Assessment of dopaminergic differentiation in 3D Lund Human Mesencephalic (LUHMES) neurospheres

Lily Ramos[1,2], Tasha Hester[2,3], Alison Bernstein[2,3]

[1]Summer Undergraduate Research Fellowship, Environmental and Occupational Health Sciences Institute, Rutgers University, 170 Frelinghuysen Rd, Piscataway, NJ 08854, [2]Department of Pharmacology and Toxicology, [3]Environmental and Occupational Health Sciences Institute, Rutgers University, 170 Frelinghuysen Rd, Piscataway, NJ 08854

Parkinson's disease (PD) is characterized by loss of dopaminergic neurons in the substantia nigra and the presence of α-synuclein-containing Lewy bodies in surviving neurons. Although animal models are widely used in PD toxicology studies, limitations of in vivo models create a need for improved in vitro methods. Here, we use Lund Human Mesencephalic (LUHMES) neurospheres as an in vitro model of human dopaminergic neurons. The goal of this study was to assess dopaminergic differentiation of 3D LUHMES neurospheres using a novel, recently published protocol. Over the differentiation time course, the expression of tyrosine hydroxylase (TH) and Ki67 was assessed by droplet digital PCR (ddPCR) to confirm successful differentiation of these cells in 3 independent experiments. TH is the rate-limiting enzyme of dopamine synthesis, and an increase in TH expression is indicative of a dopaminergic phenotype. Expression of Ki67 is a marker of proliferating cells and loss of Ki67 expression indicates that cells have stopped proliferating. In the next stage of this project, we will assess expression of additional target genes via ddPCR and confirm protein expression by western blot. The overall goal of this project is to test the effects of altered expression of candidate genes on dopaminergic differentiation and toxicity in 3D LUHMES neurospheres.