Disease Modeling and Drug Screening in Neurological Disorders Via Novel iPSC-based Technologies

Huanyu Jin, Neeraja Parameswaran, Ying Pei, Xianmin Zeng

Applied StemCell Inc., Milpitas, CA 95035

Introduction

- Human iPSC technology offers the benefits of a cell line, coupled with the advantages of using human primary cells.
  - Human disease mutations can be captured in a stable cell population.
  - iPSCs can be terminally differentiated into multiple cell lineages and genetically engineered generating cell line models with the same allelic background.
- iPSC technology and its differentiation into neuronal lineage cells has benefited research in neuroscience and neurological disorders.
- Cell-based assays using iPSCs and differentiated cells have been approved by international drug regulatory agencies for neurotoxicity screening of drug candidates.
- To aid in furthering drug development and screening for PD, we have generated a panel of iPSC lines & terminally differentiated them into neural lineage cells for neurotoxicity assays and disease modeling applications.
- We describe the utility of these lines for neurotoxicity assays, including assays to determine the specificity of different neural cell types for a small range of chemicals and drugs from the Tox21 library, as well as for neuroprotective assays with dopaminergic neurons.

Experimental Design

Generation of iPSCs lines
Control iPSCs
Engineered iPSCs to model disease
Engineered reporter lines
CRISPR Mutation Correction

Differentiation to Neural Cells
Neural stem cells (NSC)
Neurons (dopaminergic, cortical)
Astrocytes
Oligodendrocytes

Disease Modeling & Drug Screening
Neurotoxicology assays
Neuroprotection screening
CNS drug efficacy testing
Screening for new drug targets

Neurological Disease Modeling and Drug Screening With Three Panels of iPSCs

- **Control Lines:** Well-characterized, integration-free control iPSC lines generated from male and female CD34+ cells (cord blood) using episomal vectors. These lines were also used for engineering isogenic lines with disease mutations and reporter lines.
- **(A) Engineered isogenic iPSCs to model diseases:** From parental control iPSCs with knockout mutations of genes associated with neurological disorders.
- **(B) Engineered reporter lines:** Isogenic lineage-specific reporter lines engineered from parental control iPSCs with knock-in of reporters under control of endogenous neuronal promoters; and reporters inserted in safe-harbor locus under control of lineage-specific or ubiquitous promoters.

A

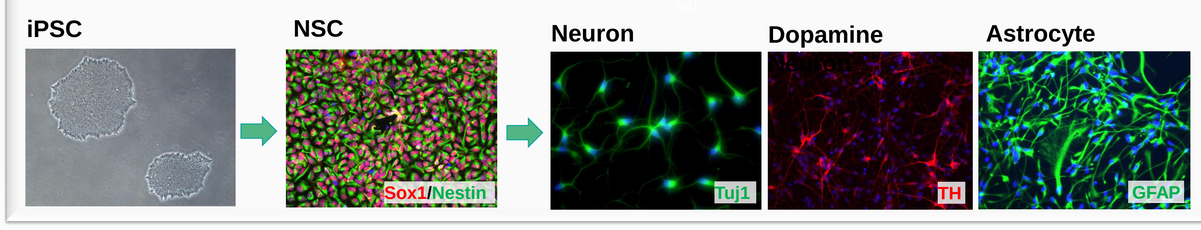
Isogenic knock-out lines	Disease
PARK2 -/-	PD
PARK7 -/-	PD
PINK1 -/-	PD
LRRK2 -/-	PD
Park2-/-; Park7-/-	PD
Park2-/-; Pink1-/-	PD
APOE -/-	Alzheimer's disease
SOD1 -/-	ALS
DICS1 -/-	Schizophrenia
CNTNAP2 -/-	Autism
BDNF -/-	CNS

B

Knock-in neural lineage-specific reporters	Description
MAP2-Nanoluc-Halotag KI	Neuron reporter
GFAP-Nanoluc-Halotag KI	Astrocyte reporter
MBP-Nanoluc-Halotag KI	Oligodendrocyte reporter

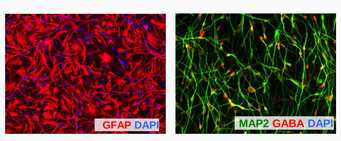
Safe-harbor knock-in lines	Description
CAG-GFP, AAVS/Chr19	Ubiquitous reporter
DCX-GFP	Neuron reporter

Differentiation to Isogenic Panels of Neurons & Glia Using Neural Stem Cells as a Stable Intermediate



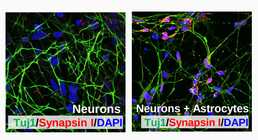
Characterization of Differentiated Astrocytes, Cortical & Dopaminergic Neurons

A



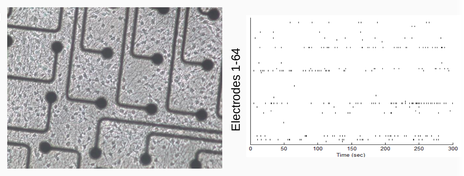
Immunocytochemical characterization of biomarkers expressed by differentiated astrocytes and neurons.

