Leveraging iPSC Technology for Disease Modeling and Drug Screening in Neurological Disorders

Luping Huang[1], Andrew Hilmer[1], Ying Pei[2], Xianmin Zeng[3]

[1]Applied StemCell Inc., Milpitas, CA 95035; [2]XCell Science Inc, Novato, CA 94945; [3]Scientific Advisory Board, 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 (Ex. Parkinson's disease (PD)).
- 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[1] for neurotoxicity assays and disease modeling applications
  - Control (normal and engineered-isogenic) lines
  - Patient-specific lines: integration-free iPSC lines from PD patients carrying various mutations[2]
  - Reporter lines (with lineage specific promoters/ ubiquitous; locus-specific KI or safe-harbor KI)
- 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

Make a panel of iPSC lines:
Control (normal & engine ered) lines
Patient-specific lines
Reporter lines

→

Generate differentiated neural cell types
NSC
Neurons
Astrocytes
Oligodendrocyte precursors

→

Model neurodegenerative diseases & run screens
Mechanism of action in a familial PD model
Neuroprotective screen with dopaminergic neurons
Toxicology assays

Parkinson's 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.
- **iPSC Lines from PD Patients[2]:** Extensively characterized integration-free lines from multiple donors of various genetic backgrounds. Their characterization includes whole genome analyses at various stages of differentiation (A).
- **Engineered isogenic lines[3]:** From parental control iPSCs with knockout mutations of genes associated with PD (B); and reporter lines with knock-in of lineage-specific reporters and in safe-harbor locus (C).

A

Gene	Mutation	Gender	Race	Age of PD	Age of sample	Family history
SNCA	SNCA triplication	Female	Caucasian	50	55	Yes
PARK2	PARK2: R42P PARK2: EX3DEL	Male	Caucasian	42	54	No
	PARK2: EX3-4DEL PARK2: 1-BP DEL, 255A	Male	Hispanic	16	50	No
	PARK2: R275W	Female	Hispanic	43	61	No
	PARK2: R42P	Female	Caucasian	44	63	N/A
LRRK2	LRRK2: G2019S	Male	Caucasian	40	52	No
	LRRK2: G2019S	Male	Caucasian	58	72	Yes
	LRRK2: G2019S	Male	Caucasian	34	57	No
GBA	GBA: N370S	Male	Caucasian	63	69	No
	GBA: N370S	Female	Caucasian	46	59	Yes
PINK1	PINK1: ILE368ASN	Male	Caucasian	66	64	Yes
	PINK1: ILE368ASN	Female	Caucasian	60	60	Yes
Control	Population control	Female	Caucasian	n/a	60	Yes

B

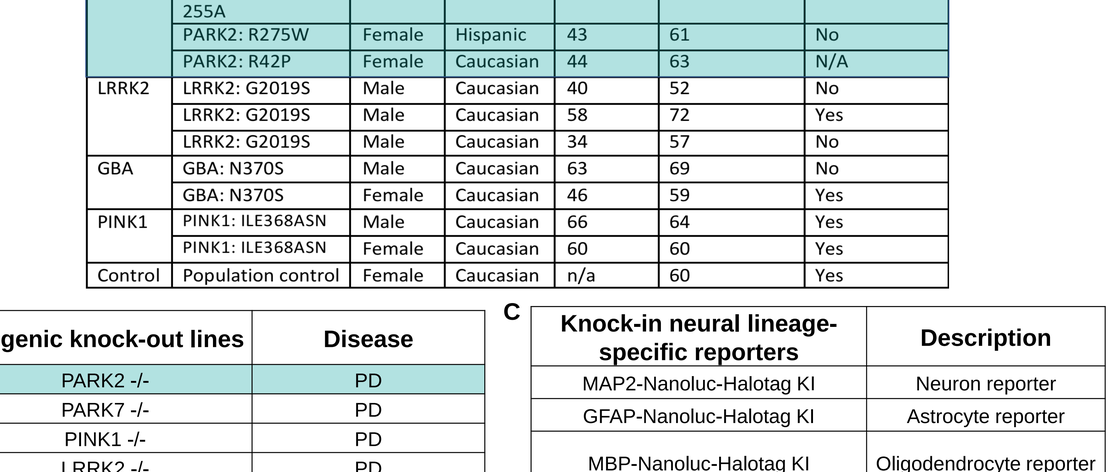
Isogenic knock-out lines	Disease
PARK2 -/-	PD
PARK7 -/-	PD
PINK1 -/-	PD
LRRK2 -/-	PD
Park2-/-; Park7-/-	PD
Park2-/-; Pink1-/-	PD
APOE -/-	Alzheimer's disease
SOD1 -/-	ALS
DICS1 -/-	Schizophrenia
CNTNAP2 -/-	Autism
BDNF -/-	CNS

