Modeling Parkinson's Disease in a Petri Dish:

Dopaminergic Neurons Derived from Stem Cell Challenged with Lewy Body Protein α-synuclein

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Introduction

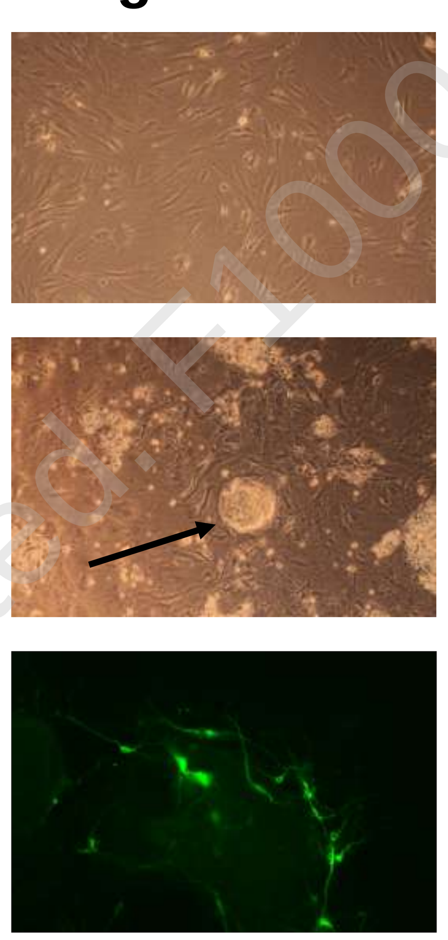
Parkinson's disease (PD) is one of the most common neurodegenerative diseases of aging, affecting A9 dopaminergic (DA) neurons while sparing adjacent A10 DA neurons. There is no cure or effective way to slow disease progression, and the causes of the disease remain obscure.

Our goal is to develop a new experimental tool using induced pluripotent stem cell (iPSC)-derived DA neurons. This novel model of PD could lay the foundation for drug discovery and it could also become a powerful tool to test environmental risk factors like pesticides on DA neurons in a petri dish.

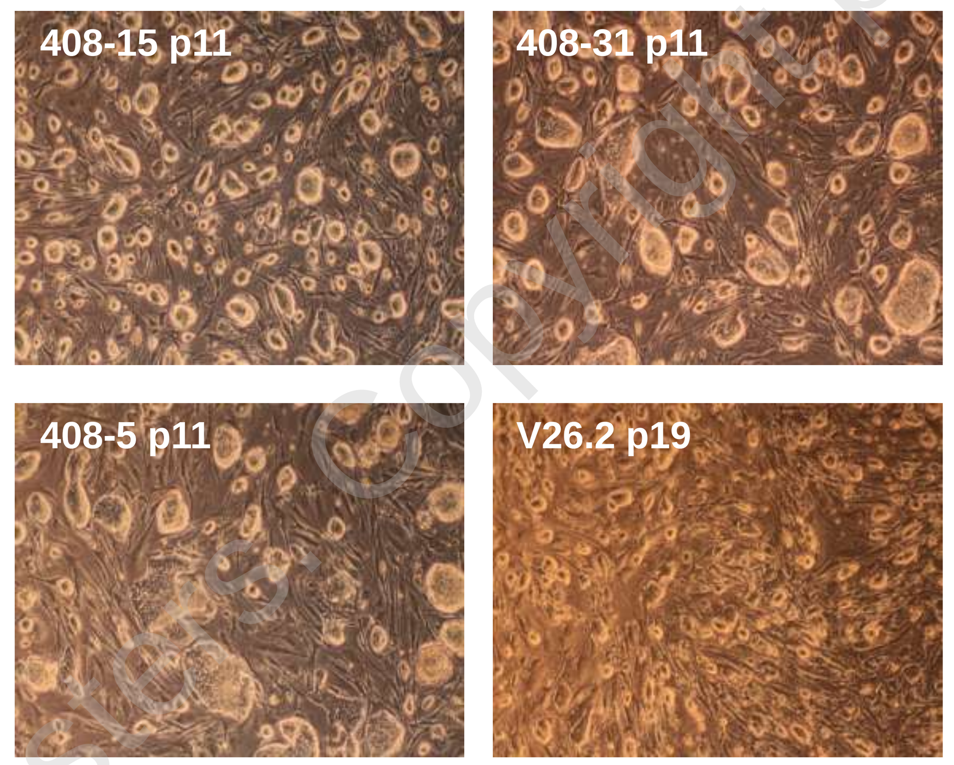
Methods

- Generate mouse iPSCs from tail fibroblasts using a retroviral system to deliver four genes encoding OCT4, KLF4, SOX2 and l-MYC [Ref 1]
- Characterize iPSC clones for pluripotency, differentiation potential, karyotpye, epigenetics
- Differentiate into dopaminergic neurons

