In Vitro Lecture and Luncheon
Sponsored by the Colgate Palmolive Company
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In Vitro Lecture Goals

• Feature important research using *in vitro* and alternative techniques to study basic mechanisms

• Illustrate how these test methods benefit animal welfare by replacing animal use whenever it is feasible

• Encourage students and postdoctoral scholars to use alternative techniques in their research
Patient-Based Cellular Model Systems to Assess Individual Risk to Neurotoxicants

2019 In Vitro Lecture
Aaron Bowman
Speaker
Patient-Based Cellular Model Systems to Assess Individual Risk to Neurotoxicants

Aaron Bowman, PhD
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Genes, environment and aging effects, and their interactions, culminate in many human diseases.
Parkinson’s Disease

Cardinal Symptoms:
Tremor (resting)
Muscular rigidity
Bradykinesia (slow movement)
Disturbances in gait and posture

Etiology:
Pesticides, metals (e.g. manganese and copper) and other environmental risk factors impinging upon backdrop of genetic risk factors, sex-effects and aging

Pathology:
Loss of substantial nigral dopamine neurons
Accumulation of alpha-synuclein in Lewy bodies

Sir William Richard Gowers, 1886
A Manual of Diseases of the Nervous System
Human Pluripotent Stem Cells
Made by “Reprogramming” Adult Skin Cells
Key Concept: Human induced pluripotent stem cells may model individuals or “sub-populations” for risk assessment of environmental/toxicological exposures.

Selection of Human Subjects
- Susceptibility Differences
- Genetic Risk Factors
- Sex Differences
- Disease-State

Types of Exposure Models
- Acute Toxicity
- Chronic Toxicity
- Multi-hit Toxicity
- Delay-effect Toxicity

Adapted from Kumar KK et al., Neurotoxicology 2012.
Key Concept: Environmental risk factors for neurological disease have been difficult to detect in epidemiological cohorts, potentially due to human variability of risk.

Occupational copper and manganese exposure are putative risk factors for Parkinson’s Disease

- Adjusting for sex, race, age and smoking status, 20 years of occupational exposure to copper or manganese was associated with Parkinson’s Disease, but not iron, zinc, or mercury alone.

- Occupational exposure for > 20 years to combinations of lead-copper, lead-iron, or iron-copper was also associated with disease.


Occupational exposure to manganese, copper, lead, iron, mercury and zinc and the risk of Parkinson's disease. Gorell JM¹, Johnson CC, Rybicki BA, Peterson EL, Kortsha GX, Brown GG, Richardson RJ.
Developing Human Mesencephalic (Nigral/Midbrain) Dopamine Neurons

Floor plate lineage differentiation at 25 days

Spontaneous and evoked release of dopamine (neurotransmitter) seen by ~45 days of differentiation, reaching steady state levels by ~55 days differentiation

Tyrosine hydroxylase  b3-tubulin  Lmx1A  Hoechst
**Key Concept:** iPSC derived neurons can be used to evaluate clinically-relevant and toxicant-relevant outcomes measures

**Examples of clinically relevant outcomes to copper or manganese exposures in hiPSC neurons**

1. Reductions of spontaneous and evoked dopamine release.

2. Increased mitochondrial fragmentation

3. Increased measures of oxidative stress such as glutathione levels, isoprostane levels and changes to redox-sensitive dyes

4. Reductions in neurite length

5. Changes in mitochondrial membrane potential

Neely ND, et al, Toxicological Sciences, 2017
Key Concept: Genetic risk factors can increase vulnerability of neuroprogenitor cells from individuals to disease-relevant environmental risk factors.

PARK2 patient neurons have elevated sensitivity to copper

1. Increased levels of mitochondrial fragmentation in familial PD patient neurons vs control subjects at 25µM Cu exposure.

2. PARK2 patient neurons show decreased ‘lowest observed adverse effect concentration’ (LOAEC) for Cu exposure induced mitochondrial fragmentation (10µM extracellular).


Mitochondrial fragmentation morphology in hiPSC-derived neurons.
Conclusion and Open Questions

Strong genetic risk factors associated with neurological disease impact susceptibility to toxicant exposures relevant to disease-associated environmental risks in individual patient-derived stem cell-based models.

Challenges to be faced for personalized toxicological risk assessment:

- Undefined modifiers of toxicity
- Multicellular/tissue-based modeling
- Chronic exposures
- Disease validation at cellular level
Developing an effective iPSC model requires knowledge about the disease. Discuss the features of an appropriate iPSC disease model by considering the following:

1. Are there characteristics of individuals, populations, sub-populations, or risk groups that would be critical in identifying whom to select for generation of a stem cell-based model system of the disease?
2. Is there a target cell type primarily impacted or contributing to pathogenesis?
3. Are there known or suspected toxicants that contribute to disease risk?
4. Are there particular developmental stages or sequential time points that are important in the pathogenesis?
5. Are there genetic-based animal models that recapitulate human pathogenesis?
Supporting Slides
Key Concept: Genetic risk factors can increase vulnerability of neuroprogenitor cells from individuals to disease-relevant environmental risk factors.

Two-way ANOVA Copper x genotype interaction ($p<0.001$)

- ** solid bars: Vehicle control
- hashed bars: 10 µM Cu 24 hour exposure
  - * $p < 0.05$
  - ** $p < 0.01$
  - *** $p < 0.001$

Subject SM derived neuroprogenitor cells with known Parkinson's Disease genetic risk factor show elevated sensitivity to copper cytotoxicity vs control subject CA derived cells.

Key Concept: Genetic risk factors can predispose individuals to disease-relevant environmental risk factors.

50µM Cu, 48 hours exposure – Flow cytometry analysis for viable cells (% viable indicated)

Key Concept: Environmental risk factors can elicit disease-relevant pathogenic effects that are more severe in presence of known genetic risk factors.

Subject SM derived neuroprogenitor cells with known Parkinson’s Disease genetic risk factor show increased mitochondrial fragmentation vs control subjects neuroprogenitor cells.

Two-way ANOVA Copper x genotype interaction (p<0.001)

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Key Concept: Genetic risk factors can increase vulnerability of neuroprogenitor cells from individuals to disease-relevant environmental risk factors.

Subject SM derived neuroprogenitor cells with known Parkinson’s Disease genetic risk factor show decreased lowest observed adverse effect concentration (LOAEC) for copper-dependent mitochondrial fragmentation vs control subjects neuroprogenitor cells.

Two-way ANOVA Copper x genotype interaction (p<0.001)
With testing advances, we continue to improve research and reduce reliance on animal models.
Thank you for your participation.