**REPORT DOCUMENTATION PAGE**

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This report focused on the differentiation of hESC- and hiPSC-derived neural progenitor cells and describes progress in these major areas: (1) characterization of induced pluripotent stem cell derived neural progenitor cells, (2) directed differentiation of hESC- and hiPSC-derived progenitor cells into dopaminergic neurons, (3) directed differentiation of neural progenitor cells into astrocytes and (4) cell culture medium development for the maintenance and differentiation of ArunA’s neural cell lines as sensor elements for neurotoxicity.

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Summary

The purpose of this project is to develop in vitro, cell based biosensors for environmental toxins. By using ArunA’s neural cell lines derived from both human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs), our goal is to provide a human neural cell based biosensor that is a more biologically relevant model of human physiology.

This report focused on the differentiation of hESC- and hiPSC-derived neural progenitor cells and describes progress in these major areas: (1) characterization of induced pluripotent stem cell derived neural progenitor cells, (2) directed differentiation of hESC- and hiPSC-derived progenitor cells into dopaminergic neurons, (3) directed differentiation of neural progenitor cells into astrocytes and (4) cell culture medium development for the maintenance and differentiation of ArunA’s neural cell lines as sensor elements for neurotoxicity.

(1) Characterization of human induced pluripotent stem cell (iPSC) derived neural progenitor cells

In our previous Q1 progress report, we had completed our first production run of our new hiPSC-derived neural progenitor cells. Since then, we have finished their characterization, including karyotype. hiPSC-derived neural progenitor cells were also able to differentiate into pan neuronal cultures positive for markers of mature neurons.

(2) Directed differentiation into dopaminergic neurons

We have been able to successfully differentiate hESC-derived neural progenitor cells into mature neuronal populations demonstrating positive protein expression of dopaminergic markers. We have evaluated multiple media formulations, and we have now repeated dopaminergic differentiation multiple times with reproducible results. Additionally we have conducted real-time PCR on our dopaminergic neuron cultures for genes relevant to Parkinson’s disease. We are now in the process of evaluating our cultures for dopamine release. Preliminary work has also begun on translating dopaminergic neuron differentiation protocols to hiPSC-derived neural progenitor cells. Based on our results, we are now in the process of developing a new dopaminergic progenitor cell line and dopaminergic differentiation kit for commercial release. We are currently testing different kit configurations.

(3) Directed differentiation into astrocytes

We have differentiated hESC-derived neural progenitor cells into astrocytes using different media, as well as additional supplements over our previously tested protocols, and for longer differentiation periods, in an attempt to improve yield and quality. Gene expression profiles obtained were favorable, but need repeating due to technical issues with real-time PCR. We also tested whether the astrocytic progenitor cells can be cryopreserved and thawed with acceptable levels of replating, and whether post thaw they can be co-cultured with neurons. Functional network based electrophysiological studies have also been initiated to characterize network behavior of our neurons in these co-culture systems.

(4) Cell culture medium development

We have developed a new basal medium to propagate both hESC- and hiPSC-derived neural progenitor cells enhance their differentiation into different mature neural cell types. We are currently evaluating neural progenitor proliferation and neural marker expression of cells cultured in this new medium. In initial differentiation studies to test our new basal medium, we have been assessing the ability to differentiate neural progenitor cells into pan neuronal cultures and astrocytes.