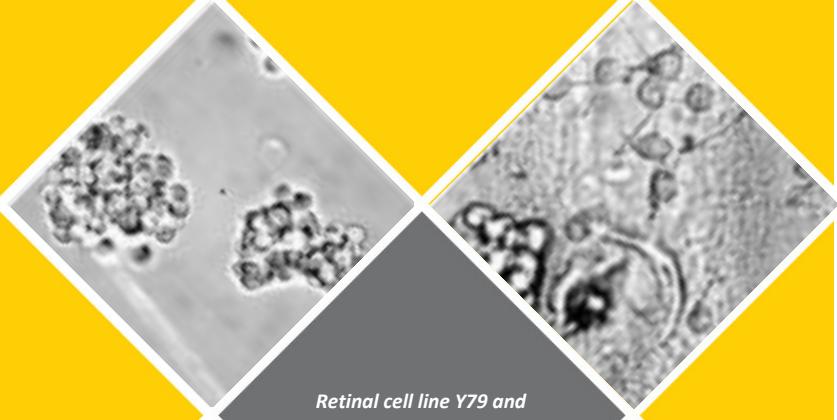
Ocular Diseases

RETINITIS PIGMENTOSA



Retinal cell line Y79 and differentiated photoceptors

Background

Photoreceptor degeneration is a cause of irreversible vision loss in incurable blinding retinal diseases including retinitis pigmentosa (RP) and atrophic age-related macular degeneration.

Among retinal pathologies, retinitis pigmentosa (RP) is a group of rare, genetic disorders that involve a breakdown and loss of cells in the retina - which is the light sensitive tissue that lines the back of the eye. Common symptoms include difficulty seeing at night and a loss of side (peripheral) vision.

Retinitis pigmentosa (RP) causes primary degeneration of photoreceptors, followed by reactive changes in the retinal pigment epithelium (RPE) and Müller glia, death of inner retinal neurons, and atrophy of the retinal vasculature.

Pathology Model

In order to test Client's compounds for their modulatory action exerted on an in vitro model of retinitis pigmentosa, differentiated retinal cell line Y79 will be challenged with oxygen glucose deprivation, in order to mimic ischemic conditions (Dvoriantchikova et al 2014).

Y79 are a retinoblastoma cell line, typically used in oncogenic application, which is well known to be capable, under certain culturing conditions (i.e. culturing on poly-D-lysine-coated substratum) to assume a photoreceptor-like morphology and to express the retina-specific interphotoreceptor-retinoid-binding protein (IRBP) mRNA in abundance. (Fassina et al 1993).

Cells will be subjected to 1 hour OGD treatment either in the presence/absence of client's compounds.

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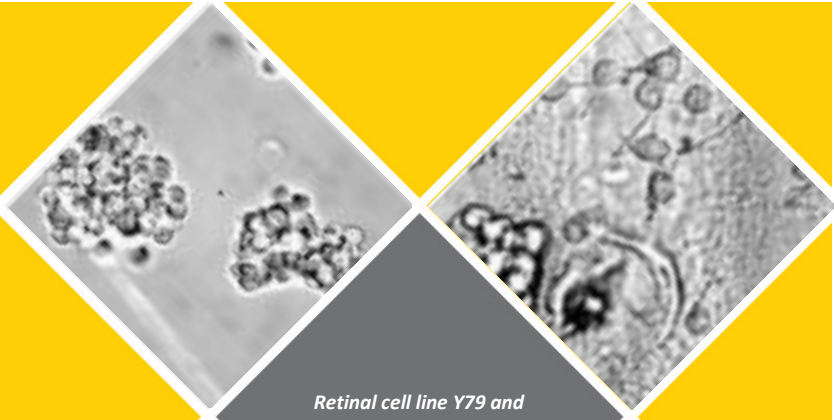

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Readouts

Cells will be quantitatively assayed for the following functional readouts:

- **Metabolic activity -** Metabolic activity will be quantitatively assayed via Vybrant® MTT Cell Assay. The MTT assay involves the conversion of the water soluble MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to an insoluble formazan. The formazan is then solubilized, and the concentration determined by optical density at 570 nm.
- **Oxidative stress -** Cells at the different experimental conditions will be quantitatively assayed via the ROS-Glo™ H_2O_2 Assay. It is a homogeneous, fast and sensitive bioluminescent assay that measures the level of hydrogen peroxide (H_2O_2), a reactive oxygen species (ROS), directly in cell culture. A derivatized luciferin substrate is incubated with sample and reacts directly with H_2O_2 to generate a luciferin precursor. Addition of ROS-Glo™ Detection Solution converts the precursor to luciferin and provides Ultra-Glo™ Recombinant Luciferase to produce light signal that is proportional to the level of H_2O_2 present in the sample.
- **Pro-inflammatory cytokine production** – Cells at the different experimental conditions will be quantitatively assayed for the production of proinflammatory cytokines IL1 beta and TNFalpha (Wang et a. I2017) via quantitative PCR.
- **Caspase activation** - Caspase 3 is implicated as an "effector" caspase associated with the initiation of the "death cascade" and is therefore an important marker of the cell's entry point into the apoptotic signaling pathway. Caspase-3 is activated by the upstream caspase-8 and caspase-9, and since it serves as a convergence point for different signaling pathways, it is well suited as a read-out in an apoptosis assay.
- Cells at the different experimental conditions are plated in a 96-well plate and treated with the challenge and the drug. According to manufacturer's protocol, Caspase-Glo 3/7 reagent is added to each well, and luminescent signal is measured at peak (2 hours), as indicated by data sheet. This kit quantifies the activity of caspase 3 and caspase 7 that share a common sequence recognized by kit substrate