**Exploration of Mitochondrial Bioenergetics using Human iPSC-derived Neurons**

**Kwi Hye Kim, Natsuyo Aoyama, Kile Mangan, and Coby Carlson**

_Cellular Dynamics International, A FUJIFILM Company, Madison, WI USA_

**Abstract**

The advent of induced pluripotent stem cell (iPSC) technology now grants the scientific community access to previously unattainable human cell types, specifically those from the brain. Moreover, iPSC technology enables us to compare cells from apparently healthy normal individuals to those cell types from disease-specific and patient-derived samples. Neurological and neurodegenerative disorders are a global health concern. Many of these disease pathologies are thought to be driven by dysfunctional mitochondria creating an imbalance in cellular bioenergetics. The ability to measure discrete changes in cellular energy and metabolism in a real-time, label-free approach is possible with Seahorse XF technology. We have used this instrument to assess the impact of various conditions (ranging from neuronal culture medium, time in culture, cell density, mitochondrial stress agents, and genetic mutations) on the bioenergetics profiles of human iPSC-derived cell types. Here we focus on the development of methods and protocols to evaluate mitochondrial stress in GABAergic, glutamatergic, dopaminergic, and motor neurons. Additionally, we have tested iPSC-derived astrocytes, macrophages, and skeletal muscle as each of these cell types are possibilities for co-culture to generate more complex and biologically relevant cell models. Finally, establishing baseline assay signals for these various cell types derived from apparently healthy normal donors paves the way for disease modeling. Examples from assaying neurons and astrocytes with the SOD1 G93A mutation will be presented.

**Measurement of Metabolism**

Agilent Seahorse XF Analyzers enable quantification of cellular bioenergetics by measuring mitochondrial respiration and glycolysis in live cells.

**Genome-Engineered Disease Modeling**

Nuclease-mediated site-specific mutations were introduced into healthy control iPSCs to generate engineered lines that harbor mutations associated with neurodegenerative or neurodevelopmental diseases. Each genome-engineered iPSC line and its isogenic control harboring a wild-type allele were used to evaluate the effects of the genetic alterations on mitochondrial bioenergetics.

**Optimization of Testing Conditions**

- **Plate Surface Preparation**: Maintaining high density monolayer neuronal cultures was critical to obtaining well-to-well consistency. Traditional PLO/laminin treatment of the surface was switched to PEI/laminin treatment to achieve a monolayer of neurons without clustering.

- **Basal Protocol Optimization**: For each cell type, optimized for the plating density to obtain OCR values >50 pmol/min, the concentration of oligomycin to obtain maximum glycolytic capacity and the amount of FCCP for maximum respiration.

**Data Normalization**

- When data normalization is desired, the number of live cells is counted after bioenergetics measurement by staining nuclei with CyQUANT™ Direct Cell Proliferation Assay kit (ThermoFisher) and analyzing using an IncuCyte™ Zoom.

**Currently Available Genome-Engineered Cells**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Targeting Gene</th>
<th>Modification</th>
<th>Cell Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parkinson’s Disease</td>
<td>Alpha-synuclein (SNCA)</td>
<td>A53T</td>
<td>MyCell® DopaNeurons</td>
</tr>
<tr>
<td>Alzheimer’s Disease</td>
<td>Amyloid Precursor Protein (APP)</td>
<td>A673T</td>
<td>MyCell® GABANeurons</td>
</tr>
<tr>
<td>Alzheimer’s Disease</td>
<td></td>
<td>A673V</td>
<td>MyCell® GABANeurons</td>
</tr>
<tr>
<td>Rett Syndrome</td>
<td>MeCP2</td>
<td>Engineered knock-out</td>
<td>MyCell® GABANeurons</td>
</tr>
<tr>
<td>Amyotrophic Lateral Sclerosis</td>
<td>Superoxide Dismutase (SOD1)</td>
<td>G93A</td>
<td>MyCell® GABANeurons</td>
</tr>
</tbody>
</table>

**Cellular Respiration in Parkinson’s Disease Model: Media Effects**

Culturing cells in BrainPhys™ Neuronal Medium (STEMCELL Technologies), which has been shown to be more physiologically relevant to neuronal cultures, was compared to traditional maintenance medium (MM) to assay the effects of media on cellular respiration. Increased basal and maximal respiratory capacity was observed in BrainPhys™ Neuronal Medium for both iCell® DopaNeurons (wild type) and MyCell® DopaNeurons harboring an alpha-synuclein mutation (SNCA[A53T]) linked to Parkinson’s Disease.

**Mitochondrial Respiration in ALS Model**

Mitochondrial respiration was measured for MyCell® GABANeurons harboring a genetic mutation (SOD1 [G93A]) implicated in Amyotrophic Lateral Sclerosis (ALS) and compared to isogenic control iCell® GABANeurons. The SOD1 [G93A] neurons had lower basal respiration and maximal respiration as compared to control neurons. Treatment with a low dose of H₂O₂ for 2 hours before the assay resulted in slightly increased respiration. Regardless of the genotype, a high concentration of H₂O₂ caused obliteration of respiration (data not shown).

**Summary**

- Measuring cellular bioenergetics (e.g., mitochondrial respiration and glycolysis) enables exploring the functionality of human iPSC-derived neuronal cell types
- Changes in cellular bioenergetics profiles can be used to identify phenotypes for disease modeling using human iPSC-derived cells harboring known genetic mutations