Developing an Assay to Measure Nrf2 Activation

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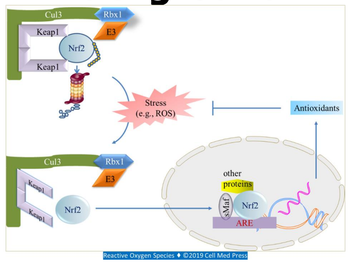
Abstract

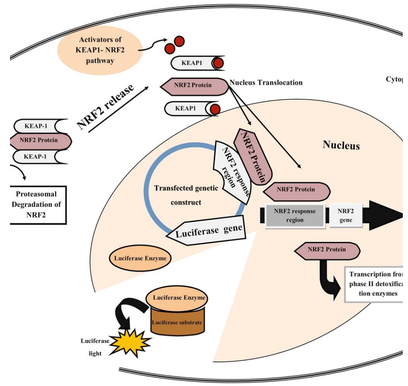
There is an oxidative stress component to a wide range of neurobiological diseases. In Alzheimer's disease (AD), secondary brain injury is associated with an imbalance between oxidant and antioxidant agents. This imbalance contributes to the pathophysiology of AD through the oxidation of macromolecules, destabilization of neuronal cells, and generation of ROS that upregulates synthesis and deposition of p-tau and Amyloid-β (Aβ). The expression of antioxidant defense enzymes can decrease damaging reactive oxygen species, so some efforts to alleviate secondary injury focus on this mechanism of reducing oxidative stress.

Background



One pathway that is activated in response to oxidative stress is the Nrf-2/ ARE pathway. Under stress conditions, the protein sensor for oxidation levels Keap1 that is bound to Nrf2 is oxidized, and Nrf2 levels are stabilized and subsequently increased in the cell. The Nrf2 transcription factor then translocates into the nucleus and binds to the antioxidant response element (ARE) promoter to turn on the expression of downstream antioxidant genes. The genes that are expressed include heme-oxygenase (HO-1) and NADPH quinine oxidoreductase 1 (NQO1). These antioxidants can then regulate the redox balance in the internal environment and reduce oxidative stress. The goal of my research is to design an assay to measure Nrf2 activation, so we can test drugs shown to reduce oxidative stress *in vitro.*

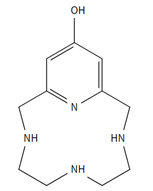
Luciferase Assay



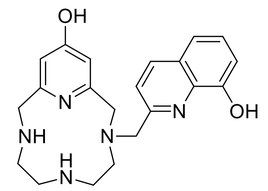
The luciferase assay is used to measure the activation of Nrf2-ARE under conditions that elevate ROS in the cell using the reporter gene luciferase.
When Nrf2 is activated under oxidative stress conditions, it translocates into nucleus, binding to ARE and turning on the luciferase gene.

Hypothesis

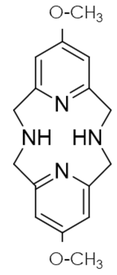
The compounds produced in Dr. Green's lab will modify the activity of Nrf2.



L2



PK108



$OMe_2Py_2N_2$

The drugs have been designed to be multimodal: to bind to metal ions and to quench ROS. Reducing ROS levels should decrease the activation of Nrf2.

Activation of Nrf2 After Exposure to Known Stimulants

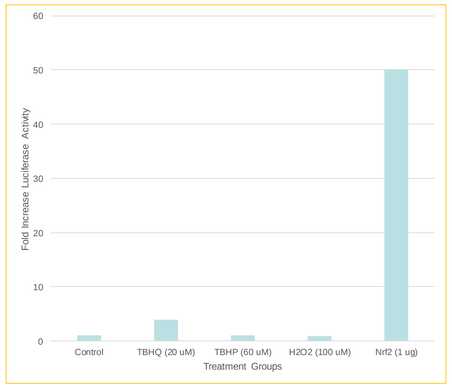


Fig. 1: 293HEK cells were treated with TBHQ, a known activator of Nrf2; and TBHP and H_2O_2, known sources of ROS. As a control, cells were transfected with the expression vector for Nrf2. TBHQ increased luciferase activity 4-fold, while TBHP and H_2O_2 had no effect.

The Effect of L2 and PK108 on Nrf2 Activation

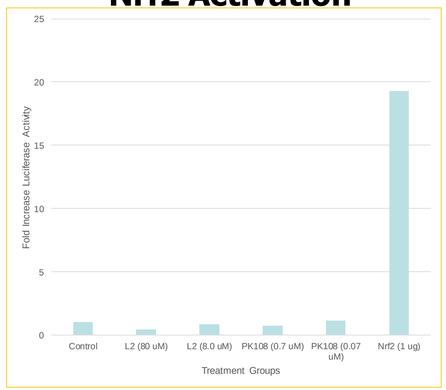


Fig. 2: 293HEK cells were treated with L2 and PK108 at different concentrations. As a control, cells were transfected with the expression vector for Nrf2. L2 and PK108 had no effect on Nrf2 activation.

The Effect of L2 and $OMe_2Py_2N_2$ on Nrf2 Activation in Cells Overexpressing Nrf2

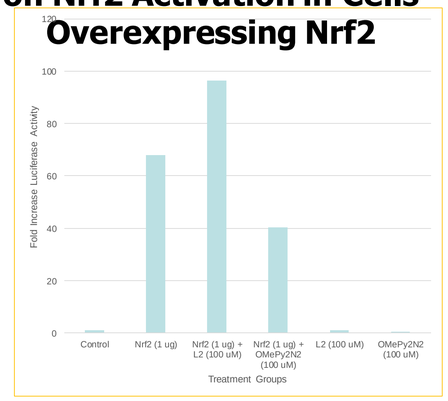


Fig. 3: 293HEK cells were transfected with Nrf2 and subsequently treated with L2 and $OMe_2Py_2N_2$. L2 induced Nrf2 activation, while $OMe_2Py_2N_2$ decreased Nrf2 activation. L2 and $OMe_2Py_2N_2$ alone had no effect.

Conclusion

- The Luciferase Assay can be used successfully to measure Nrf2 activation
- Treatment with TBHQ, a known activator of TBHQ, increased Nrf2 activation
- The compounds, L2, PK108 and $OMe_2Py_2N_2$, on their own did not appear to modulate Nrf2 activity in 293HEK cells
- L2 increased Nrf2-mediated gene expression in cells that were overexpressing Nrf2