Neurotoxicity and Drug Screening Assay Characterization in Healthy and Progranulin R493X HZ KO Human iPSC-derived Induced Excitatory Neurons

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Abstract

Neurogenin-2 (NGN2) forward programming of human induced pluripotent stem cells (iPSCs) offers a robust method for generating scalable quantities of neurons with low lot-to-lot variability. Using this methodology, we generated highly pure excitatory glutamatergic neurons (iCell Induced Excitatory Neurons) at commercial scale from iPSC lines with an apparently healthy normal (AHN) background or a heterozygous (HZ) and pathogenic R493X nonsense mutation in the progranulin gene (GRN) to model frontotemporal dementia (FTD). These induced cells are highly pure neurons (>90% βIII-Tubulin-positive) and express excitatory glutamatergic genes, including vesicular glutamate transporters (VGLUT) and AMPA receptor subunits (GRIA). We verified that these characteristic markers are expressed consistently across lots and confirmed that a reduction in granulin monomers in the GRN R493X cell line was observed. In the current study, we evaluated the suitability of these induced excitatory neurons for high-throughput neurotoxicity and drug screening experiments, including neurite outgrowth (Incucyte), multielectrode array (MEA), calcium imaging, and cell survival assays. Within each assay we established a baseline comparison between the AHN and GRN R493X HZ KO induced excitatory neurons to identify and characterize differences in phenotypes. Notably, differences in MEA activity development were detected, with GRN R493X HZ KO displaying aberrant network synchrony compared to AHN neurons. These baseline metrics of survival, neurite outgrowth, and activity were then challenged via treatment with a panel of neurotoxic compounds or chemotherapeutic agents to determine dose responses across high throughput assays. These studies demonstrate the high-throughput utility and biological relevance of induced excitatory neurons across numerous neurotoxicity assays, suggesting these cells offer a platform for early drug screening and disease modeling.

Characterization of iPSC-derived Induced Neurons

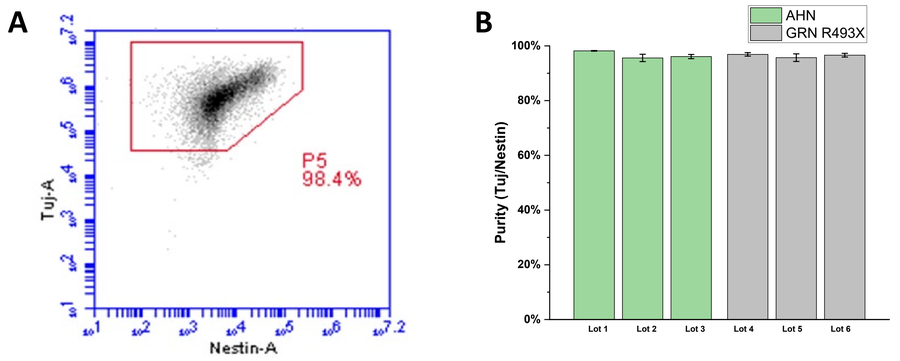


Figure 1. Purity of iPSC-derived Induced Neurons by Flow Cytometry. Cryopreserved AHN and GRN R493X iCell Induced Excitatory Neurons were thawed and evaluated for purity by flow cytometry. **(A)** Representative flow cytometry plot showing highly pure βIII-Tubulin+ neurons (Tuj+/Nestin-). **(B)** Neuronal purity of the cells is highly consistent across lots. **(C)** Immunocytochemistry of iCell Induced Excitatory Neurons (βIII-Tubulin, green) and iCell Astrocytes 2.0 (GFAP, red) co-cultures.

Evaluation of Neurite Outgrowth Kinetics for Induced Neurons

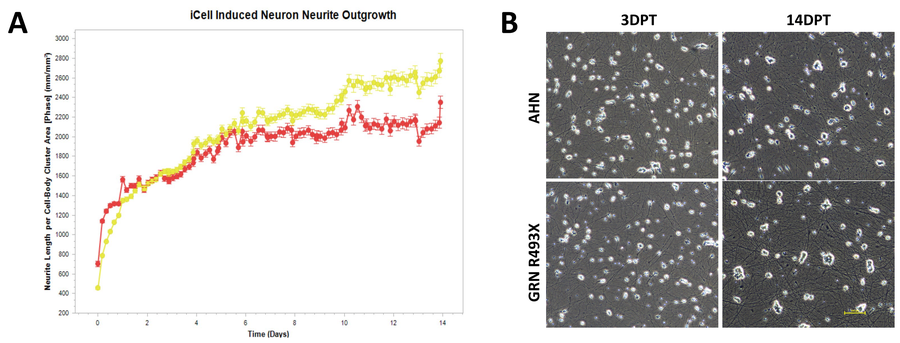


Figure 2. **(A)** iCell Induced Excitatory Neurons from AHN and GRN R493X donors were cultured and monitored for neurite outgrowth (Incucyte) and data analyzed using IncuCyte SX5 Neurotracker. A clear deficiency in neurite outgrowth can be observed in GRN mutant neurons when compared to AHN neurons after day 7 in culture. **(B)** Representative images of AHN and GRN R493X iCell Induced Excitatory Neurons at 3- and 14-days post-thaw (DPT) demonstrate robust neurite outgrowth and low incidence of cell clustering throughout. Plating efficiency and survival of neurons is consistent through 14 days in culture with no observable impurities.

