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Case Study: Module 4 Small Molecule and Genetic Probes (Part 1)

Question/Biological Problem

Many risk factors are associated with the onset of age-related macular degeneration, ranging from a familial history of AMD to environmental factors, to cardiovascular health issues. Scientists have noted increasing difficulty in pinpointing the main contributor in AMD progression until recently, with studies showing some correlation to specific genes located in microglial cells – AMRS2 and HTRA1 genes. AMD is the deterioration of the macula, the eye's retinal region, causing severe loss of vision and blindness in older populations.

Several photoreceptors linked to AMRS2/HTRA1 genes display polymorphisms leading to higher associated risk for AMD. Studies have cited this as, "a change in a version of the gene that is produced to result in an amino acid change from alanine to serine at position 69 of the AMRS2 protein" (*HTRA1 - an Overview | ScienceDirect Topics*). Researchers further suggest through data trends, "that the AMRS2 protein is located in the mitochondria and involved in modulating oxidative stress and… apoptosis", although a direct link has not yet been established (*HTRA1 - an Overview | ScienceDirect Topics*). Reduced levels of the genes may present a higher risk for AMD, as both remain critical targets for further study.

Biological Question:

My aim is to investigate the role of ASRM2/HTRA1 protein levels in the retina, in mitochondria-rich retinal photoreceptor cells.

Research Model: To study the function of ASMR2/HTRA1 and its variants, displaying the polymorphisms described above, transfected in human monocyte cell line.

Research Plan:

- To see how expression of ASMR2/HTRA variants affects the cells physiology using a small molecule called DCFH, that will measure ROS (H202) intracellular concentrations. ROS will be produced and measured. DCFH targets oxidative stress in cells.
- DCFH permeates the cell membrane and once oxidized by ROS, DCFH becomes fluorescent DCF.
- In order to locally visualize this expression, they will be transiently released into the cytoplasm, paired with the use of another fluorophore, Mitotracker, to target the mitochondria where these genes are believed to be.
- Levels of the expressed cells with mutant gene vs. control cells with no expression gene (wild type gene) will be measured using a fluorescent plate reader, such as the Spectramax microplate reader (*MAXimize Your DISCOVERY*).
- DCF fluorescence is directly proportional to ROS levels. If DCF fluorescence levels show an increase, we can deduce higher levels of wild type gene ASMR2/HTRA are present. This suggests oxidative stress/apoptosis in the mitochondria is also present. Lower DCF fluorescence due to lower ROS levels therefore signify reduced levels of the ASMR2/HTRA1 proteins or mutant proteins exist. This suggests lower levels of oxidative stress/apoptosis present in the cell (*FAQ: Intracellular ROS Assay | Cell Biolabs*).

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Sources

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