PD in a petri-dish: Overcoming a major bottleneck in modeling Parkinson's disease

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Background

Parkinson's disease (PD) is one of the most common neurodegenerative diseases of aging, affecting 1-2% of the population over 65 years of age. It is estimated that the number of prevalent cases of PD world-wide will double by the year 2030. There is no cure or effective way to slow disease progression, and the causes of the disease remain obscure.

Problem

Virtually every cellular or animal model for PD developed to date has clear and unequivocal shortcomings. The goal is of this collaborative effort is to develop new experimental tools using induced pluripotent stem cell (iPSC)-derived neurons from patients with PD.

Hypothesis

The underlying hypothesis of this collaborative CIRM-funded initiative is that differentiated iPSC lines from patients with known genetic and sporadic forms of PD will recapitulate *in vitro* one or more of the key molecular aspects of neural degeneration associated with PD and thus provide an entirely novel human cellular system for investigation of PD-related disease pathways and for drug discovery.

Skin biopsy from a patient, isolation and growth of skin cells

Nuclear reprogramming

Directed Neuronal differentiation

Study of disease mechanisms and 'in vitro clinical trials' for drug discovery

DNA and tissue bank at the Parkinson's Institute

- **Neurogenetics program at the Parkinson's Institute has enrolled over 900 subjects with PD and matched controls over the last 8 years and identified ~120 cases with family history of PD**
- **PD patients have been diagnosed and followed by trained movement disorder specialists at our Clinical Center**
- **Standardized clinical data are collected: testing for memory, olfaction and heart rate variability and questionnaires on psychiatric changes, sleep disturbances, exposure history and residential and occupational history**
- **All samples are genotyped for common mutations in PD genes**

Skin biopsies and fibroblast culture

We have collected 59 skin biopsies from both familial and sporadic cases of PD as well as healthy matched control subjects over the last 2 years:

59 individuals enrolled:

SNCA triplication case
GBA N370S homozygote
Digenic LRRK2/GBA compound heterozygote
3 PARKIN YOPD cases
17 LRRK2 p.G2019S cases
1 multiple system atrophy (MSA) case
1 progressive supranuclear palsy (PSP) case
18 mutation negative PD (age range 48-77)
16 controls (age range 25-92)

Fibroblast explant culture [Ref. 1] takes 6-8 weeks, 15-20 Mio cells are cryopreserved per subject

Skin biopsy

Skin explant culture

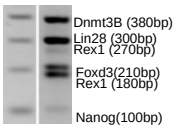
iPSC reprogramming

Using a retroviral system to deliver four genes encoding OCT4, KLF4, SOX2 and c/l-MYC, we have successfully derived 39 iPSC clonal lines from a subset of these fibroblast lines.

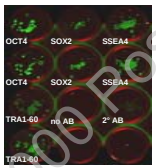
iPSC "quality score chart"

- ✓ **Expression of pluripotency markers by Q-PCR and IHC**
- ✓ **Differentiation potential in three germ layers**
- ✓ **Karyotyping and identity testing**
- ✓ **Epigenetic modification of pluripotency genes (in progress)**

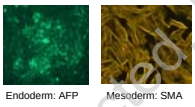
RT-PCR characterization



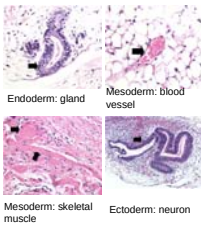
Immunostaining



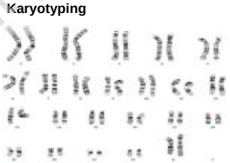
Embryoid body assay



Teratoma assays



Karyotyping



PI-1818 C2-13

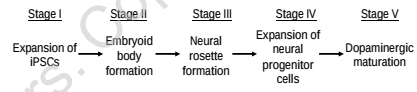
Normal karyotype, 46, XX

Identity testing

Sample	1818_FB	IPSC 1818 2-13
Amel	X X	X X
D13S325	166 170	166 170
D14S1434	239 243	239 243
D15S652	148 151	148 151
D17S974	259 263	259 263
D18S1364	316 332	316 332
D1S3721	342 358	342 358
D3S1259	352 354	352 354
D5S1453	157 157	157 157
D6S2439	277 277	277 277
D7S1802	191 200	191 200
D8S1108	121 121	121 121

Neuronal differentiation into dopaminergic neurons

We have adapted and optimized embryoid body (EB) mediated neuronal differentiation in combination with dual SMAD inhibition. The goal is to fulfill criteria for an optimal culture of mDA neurons: a) purity of differentiated cultures, b) correct region identity of derived neurons, c) reproducibility of the method, and d) throughput of method.

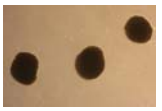


Media Name	EB 4 days	NIM 4 days	NPC 2-3 days	DA1 10 days	DA2 20-40 days
Basal Media		DMEM/F12 DMEM/F12	Neurobasal medium	Neurobasal medium	Neurobasal medium
Media supplement	β-ME	Bottenstein N2 FGF2	B27 FGF2	B27 FGF8	B27 BDNF GDNF
Test components	Dorsomorphine SB431524			Purmorphamine SAG Sonic Hedgehog	

Ref. [2]

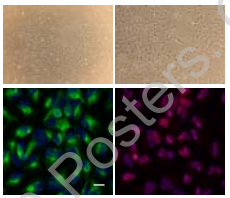
5-stage neuronal differentiation protocol

Stage 1: Expansion of iPSCs

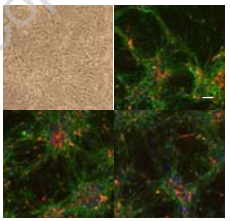


Stage 2: EB formation

Stage 3: Neural rosette formation

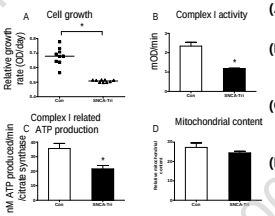


Stage 4: Expansion of neural progenitors



Stage 5:Dopaminergic maturation

Mitochondrial phenotype in patient-derived cell lines



(A)Growth rate of SNCA-Tri was decreased significantly by 24%

(B)Reduction of mitochondrial complex I activity in SNCA-Tri by ~49 %, * $p<0.0084$.

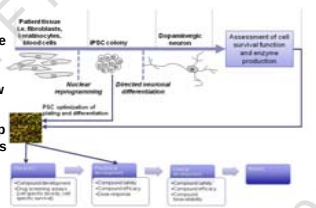
(C)Complex I-linked ATP production in patient cells was decreased by 39 %, * $p<0.0493$.

(D)No significant difference in mitochondrial content by Q RT-PCR of mitochondrial DNA. Ref. [3]

Future Directions

The future goal of this initiative is to take the iPSC "PD in a petri-dish" model to a drug screening tool to discover new therapeutics for PD.

On its way, this model will help better understand mechanisms of PD and also serve as a model to explore potential environmental toxins.



Adapted for PD drug discovery from Ebert and Svendsen 2010

Acknowledgments

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References

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[2] Mak et al. invited for Stem Cell International
[3] Mak et al. in press in Journal of Parkinson's disease