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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 cell lines. In this report, we detail progress in development for the following areas: (1) neural progenitor isolation from induced pluripotent stem cells, (2) directed differentiation of progenitors into dopaminergic neurons, motoneurons and astrocytes using defined medium conditions, (3) cell-based methods to detect botulinum toxin, and (4) HTS amenable assays for proliferation, differentiation, cell migration, mitochondrial function, reactive oxygen species generation and apoptosis as sensor elements.

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Human Neural Cell-Based Biosensor

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Stice Q2 2011 DoD Progress Report v2

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 cell lines. In this report, we detail progress in development for the following areas: (1) neural progenitor isolation from induced pluripotent stem cells, (2) directed differentiation of progenitors into dopaminergic neurons, motoneurons and astrocytes using defined medium conditions, (3) cell-based methods to detect botulinum toxin, and (4) HTS amenable assays for proliferation, differentiation, cell migration, mitochondrial function, reactive oxygen species generation and apoptosis as sensor elements.

(1) Neural progenitor isolation from induced pluripotent stem cells

Our previous studies identified novel markers that could be used to isolate neural progenitors from induced pluripotent stem cells (iPSCs). We also developed methods to generate iPSCs. Using these methods, we have generated a new iPSC-derived neural progenitor line. We are now amplifying this line into working stocks for beta testing and potential commercial distribution.

(2) Directed differentiation using defined media conditions

We are continuing development of methods for directed differentiation of neural progenitors into dopaminergic, motoneurons and astrocytes. Substantial progress has been made with astrocyte differentiation procedure; recent work continues to optimize conditions necessary for establishing a robust, reliable differentiation protocol. Work also continues on optimizing the differentiation protocols for dopaminergic and motor neurons. We have recently set up a new phenotype screening assay for dopaminergic differentiation in addition to the standard markers tested for this cell type. We are also in the midst of beta testing a dopaminergic progenitor cell line developed for potential commercial distribution.

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

There has been substantial progress in the development of an hN2™ cell based sensor for botulinum toxin (BoNT). We established the expression of SNAP-25, the target for BoNT, in hN2™ cells and demonstrated that treatment of hN2™ with BoNT-A caused SNAP-25 cleavage of in a dose-dependent fashion. This cleavage was specific to the BoNT treatment since it blocked by co-treatment with inhibitors of BoNT action. The manuscript of these findings has been submitted for publication and is under review.

(4) Development of HTS amenable assays as sensor elements for neurotoxicity

We continue to make rapid, substantial progress in developing fluorescence based assays as sensor elements in cell-based biosensors.

Alamar Blue assay: We have optimized conditions for using both hNP1™ and hN2™ cells with the Alamar Blue assay, which measures mitochondrial reductase activity in both cell types. We have also assembled a small set of known neurotoxins to complete proof-of-concept studies for this assay.

Cellular ATP assay: We are in the midst of developing a cellular ATP assay for both hNP1™ and hN2™ cells. We are currently exploring the ability of this assay to measure both proliferation potential and cellular metabolism, thus increasing the assay's utility.

ROS assay: The development of a reactive oxygen species (ROS) assay is currently on hold – the novel dyes under development show instability during shipping, making further assay development infeasible. Additional optimization of the dye chemistry is needed to overcome this problem.

Neurite outgrowth assay: We previously established a neurite outgrowth assay for detecting neurotoxicants using the already differentiated, mixed neuronal hN2™ cell line. Now, we are expanding the utility of this assay using the ImageXpress high content imaging platform. We are currently testing the assay's ability to measure not only toxicant effects on neurite outgrowth in the ***differentiated*** hN2™ neuronal cells and but also growth factor effects on neurite outgrowth on the ***differentiating*** hNP1™ neural progenitors. The expanded capability would allow detection of both positive and negative modulators of neuronal differentiation.

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Cell migration assay: We have developed an HTS-amenable cell migration assay using hNP1™ neural progenitors. Previous studies show that the assay can identify inhibitors and stimulators of migration. We have assembled a small set of known neurotoxins to complete proof-of-concept studies for this assay. We are also exploring the ability of this assay to measure proliferation using high content imaging, thus expanding the assay's utility. Our progress to date will be presented in poster format at International Society for Stem Cell Research 9th Annual Meeting, June 15 - 18, 2011, Toronto, Canada. We are in the process of marketing our neural progenitors with the migration assay as a screening tool for drug discovery as well as toxicology investigators.