C



Neurons and astrocytes can be co-cultured for complex neuronal models to evaluate the interactions between neurons and glia.

D



Electrophysiological activity of neurons can be measured using neurons cultured on MEA plates for up to 3 months; Raster plot shows neuronal activity across 64 electrodes on day 49.

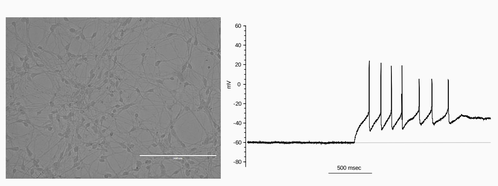
B

SYMBOL	NSC	NEURONS	ASTROCYTES	DESCRIPTION
ATP1B	2	489	165	
DCX	467	7918	1026	
GREM2	11	685	182	
LHX1	15	1878	36	
LOC150568	284	640	317	
MAB21L2	2	2660	7	
MAP6	1266	4210	1004	
MYT1	70	8591	578	
NEUROD1	53	328	190	
NEUROG1	34	570	137	NEURONS
NEUROG2	207	8367	131	
PORK1	245	450	96	
PLXNA2	23	780	328	
POU4F2	-5	1386	69	
RGS1	8	13	9	
SEMASA	212	482	282	
SLC17A6	12	2357	161	
SLC17A8	-15	316	76	
TUBB3	20296	54556	35715	

SYMBOL	NSC	NEURONS	ASTROCYTES	DESCRIPTION
AQP4	-23	-5	1082	
CCL2	396	63	2991	
CD44	29	43	8716	
CRYAB	-21	5	9661	
GFAP	-2	1	30956	
HOPX	-14	96	777	
LGALS3	33	8	1203	
NFIA	-43	981	2110	ASTROCYTES
NFIX	-6	720	7849	
PMP2	12	606	3186	
PRRX1	23	19	3973	
S100A6	137	142	1952	
SPARCL1	1092	40	13584	
TNC	164	589	4838	

Whole genome profiling of differentiated neurons and astrocytes was used to confirm expression of lineage specific markers.

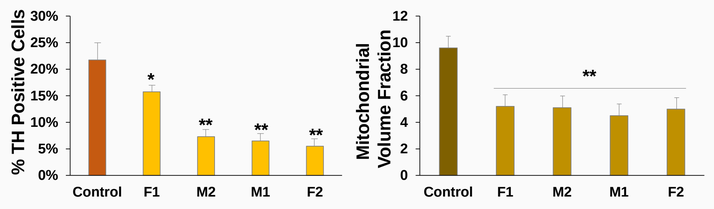
E



Single pulse current (patch clamp) recording of dopaminergic neurons derived from iPSCs indicate neurons are excitable upon injection of current.

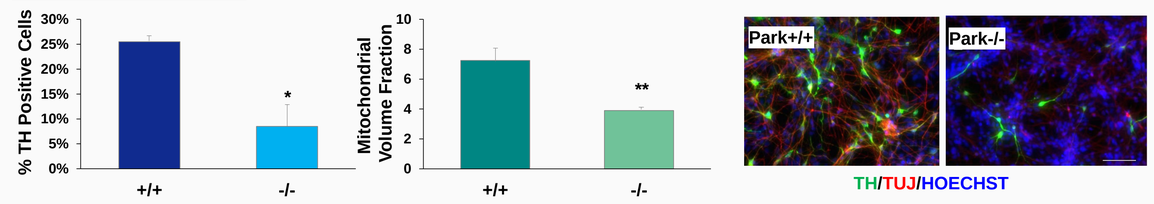
Isogenic Knockout Lines Recapitulate Phenotypes of Abnormal Neurons Derived from Patient Lines (Ex. Park2 -/-)

A **Patient lines carrying Park2 mutations**



(A) The number of tyrosine hydroxylase (TH+) neurons derived from patient-derived iPSCs with Park2 mutations, and the mitochondrial volume fraction in TH+ dopamine neurons was significantly lower as compared to control iPSC (Y09) lines.

