Scintillant BIOSCIENCE

Advancing Therapeutics for Neurodegenerative Disease Through Human iPSC-Derived Neurons: A Case Study

The Challenge

Developing therapeutics for neurodegenerative diseases has historically been a formidable challenge. Validated drug targets for these conditions are scarce, and animal models do not predict human therapeutic efficacy reliably. However, recent innovations in disease models using human neurons derived from induced pluripotent stem cells (iPSCs) offer a promising yet complex avenue for drug discovery. One strategy is identifying phenotypic differences between normal neurons and those harboring a gene mutation associated with a neurodegenerative disease. Then, therapeutic agents can be screened for those that can reverse such disease-related traits. This approach is powerful as it is not dependent on the mechanism of action and can be used to query compounds against various targets.

The Solution

Scintillant Bioscience conducted contract research for a biotechnology startup to compare medium spiny neurons (MSNs) derived from human iPSCs. These MSNs originated from a healthy donor and a donor with a known Huntington's disease mutation expressing 109 CAG (polyglutamine) repeats in the Huntingtin gene. MSNs are particularly vulnerable and among the first to degenerate in Huntington's disease. We procured normal and mutant iPSC-derived neurons from BrainXell in Madison, Wisconsin, and employed a range of laboratory techniques to tackle this challenge:

- Neurons were cultured in 96-well plates for live-cell and fixed-cell staining, followed by high-content imaging at different time points, spanning up to 3 weeks in culture. We also cultured neurons in 6-well plates to provide cell lysates for RNA-seq and proteomics experiments. Multiple iterations of these experiments were conducted, including testing the client's compounds at various doses to assess their potential to rescue mutant phenotypes.
- Immunocytochemistry experiments were conducted using MAP2, beta3-tubulin, GAD67, and DARPP-32 antibodies to confirm cell identity as medium spiny neurons and to serve as phenotypic markers. Various additional phenotyping markers, including LC3B for autophagy, Huntingtin protein antibodies, LysoTracker, and MitoTracker dyes, were also employed, both with and without other stains, including Calcein-AM and Hoechst 33342.
- We utilized our high-content imaging instruments to automate the capture of multiple images per well, across multiple fluorescence channels, at different time points in all wells of 96-well plates.
- We conducted functional calcium-imaging experiments to assess differences in response to various stimuli between normal and mutant neurons, including depolarization with high extracellular potassium and the application of neurotransmitters, including glutamate and acetylcholine.
- Our data scientist rigorously analyzed hundreds of images and calcium-imaging traces to identify potential phenotypic differences between normal and mutant MSNs. This analysis included evaluating staining patterns for protein localization and staining intensities for protein abundance, in addition to analyzing cell and organelle morphologies, and neurite outgrowth at different culture time points.

The Results

Several statistically significant phenotypic differences between normal and mutant MSNs were identified (as one example, see images on the next page for differences in cell-body clustering and neurite morphology at day 15 in cell culture). Some select compounds partially rescued mutant phenotypes, aligning them more closely with the normal neurons. This data remains confidential to protect the competitive position of our

client's on-going preclinical research. However, these data have significantly contributed to securing SBIR funding for this promising drug-development program.