C

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

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



Familial PD Model Using a Combination of Patient-specific & Isogenic Parkin (Park2) Lines

A

Park2 integration-free patient & control iPSC lines

Line	NINDS Catalog ID	Mutation	Gender	Race	Age of onset	Age of sample
Park2	ND30171 (P)	Park2: R42P Park2: EX3DEL	Male	Caucasian	42	54
	ND29543 (I)	Park2: EX3-4DEL Park2: 1-BP DEL, 255A	Male	Hispanic	16	50
	ND29369 (B)	Park2: R275W	Female	Hispanic	43	61
	ND31618 (S)	Park2: R42P	Female	Caucasian	44	63
Control	ND34791 (Y)	Population control	Female	Caucasian	n/a	60

B

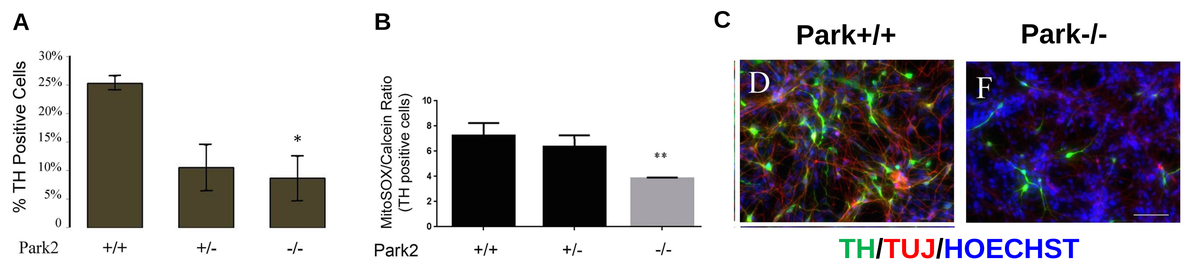
Isogenic Park2 iPSC lines

PARK2 +/+	WT/parental line XCL1
PARK2 +/-	Mono-allelic/heterozygotes
PARK2 -/-	Bi-allelic/homozygotes

Impaired dopaminergic differentiation and accumulation of SNCA in Park2 patient lines

(A) The number of TH+ neurons derived from patient-derived iPSCs is significantly lower as compared to control iPSC (Y09); (B) Western Blot for α-synuclein identified aggregates in patient iPSC-derived neurons and none in control line, Y09; (C) Mitochondrial volume fraction in TH+ dopamine neurons was significantly lower in patient lines.

Park2$^{-/-}$ Lines Recapitulate Abnormal DA Neuron Phenotypes



(A) Decreased TH+ cells in derived neurons from Park2 -/- knockout iPSC lines; (B) Decreased mitochondrial volume fraction in TH+ dopamine neurons in Park2 -/- lines; (C) Immunostaining for TH (green) and neuronal marker, Tuj1 (red) shows decreased TH+ neurons in Park -/- lines

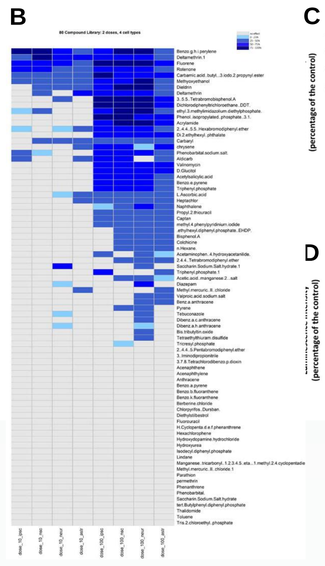
Screening Drugs for Neuroprotection & Neurotoxicity

