoxicity.

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Quarterly Report
Human Neural Cell-Based Biosensor

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Summary

As a more biologically relevant model of human physiology, human neural progenitor cells have a strong potential as in vitro, cell-based biosensors for environmental toxins. The overall goal of this project is to develop a human neural cell based biosensor using ArunA's neural cell lines. ArunA's neural cell lines, hNP1™ Human Neural Progenitor Cells and hN2™ Differentiated Neuronal Cells, are derived from human embryonic stem cells (hESC), and more recently ArunA's neural cells lines are being derived from human induced pluripotent stem cells (hiPSC). This report describes progress in these major areas: (1) production of induced pluripotent stem cell derived neural progenitor cells, (2) directed differentiation of progenitors into dopaminergic neurons and astrocytes and (3) assay development of ArunA's neural cell lines as sensor elements for neurotoxicity.

(1) Production of induced pluripotent stem cells (iPSCs)

We have successfully been able to expand our new iPSC-derived neural progenitor cells and have completed our first production run. We are currently characterizing their karyotype and expression of neural progenitor cell phenotype markers.

(2) Directed differentiation

Successful development continues on the directed differentiation of neural progenitor cells into dopaminergic neurons and astrocytes. Initial work to optimize differentiation protocols (media formulations, etc.) for hNP1™ derived dopaminergic neurons has been completed. Populations of hNP1™ cells were successfully differentiated into mature neuronal populations demonstrating positive protein expression of dopaminergic markers. Current work is now concentrating on reliably reproducing these results, as well as translating these culture methods to iPSC-derived neural progenitor cells. For astrocyte differentiation of hNP1™ cells, media conditions are being further refined and gene and protein expression is being more fully characterized at various timepoints. Preliminary work has also begun on translating astrocyte differentiation protocols to iPSC-derived neural progenitor cells. Work continues on the written manuscript of these findings on astrocyte differentiation for future publication.

(3) Assay development of hNP1™ and hN2™ cells as sensor elements for neurotoxicity

Progress this quarter on assay development has focused on 1) high content image analysis of neurogenesis and 2) high throughput automated patch clamp systems.

High content image analysis of neurogenesis: We have continued our development of high content image analysis of neurogenesis using an ImageXpress® high content imaging platform. Using this assay platform allows us to monitor neural progenitor cell proliferation, as well as differentiation in terms of neurite outgrowth in both our hNP1™ and hN2™ cells. This assay allowed for the detection of neurotoxicants as well as positive and negative modulators of neuronal differentiation, with output parameters including the number of neurites, length, branches, etc. per cell or per field. Results were recently presented in both oral presentation and poster format at the Society of Toxicology Annual Meeting, March 11-15, 2012, San Francisco, CA. In continued HCI studies, we are currently expanding our test set of known inducers of apoptosis and mediators of neuronal toxicity to market our hNP1™ and hN2™ cell lines with high content imaging platforms as a screening tool for drug discovery and toxicology studies.

High throughput automated patch clamp systems: Development has resumed on the large scale screening of compounds for effects on ion channel activity through direct electrophysical measurements using an IonWorks Barracuda™ system. Using this high throughput automated patch clamp system, the electrophysiological and pharmacological properties of endogenous ion channels in hN2™ cells can be characterized by measuring ligand and voltage-gated ion channels in 384 parallel recording sites. Such a platform technology can accelerate drug discovery, as well as evaluate neurotoxicity of compounds that are ion channel targets. Previous results were presented at the Society for Neuroscience Annual Meeting, November 12-16, 2011, Washington, D.C. Results from continued studies are expected to be presented in the upcoming annual meeting of the International Society for Stem Cell Research in June 2012.