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14. ABSTRACT Human neural progenitor cells have a strong potential for use as 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 progenitor cell line, hNP1™. In this report, we detail the progress in development for the following areas: (1) methods to isolate and enrich for neural progenitors using cell surface markers, (2) methods to directed differentiation of progenitors into dopaminergic neurons, motoneurons and astrocytes using defined medium conditions, (3) development of cell-based methods to detect botulinum toxin, and (4) development of fluorescence based assays for proliferation, mitochondrial function and reactive oxygen species generation as sensor elements.						
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425 River Road
Athens, GA 30605

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Office of Naval Research (ONR)
Director, Naval Research Lab
Attn: Code 5596
4555 Overlook Avenue, SW
Washington, D.C. 20375-5320

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Submitted by:
Dr. Steven L. Stice, Principle Investigator
ArunA Biomedical, Inc.
425 River Road
Athens, GA 30602
Phone: 706-583-0071
Fax: 706-262-2821
Email: ssstice@arunabiomedical.com

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Summary

Human neural progenitor cells have a strong potential for use as 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 progenitor cell line, hNP1™. In this report, we detail the progress in development for the following areas: (1) methods to isolate and enrich for neural progenitors using cell surface markers, (2) methods to directed differentiation of progenitors into dopaminergic neurons, motoneurons and astrocytes using defined medium conditions, (3) development of cell-based methods to detect botulinum toxin, and (4) development of fluorescence based assays for proliferation, mitochondrial function and reactive oxygen species generation as sensor elements.

(1) Isolation and enrichment of mature neural cells using cell surface markers

Our previous studies using flow cytometry showed that our hNP1™ neural progenitors express three particular cell surface markers that could be used as tools for isolation and enrichment of human neural progenitor cells from a mixed population of cells, such as induced pluripotent stem cells. To corroborate our flow cytometry results, we examined the subcellular localization of these proteins by immunofluorescence and found that ~100% of the hNP1™ cells were positive for all the markers with the expected plasma membrane localization. We think that any quantitative differences between immunofluorescence and flow cytometry results (e.g., ~100% versus ~75%, respectively, for one of the markers) reflect differences in detection sensitivity between the two methods, warranting some additional optimization of the flow cytometry procedure. We are now working on optimizing cell sorting methods using these cell surface epitopes with magnetic beads to determine which sorting marker will work best going forward. Studies using one of the markers have been completed and will be submitted for publication shortly.

(2) Directed differentiation using defined media conditions

We are continuing development of methods for directed differentiation of neural progenitors into dopaminergic, motoneurons and astrocytes. Dopaminergic neurons are a target cell type for mitochondrial toxins, such as rotenone, MPTP, etc. and will be a good model system for environmental neurotoxins and neurodegenerative disorders, such as Parkinson's disease. Motor neurons are the target cell type for botulinum toxin and would be a useful cell type for the detection of this potential bioterrorism agent. Astrocytes, a type of glia, can be used to promote synapse formation and to support the long-term survival of neurons in a cell-based biosensor. We are now setting up screening assays to measure differentiation efficacy and optimizing differentiation protocols to improve yields for the target cell types.

(3) Development of cell based methods to detect botulinum toxin

The development of an hN2™ cell based sensor for botulinum toxin has begun. hN2™ cells are derived from hNP1™ progenitors using a proprietary differentiation method. Early experiments demonstrate that we are able to detect SNAP-25 in hN2™ cells using Western blotting and that treatment of hN2™ with botulinum toxin A causes the cleavage of SNAP-25. Further investigations into method development and dosage sensitivity are underway.

(4) Development of fluorescence based assays as sensor elements for neurotoxicity

Currently, we are developing fluorescence based assays to examine mitochondrial health, reactive oxygen species generation and cell migration in our neural progenitor and differentiated neural cells. These assays would serve as sensor elements in cell-based biosensors. All three assay methods continue to show promise as potential sensor elements.

We plan to adapt the Alamar Blue assay for use with both hNP1™ and hN2™ cells. The Alamar Blue assay measures the activity of mitochondrial reductases, providing an indicator of mitochondrial health for the non-proliferative hN2™ cells and both mitochondrial health and cell number for the proliferative hNP1™ cells. We have optimized conditions for use with hNP1™ and will next test a small set of known neurotoxins to complete proof-of-concept studies. Adaptation using hN2™ will begin shortly.

In addition, we are continuing development of a reactive oxygen species (ROS) assay as described in the last progress report (Q3 2010). Our results to date continue to suggest that certain growth factors reduce cellular levels of ROS in neural progenitors and confer a neuroprotective effect. Testing of select toxins with this assay is underway.

In collaboration with Platypus Technologies, we have also begun development of a cell migration assay using neural progenitors. Migration of neural progenitors to their proper location in the central nervous system is a critical process for normal development and can be affected by environmental toxins. Early results demonstrate that cytochalasin D, an actin polymerization inhibitor, can block hNP1™ migration. Our preliminary results also show that certain individual growth factors and combinations thereof can have potent chemokinetic effects on hNP1™ migration. Further development of this assay and testing with cell migration stimulators and inhibitors is underway. Our progress to date will be presented in poster format at the American Society for Cell Biology meeting in Philadelphia, PA on December 14th 2010.