Characterization of Progranulin Disease Model via Multielectrode Array (MEA)

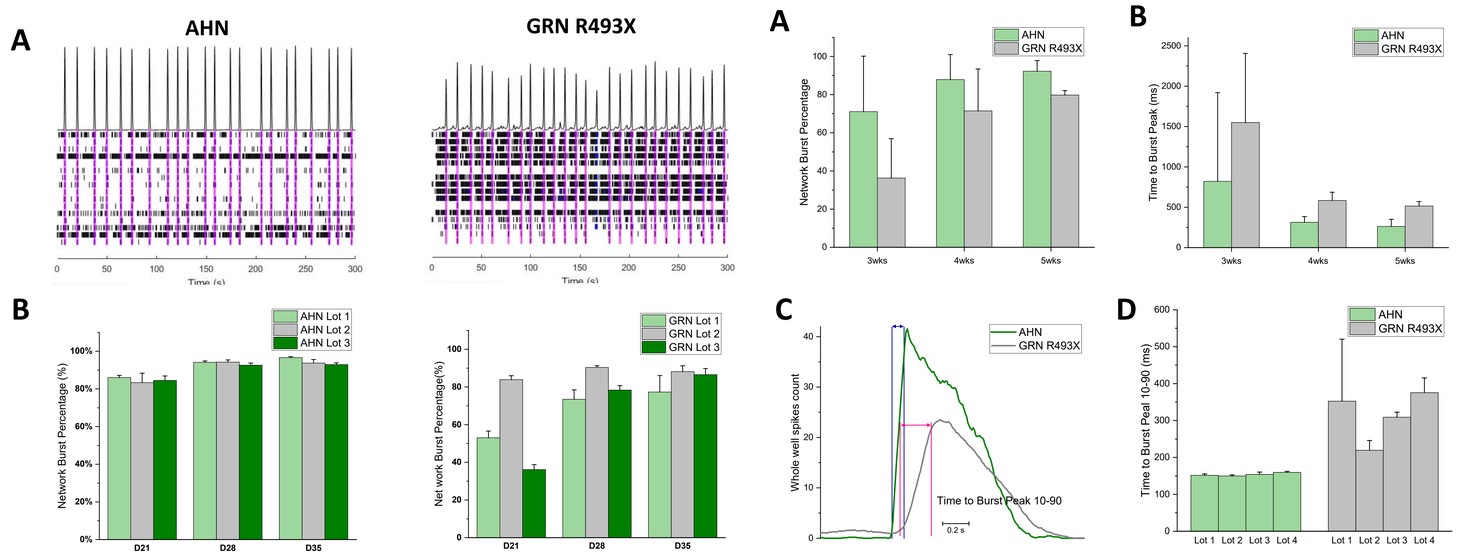
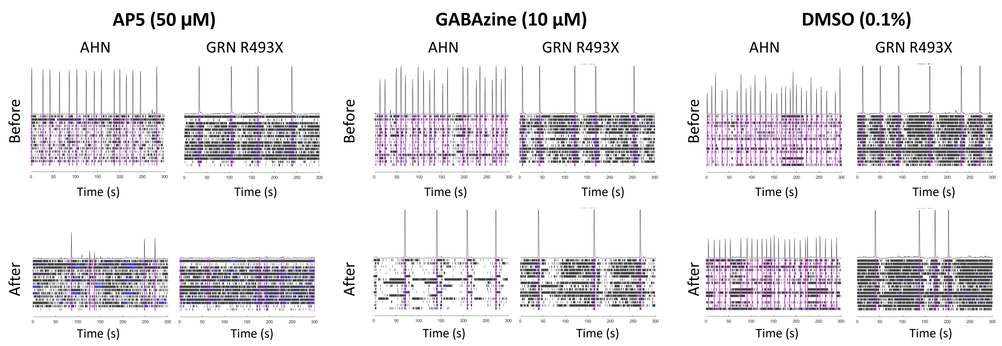


Figure 3. MEA to Monitor Development of Synchronous Networks. Three lots of iCell Induced Excitatory Neurons from either AHN or GRN R493X mutant were co-cultured with iCell Astrocytes 2.0 at a 6:1 ratio (140,000 cells/well total). Network activity was recorded throughout a 36-day culture using Axion Maestro Pro MEA system. **(A)** Representative raster plots on D28 of culture. **(B)** Cells from both AHN and GRN R493X mutant line form synchronous bursting networks with a high network burst percentage. Neural activity development was consistent across lots from both lines.