Mouse Tail Fibroblasts → Induced Pluripotent Stem Cells → Dopaminergic Neurons



Results



p=passage number

Figure 1. Three miPSC clones and one control mESC line

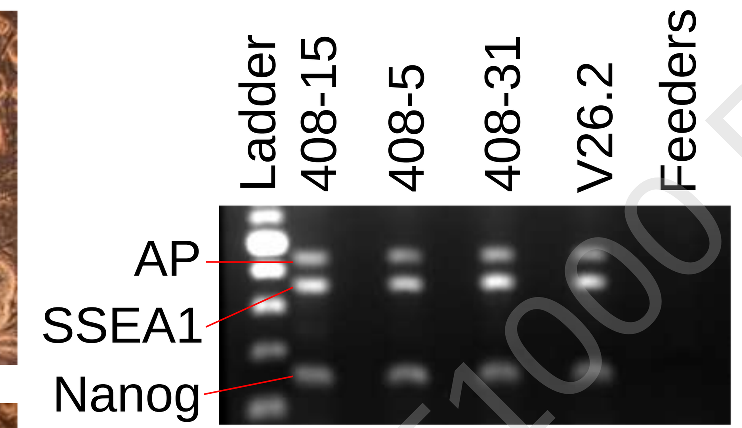


Figure 2. RT-PCR showing pluripotency markers for all clones

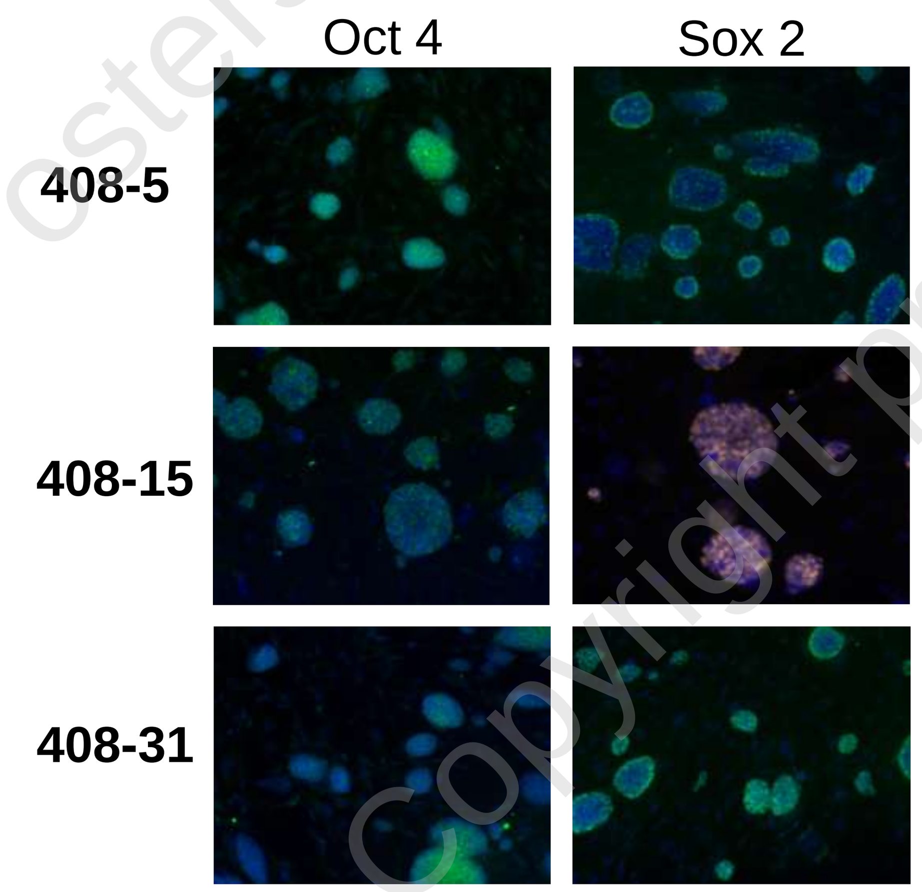
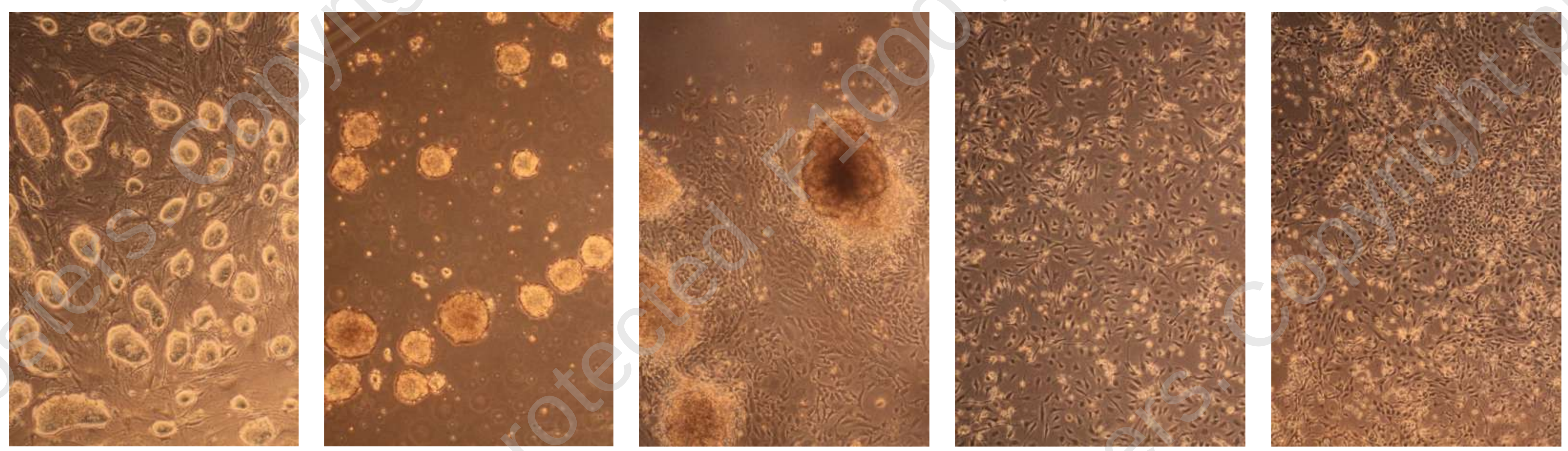
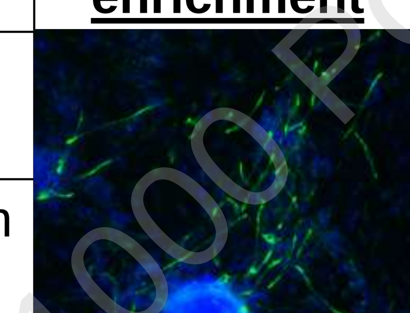