A

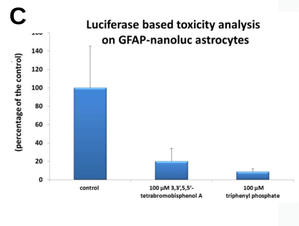
Names
Neurotransmitter/MAO inhibitor
Rasagiline
Selegiline
Nicotine
Topiramate
Amantadine
Zonisamide
Taurine
Antioxidant/Mitochondrial stabilizer
Resveratrol
N-Acetyl cysteine
Lipoic acid
Epigallocatechin gallate
Creatine
Anti-inflammatory
Rolipram
NS 398
Indomethacin
7-Nitroindazole
3-Aminobenzamide
Phenanthridone

Names
Neurotransmitter/MAO inhibitor
Donepezil
Caffeine
Theophylline
Phenelzine
Tranylcypromine
Pramipexole
Pergolide
Apomorphine
Lithium chloride
Acamprosate
Levetiracetam
Remacemide
Riluzole
Modafinil
Antioxidant/Mitochondrial stabilizer
Ascorbic acid
O-Acetyl-L-carnitine
Coenzyme Q_{10}
Uric acid
Evitaminum
Folic acid
Ropinirole
Anti-inflammatory
Plicamycin
Minocycline
Dimebolin
Estradiol
Clioquinol

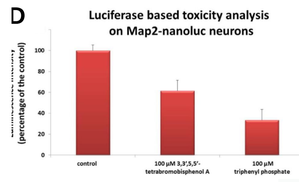
B



C



D



(A) 18 out of 44 compounds screened were found to be neuroprotective in derived-DA neurons, most of which have been used in human trials; (B) 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[4]; (C-D) Luciferase-based toxicity analysis in astrocytes and neurons derived from lineage-specific reporter iPSC lines[4] for high throughput screening of compounds.

Conclusions

- We have developed a panel of lines including control, patient-specific, isogenic and reporter lines, which provide a unique advantage for disease modeling.
- We have established robust methods for generating neurons and glia from iPSC using a NSC gateway concept. Our strategy enables us to derive neurons and glia from virtually all lines.
- We have shown that iPSC-derived neuronal and glial cells can be used for modeling neurodegenerative diseases as well as for neurotoxicological and neuroprotective screens.
- Human neural cultures may better mimic human neurodegenerative disorders.

References

1. Efthymiou A, Shaltouki A, Steiner JP, Jha B, Heman-Ackah SM, Swistowski A, Zeng X, Rao MS, Malik N. Functional screening assays with neurons generated from pluripotent stem cell–derived neural stem cells. Journal of biomolecular screening. 2014;19(1):32-43.
2. Momcilovic O, Sivapatham R, Oron TR, Meyer M, Mooney S, Rao MS, and Zeng X. Derivation, Characterization, and Neural Differentiation of Integration-Free Induced Pluripotent Stem Cell Lines from Parkinson's Disease Patients Carrying SNCA, LRRK2, PARK2, and GBA Mutations. PLoS One. 2016 May 18;11(5):e0154890
3. Shaltouki A, Sivapatham R, Pei Y, Gerencser AA, Momčilović O, Rao MS, Zeng X. Mitochondrial alterations by PARKIN in dopaminergic neurons using PARK2 patient-specific and PARK2 knockout isogenic iPSC lines. Stem cell reports. 2015 May 12;4(5):847-59.
4. Pei Y, Peng J, Behl M, Sipes NS, Shockley KR, Rao MS, Tice RR, Zeng X. Comparative neurotoxicity screening in human iPSC-derived neural stem cells, neurons and astrocytes. Brain research. 2016 May 1;1638:57-73.
5. Swistowski, A, Peng, J, Liu, Q, Mali, P, Rao, MS, Cheng, L, and Zeng, X. Efficient generation of functional dopaminergic neurons from human induced pluripotent stem cells under defined conditions. Stem Cells. 2010 Oct;28(10):1893-904.
6. Shaltouki, A., Peng, J., Liu, Q., Rao, MS., and Zeng, X. Efficient generation of astrocytes from human pluripotent stem cells in defined conditions. Stem Cells. 2013 May;31(5):941-52.