Figure 4. "Time to Burst Peak" is a Reliable MEA Parameter. iCell Induced Excitatory Neurons AHN show stronger synchronized network bursts compared to iCell Induced Excitatory Neurons GRN R493X using MEA metrics **(A)** Network Burst Percentage and **(B)** Time to Burst Peak. **(C)** Representative whole well spikes count traces of an individual network burst shows differences in "time to burst peak" between AHN and GRN R493X neurons. "Time to Burst Peak 10-90" was used to minimize the variation from burst to burst. **(D)** This metric was reliable between 4 lots of AHN and 4 lots of GRN R493X iCell Induced Excitatory Neurons co-cultured with a single lot of iCell Astrocytes 2.0.

Response to Known Modulators of Excitatory and Inhibitory Receptors

Figure 5. iCell Induced Excitatory Neurons Respond to NMDA and GABA Antagonists. iCell Induced Excitatory Neurons were co-cultured with iCell Astrocytes 2.0 for 5 weeks and MEA activity measured using the Axion Maestro Pro MEA system. Baseline (before) readings were recorded followed by the application of AP5 (50 µM, NMDA antagonist), GABAzine (10 µM, GABA-A receptor antagonist), or DMSO (0.1%, control) for 5 min. Representative raster plots for each condition are displayed. Both AHN and GRN R493X HZ KO cells responded as expected, with reduced activity in response to AP5 and increased activity to GABAzine (increased network burst duration).



Calcium Waveform Oscillations from 3D Neurospheres

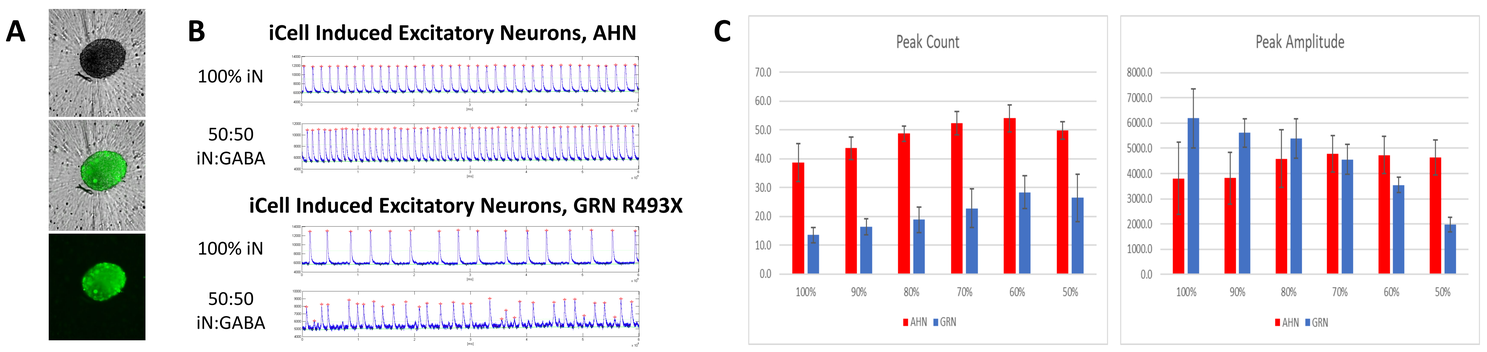


Figure 6. Formation and Functional Testing of iCell Induced Excitatory Neurons as 3D Spheroids. iCell Induced Excitatory Neurons can be combined with other iCell products, including iCell GABANeurons and iCell Astrocytes 2.0, in isogenic 3D cell culture. **(A)** Representative images (brightfield and fluorescence) of cells as iCell Neurospheres in ULA 3D spheroids plates after loading with Cal6 calcium indicator dye. Spheroids were formed by mixing induced excitatory neurons with GABAergic inhibitory neurons at different ratios (e.g., 100% = all excitatory and 70% = 14K excitatory and 6K inhibitory). 20,000 neurons were combined with 5,000 iCell Astrocytes 2.0. After 3-4 weeks of culture in complete BrainPhys medium, functional calcium oscillation assay was performed. **(B)** Representative Ca^{2+} traces from 10 min baseline recordings are pictured. **(C)** Analysis of Ca^{2+} traces for peak count and peak amplitude demonstrate the impact of varying the Excitatory/Inhibitory ratio of neurons. The GRN R493X disease model showed the largest variation in peak amplitude with higher numbers of GABA neurons.

Summary and Future Directions

Human iPSC-derived iCell Induced Excitatory Neurons provide a robust, consistent and functional population of human glutamatergic neurons. This highly pure population of cells displays stable neuronal morphology and functional electrophysiological properties on MEA. iCell Induced Excitatory Neurons engineered to contain a Progranulin R493X HZ KO mutation can be manufactured to the same scale and purity as the apparently healthy normal (AHN) cells and display a potentially divergent phenotype with aberrant neurite outgrowth kinetics and Time to Burst Peak metrics on MEA. These cells offer an accessible and human disease-relevant heterozygous progranulin knockout model for use in FTD/ALS drug discovery research.