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3D Cytoarchitectural Characterization of a hiPSC-Derived Midbrain Organoid Model of Tauopathy

Background: Progressive Supranuclear Palsy (PSP) is a primary tauopathy characterized by abnormal tau accumulation in neurons and glia. Human induced pluripotent stem cell (hiPSC)-derived organoids are powerful tools to model neurodegenerative diseases, including PSP. Organoids contain diverse cell populations and complex interactions, yet standard immunohistochemistry (IHC) fails to capture their full cytoarchitecture. Tissue clearing renders samples optically transparent for 3D imaging, revealing structural disease correlates.

Methods: Organoids from PSP and matched control hiPSC lines were generated using a modified Sarrafha et al. (2021) protocol and cultured for 10 months. Fixed organoids were delipidated with aqueous detergents and immunolabeled for tyrosine hydroxylase (TH), microtubule-associated protein 2 (MAP2), and glial fibrillary acidic protein (GFAP). Refractive index matching was achieved with EasyIndex. Imaging was performed using confocal and light sheet microscopy, and Imaris 10.2 was used for 3D analysis.

Results: Adjustments to incubation time, temperature, and EasyIndex equilibration improved antibody penetration and visualization. Light sheet microscopy enabled rapid volumetric imaging, providing an overview of total cell count and layering but with limited resolution. Confocal microscopy offered higher resolution, but with longer image acquisition time and limited volume coverage. Cellular reconstruction using the Imaris Filament tracer package revealed complex, long-range neuronal branching in TH- and MAP2-positive neurons.

Conclusions: 3D visualization of cleared, immunolabeled organoids demonstrates complex cytoarchitecture. Future work will focus on quantitative analysis of reconstructed neurons to assess morphological variability across disease and control lines. This protocol will be used for precise characterization of our organoid model, yielding insights into disease pathogenesis.