Figure 3. Immunohistochemistry showing pluripotency markers for all clones and control

Stage I	Stage II	Stage III	Stage IV	Stage V
iPSC expansion	Embryoid Body	Nestin+ Selection	Nestin+ Expansion	Neuron Differentiation



	Stage I	Stage II	Stage III	NCAM enrichment	Stage IV	Stage V
Days in Culture	3-4	4	6-8		4-6	10-15
Media name	mESC	mESC	Insulin Transferrin Selenium		N2	N2
Media Supplement	LIF	Dorsomorphin SB431542	Fibronectin	**Figure 4.** Pre-NCAM separation staining for Nestin+ population	Progesterone Putrecin Ascorbic Acid FGF8b, FGF2, SAG	Ascorbic Acid [Ref 2]

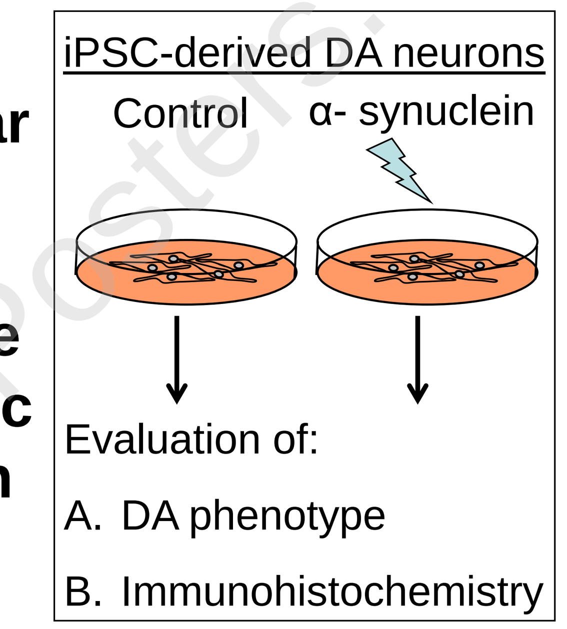
Discussion

We have generated 36 mouse iPSC lines, three are being now fully characterized. We are currently optimizing our protocol to develop a high yield of DA neurons. We believe that starting EB formation with small aggregates from a pristine miPSC is important, and that adding inhibitors like Dorsomorphin and SB431542 increases the number of nestin+ cells [Ref 3-4]. The preparation of ITSF and N2 media fresh from individual components weekly is ideal. During stage five it is important to split cell when confluent before beginning final maturation.

Future Work

Hypothesis: Extracellular alpha-synuclein is toxic for neuronal cell cultures. DA neurons are more susceptible to toxic effect of alpha-synuclein

Experiment: Challenge DA neurons with recombinant monomeric alpha synuclein under physiological conditions.



Dose-response and time course studies
- 70uM α-synuclein
- 24 hours

References

1 Takahashi et al. 2006 Cell p.663-676
2 Mckay et al. 2001 Science p.1389-1394
3 Morizane et al. 2011 JNR p.117-126
4 Kim et al. 2010 Stem Cell Rev Rep p.270-281

Acknowledgement

CIRM Grant TB1-01195 Stem Cell Internship for Laboratory Based Learning (SCILL) and thanks to Sally Mak for her insight and fruitful discussions.