Using LUHMES cells as a model system to study dopaminergic neuron cell biology and Parkinson's disease

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Abstract

Dopaminergic neurons play significant roles in motor, reward, and motivational behavior related circuits throughout the brain. To date, there are few continuous *in vitro* models available to laboratories in research, industry, and academia for studies related to basic dopaminergic cell biology or high throughput screening. Here, we propose the use of a human model system, LUHMES cells (ATCC® CRL-2927™), to study dopaminergic neuron cell biology and Parkinson's disease. LUHMES cells are neuronal precursors derived from fetal ventral mesencephalon. Neuronal differentiation is governed by the termination of *v-myc* expression using low levels of tetracycline. During our characterization, we found that tetracycline induced differentiation resulted in consistent neurite outgrowth in LUHMES cells within two to four hours. One day post differentiation, cells displayed similar morphology, with several long processes protruding from the cell soma. Growth cones were often observed in early differentiated cultures. Immunocytochemistry in early differentiated cultures (Days *in vitro*, DIV 2-3) revealed low level expression of tyrosine hydroxylase; however, these levels were increased significantly by 7 DIV with many neurons expressing tyrosine hydroxylase. We also investigated dopamine transporter expression. Differentiated LUHMES cultures were positive for neuronal markers such as βIII tubulin and devoid of expression of traditional glial markers including GFAP and IBA-1. Both undifferentiated and differentiated LUHMES cells were easily transfected using basic eGFP constructs, although greater efficiencies were observed with the use of viral constructs. In summary, LUHMES cells are a suitable and robust *in vitro* model system for studying dopaminergic neuron cell biology and mechanisms underlying Parkinson's disease.

Introduction

Functions of the dopaminergic system include the sensation of pleasure, motivation and reward, motor function, and compulsion. For studies in culture, an ideal *in vitro* model should capture multiple aspects of the desired *in vivo* system, yet be quick and easy to generate as well as cost efficient. Researchers often use primary neurons or neurons derived from IPSCs, however:

Limitations to using primary neurons include:
- Requires very early embryonic time points (usually E11-E14)
- Necessitates highly skilled personnel.
- Variable in health and quality.
- Low yield

Limitations to generating neurons derived from IPSC:
- Time

LUHMES cells are neuronal precursors derived from embryonic ventral mesencephalon that can be differentiated in as little as 4 days to fully post-mitotic dopaminergic neurons. Prior to differentiation, a homogenous population of over 80 million cells may be generated within 1 week to be used for a variety of assays that are applicable to dopaminergic biology and generalized neuroscience studies.

Results

LUHMES grow rapidly *in vitro* and differentiate into neurons

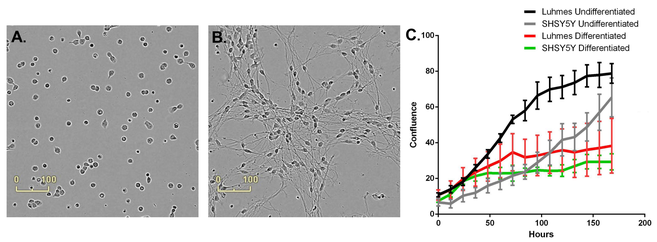


Figure 1: LUHMES cells differentiate into neurons in the presence of tetracycline, dbcAMP, and GDNF. Addition of 1 µg/mL tetracycline, 1 mM dbcAMP, and 2 ng/mL GDNF transforms undifferentiated LUHMES cells A) into differentiated cells B) within 4 days *in vitro*. C) LUHMES cells grow to confluence in approximately 1 week. Approximately 80 million undifferentiated cells were generated during 1 week in culture. All images and growth curves were captured using an IncuCyte™ FLR (Essen Biosciences Inc). Images were taken at 10x magnification.

