

## CeBioND

### Cellular Bioenergetics in Neurodegenerative Disease

#### High throughput screening and validation of mitochondria stabilizers

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Mitochondrial and bioenergetic dysfunctions are implicated in most neurodegenerative diseases, therefore one of the aims of the Cellular Bioenergetics in Neurodegenerative Diseases consortium (CeBioND) was to screen for molecules that stabilize mitochondrial functions. First, a cell-based assay for high throughput screening (HTS) using differentiated neuroblastoma SH-SY5Y cells was established. During the last step of differentiation cells were cultured in pyruvate in order to force them to use mitochondria for ATP production. Next, a pilot screen using the Prestwick library with 1200 FDA approved small molecules (10  $\mu$ M for 24 h) was performed. 15 hits with  $>2 \sigma_{\text{DMSO}}$  on ATP levels were tested in three different concentrations (3, 10 and 30  $\mu$ M) and out of these one molecule was a true hit. This molecule, the flavonoid luteolin, was further validated using nine different concentrations (0- 80  $\mu$ M). All concentrations from 0.625  $\mu$ M up to 40  $\mu$ M resulted in increased ATP levels and no cytotoxicity (ToxGlo assay).

To validate luteolin in a more complex neuronal model, primary mouse cortical neurons were treated with luteolin (1-20  $\mu$ M). Interestingly, the Seahorse respirometry assay revealed that 2.5  $\mu$ M luteolin increased both basal and maximal respiration and oligomycin-sensitive ATP production, from 6 -16 h of treatment. To exclude the possibility that luteolin entail increased reactive oxygen species (ROS) production, we used the fluorescent probe MitoPY1 which indicated that 2.5  $\mu$ M luteolin did not stimulate H<sub>2</sub>O<sub>2</sub> production in basal conditions or after inhibition of complex III with antimycin A (2  $\mu$ M). Transmission electron microscopy (TEM) analysis of neurons exposed to luteolin did not reveal any changes in mitochondrial profile numbers or length, reflecting lack of mitochondrial biogenesis or dynamics alterations. Protein analysis of mitochondrial complexes subunits also corroborated these observations. Remarkably, 2.5  $\mu$ M luteolin increased the number of mitochondrial-endoplasmic reticulum contacts per individual mitochondrion and further experiments revealed that IP3R-dependent Ca<sup>2+</sup>-shuttling drives ATP synthesis in treated neurons. In summary, CeBioND presents a cell-based assay for HTS of molecules affecting mitochondrial functions and report the identification of luteolin as a mitochondrial stabilizer.