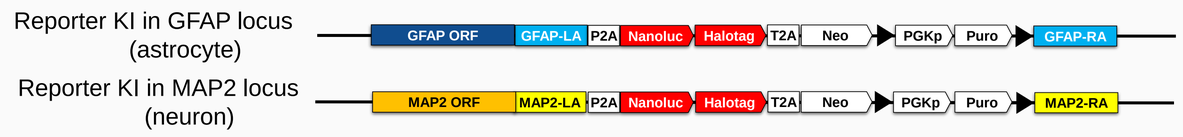
B **Park2 -/- iPSC lines**



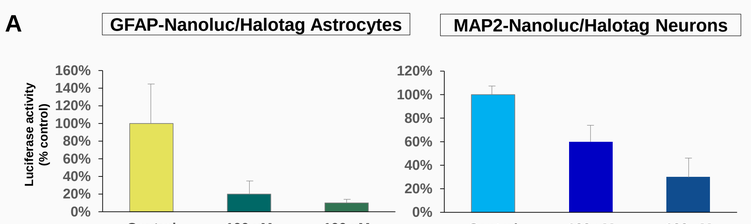
(B) The number and mitochondrial volume fraction of TH+ dopamine neurons derived from Park2 -/- iPSCs was also significantly lower as compared to wildtype (parental; +/+) cell line. Immunocytochemical staining for dopamine marker (TH; green) showed fewer TH+ cells indicating decreased dopamine neurons among the total population of differentiated neurons (Tuj1; red).

% TH+ Cells was calculated as percentage of total number of cells counted.

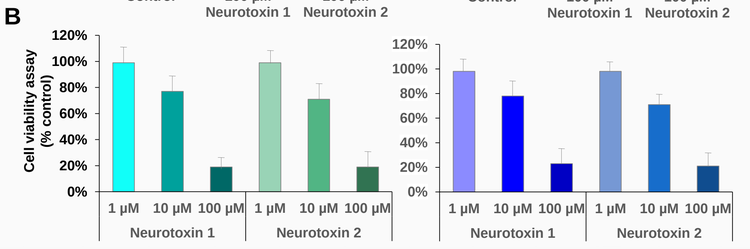
Lineage-Specific Reporter Knock-in iPSC Lines for Neurotoxicity Screening



A



B



(A) Luciferase-based cell viability was significantly reduced by up to 90% in astrocytes and neurons derived from lineage-specific reporter iPSC lines when exposed to two neurotoxins. Luciferase activity was measured as % of control (DMSO-treated cells).

(B) Cell loss of ~80% was seen in astrocytes and neurons after exposure to different concentrations of neurotoxins. Cell viability was evaluated using MTT assay (MTT tetrazolium salt) and cell survival was expressed as % of absorbance of viable cells normalized to control (DMSO-treated cells).

Screening Drugs for Neuroprotection & Neurotoxicity

Table 1. Drugs that were neuroprotective in iPSC and differentiated neuronal cells, and used for human clinical trials.

Neurotransmitter/ MAO Inhibitors:	Rasagiline, selegiline, nicotine, topiramate, amantadine, zonisamide, taurine
Antioxidant/ Mitochondrial Stabilizers:	Resveratrol, N-acetyl cysteine, lipoic acid, epigallocatechin gallate, creatine
Anti-Inflammatories:	Rolipram, indomethacin, 7-nitroindazole, 3-aminobenzamide, phenanthridone

Table 2. Drug that were <u>not neuroprotective in iPSC-based models</u> but were neuroprotective in conventional cell lines and animal models:

Neurotransmitter/ MAO Inhibitors:	Donepezil, caffeine, theophylline, pergolide, apomorphine, riluzole, pramipexole
Antioxidant/ Mitochondrial Stabilizers:	Ascorbic acid, coenzyme Q10, uric acid, folic acid, ropinirole
Anti-inflammatories:	Minocycline, estradiol, clioquinol, plicamycin

Dopaminergic (DA) neurons derived from control iPSC lines were used to evaluate neuroprotection of compounds previously shown to be neuroprotective in rodent and cell line models (Table 1 and 2), when challenged with rotenone or MPP+. Cell viability was measured using the MTT assay. Only 18 out of the compounds (Table 1) were found to be neuroprotective in these iPSC-derived DA neurons, and these same compounds have been used in human Parkinson's disease neuroprotection clinical trials.

(A) Screening for 80 compounds in the Tox21 library showed differential toxicity in isogenic iPSCs, NSCs, Neurons, and Astrocytes, using MTT assay in 96-well plates and at two doses for each compound.

Conclusions

- We have developed a panel of lines including control, and engineered isogenic and reporter lines, which provide a unique advantage for disease modeling and drug screening.
- We have established robust methods for generating neurons and glia from iPSC using a NSC gateway concept from virtually all lines.
- Human neural cultures may better mimic human neurodegenerative disorders and therefore are a more relevant model to screen for drug efficacy and toxicity.
- We have shown that iPSC-derived neuronal and glial cells can be used for modeling neurodegenerative diseases as well as for neurotoxicological and neuroprotective drug screening.

www.appliedstemcell.com | 1-408-773-8007 | info@appliedstemcell.com