LUHMES cells express neuronal markers following differentiation

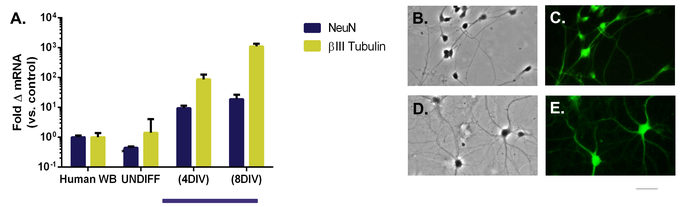


Figure 2: LUHMES cells express neuronal markers following differentiation by qPCR and immunocytochemistry. A) Two neuronal markers, NeuN and βIII tubulin, are upregulated significantly following either 4 or 8 days of differentiation when compared to undifferentiated LUHMES cells or RNA isolated from whole human brain. B-E) Immunocytochemistry for βIII tubulin (Covance 1:500) in 8 day differentiated LUHMES cells B) and C) and primary neurons D) and E). Blue bar indicates differentiated cells in A) cDNA for qPCR was generated from 25 ng of RNA using qscript™ cDNA synthesis kit (Quanta Biosciences). All qPCR reactions were carried out using SYBR® Green (Life Technologies) on a CFX96 Touch™ (BioRad). All experiments were performed in triplicate or greater. * = p≤0.05. Images B)-E) were taken at 20x magnification. Scale bar represents 20 µm.

LUHMES cells express dopaminergic markers following differentiation

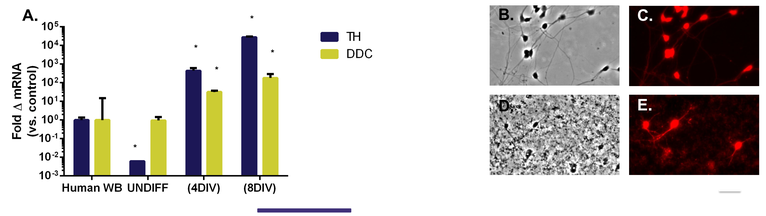


Figure 3: LUHMES cells express important dopaminergic markers by qPCR and immunocytochemistry. A) Two dopaminergic markers, tyrosine hydroxylase and dopamine decarboxylase, are upregulated significantly following either 4 or 8 days of differentiation when compared to undifferentiated LUHMES cells or RNA isolated from whole human brain. B)-E) Immunocytochemistry for tyrosine hydroxylase in 8 day differentiated LUHMES cells B) and C) and primary neurons D) and E). Blue bar indicates differentiated cells. cDNA for qPCR was generated from 25 ng of RNA using qscript cDNA synthesis kit. All qPCR reactions were carried out using SYBR Green on a CFX96 Touch. All experiments were performed in triplicate or greater. *=p≤0.05. Images were taken at 20x magnification. Scale bar represents 20 µm.

LUHMES cells express markers important for the study of Parkinson's disease

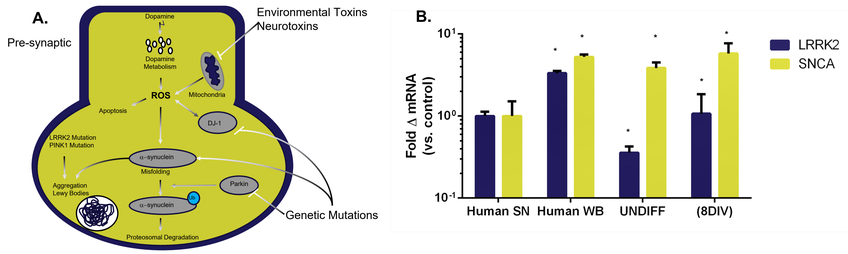
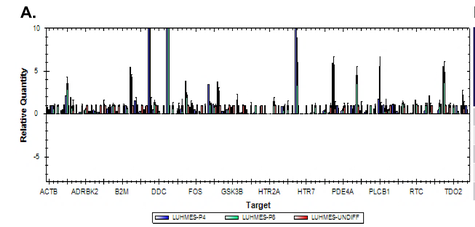


Figure 4: LUHMES cells express important Parkinson's related markers by qPCR A) Illustration of mechanisms hypothesized to be involved in the neurodegeneration that occurs in Parkinson's. B) RNA transcripts for two proteins, LRRK2 and α–synuclein, are expressed in LUHMES cells as assessed by qPCR. cDNA for qPCR was generated from 25 ng of RNA using qscript cDNA synthesis kit. All qPCR reactions were carried out using SYBR Green on a BioRad CFX96 Touch. All experiments were performed in triplicate or greater, *=p≤0.05.

LUHMES express RNA for many proteins important in dopaminergic biology



B.

GENE	LUHMES – P4	LUHMES – P8
DRD1	N.E	N.E
DRD2	↑	↑
DRD3	N.E	N.E
DRD4	↓	↑
DRD5	N.E	N.E

GENE	LUHMES – P4	LUHMES – P8
ADCY1	↑	↑
ADCY2	N.E	N.E
ADCY3	(-)	↑
ADCY5	↑	↑

GENE	LUHMES – P4	LUHMES – P8
SLC18A1	(-)	(-)
SLC18A2	(-)	↑
SLC6A3	↑	↑

N.E - No expression
(-) - No change relative to undifferentiated LUHMES
↓↑ - Up/down regulation > 2 fold

Figure 5: qPCR profiling of undifferentiated, 4 and 8 days differentiated LUHMES cells for dopaminergic and serotonergic markers. A) Dopaminergic and Serotonergic RT2 Profiler PCR arrays (QIAGEN®) were run and analyzed according to manufactures instructions. B) Differentiated LUHMES cells express dopamine receptor 2 and 4. DRD2 was upregulated more than 500 fold in P4 and P8 differentiated LUHMES when compared to undifferentiated LUHMES. RNA for multiple isoforms of adenylate cyclase were also present in differentiated LUHMES cells. Additional markers that were upregulated include CYP2D6, SLC18A2, and SLC6A3. All experiments were performed in triplicate or greater, *=p≤0.05.

LUHMES are electrically active and express functional glutamate receptors

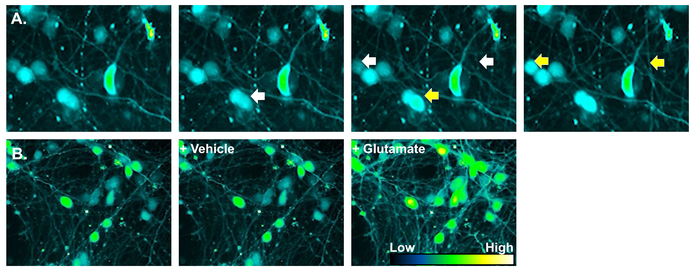


Figure 6: LUHMES cells demonstrate neuronal activity using fluorescent calcium indicator dyes. A) Differentiated, untreated LUHMES cells or primary neurons (not shown) were loaded with Fluo-4AM (ThermoFisher™) for 15 min at 37ºC. Cell bodies and processes were then assayed for spontaneous neuronal activity over 30 seconds as indicated by changes in fluorescent intensity (see scale in B, panel 3). Increases in fluorescence intensity were observed within neuronal processes and cell bodies. A) White arrows (frame two) compared with corresponding yellow arrows (frame three), as well as white arrows (frame three) compared with yellow arrows (frame four), indicate differences in intensity. Similar results were obtained in primary neurons. B) Differentiated LUHMES cells before and after the addition of vehicle or 25 µL of 1 mM glutamate (final concentration, 50 µM) demonstrate the presence of functional glutamate receptors. Original videos taken at 10X magnification. Videos were optically zoomed 3.5x and edited using Adobe Premier Pro cs5 software. Videos can be found online at the following URL: https://www.atcc.org/resources/webinars/2014-webinars/using-luhmes-cells-as-a-model-system-to-study-dopaminergic-neuron-cell-biology.

Conclusion

- LUHMES cells are precursor cells isolated from 8 week embryonic ventral mesencephalon.
- Undifferentiated LUHMES cells grow rapidly in culture while also being easily differentiated into postmitotic neurons upon the addition of minimal growth factors.
- LUHMES cells express RNA and protein for mature neuronal, dopaminergic, and Parkinson's related markers.
- LUHMES cells have spontaneous activity as well as functional glutamate receptors as measured by fluorescent calcium indicator dyes.
- Expression of the dopamine 2 receptor subtype makes this line useful for studies related to various neuropsychiatric disorders, including Schizophrenia.

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