2024 Multidisciplinary Research Program in Medicine Project*: Role of Cell-Cell Contacts in RPE Atrophy in Age-Related Macular Degeneration*

Hypothesis or Research Question(s): Loss of cell-cell contacts between retinal pigment epithelial cells (RPE) promotes cell and molecular changes leading to RPE atrophy, a pathological, early feature of AMD. We hypothesize that overactive enzymes (e.g. Granzyme B) and oxidative stress due to blue light stimulation promote the loss of cell-cell contacts in RPE in vitro and in vivo.

PROJECT BACKGROUND & SUMMARY

Rationale: Age-related macular degeneration (AMD) is a common eye condition that can cause blindness among people 50 and older. AMD causes damage to the back of the eye, in an area called the macula, which is needed for everyday task such as reading, driving, cooking meals etc. The vast majority (90%) of people with AMD have the 'dry' form. Dry AMD advances so slowly that blindness does not occur for years; but in the late stage (i.e.,"Geographic Atrophy" (GA)) blindness does occur. In 2023, two new drugs, "Syfovre," & "Zimura" were FDA-approved for patients with GA. These drugs target and inhibit the complement cascade (part of the body's immune response). While these drugs show promise, they do not stop other factors that cause dry AMD, such as the retinal pigment epithelial cell (RPE) to die.

Proposed Multidisciplinary Research Approach: A key feature of dry AMD is the degeneration, or atrophy, of the RPE cell. We will study the cellular & molecular changes in the RPE cell as it degenerates in two models: 1) cell culture model of RPE and 2) mouse model of dry AMD. We will pair this cell & molecular approach with a bioengineering approach to image the RPE cell in culture and the live, anesthetized mouse. High-resolution imaging of the RPE with custom optical coherence tomography-based equipment designed for mouse and RPE cells in cell culture are active areas of our research programs. A key factor that causes the RPE to degenerate is the loss of the cell-cell contacts between neighbouring RPE cells. Our earlier work showed that the cell-cell contacts degrade due to overactive enzymes that accumulate with aging and exposure to blue light (e.g., blue light from sunlight and electronic devices). We will study the effects of the overactive enzyme (Granzyme B) and blue light-induced RPE atrophy, and whether inhibitors of the over-active enzymes or filters that block blue light, can slow the loss of the cell-cell contacts, and thereby rescue the RPE from dying. This multidisciplinary approach is valuable for future translational studies on AMD patients, as the methods for in vivo ophthalmic imaging developed in mice will be translated to the clinic to allow physicians to track key changes in the outer retina in dry AMD patients. The in vivo imaging devices will provide an important quantification tool to assess whether future drugs developed for AMD can slow the degeneration or atrophy of the RPE cell. The RPE images acquired from in vivo imaging and cell culture microscopy will provide a dataset for developing deep neural networks to discern key image features that will become the "signature" that represents RPE atrophy associated with AMD.

Expected Outcomes: The expected outcomes of the proposed study include 1) data collection of cellular and molecular changes that characterize RPE atrophy and 2) development of hardware and software to support bioengineering methods of in vivo ophthalmic imaging first in mouse models and later for AMD patients.

BENEFIT TO THE STUDENTS

GAIN UNDERSTANDING OF CONDUCTING HIGH QUALITY RESEARCH- TS will learn how to conduct a high quality research project by several methods: TS will read selected, relevant scientific papers and discuss/critique experimental design with both supervisors and team members. TS will learn the ethical considerations of using animals in research and biosafety methods for cell culture. TS will take online

UBC biosafety and animal ethics courses. TS will learn basic statistics to identify the value of a power calculation to estimate sample size in animal studies, how to develop a testable hypothesis, and appropriate statistical tests for studies. TS will learn to problem-solve and trouble-shoot experimental methods and data collection as the situation requires. TS will zoom with at least one, most likely both supervisors and/or their team members, once every week to provide ample time to discuss and ask questions towards the research project.

DEVELOP NEW SKILLS- In this multidisciplinary project TS will develop skills in cellular/molecular methods as well as in optics, microscopy, and in-vivo retinal imaging. Cellular/molecular skills will include confocal microscopy, advanced image analysis, protein assays (immunohistochemistry, ELISA, western blots). Bioengineering/optics skills will include understanding the basics of optics of the eye and lens, optical coherence tomography, principles of confocal microscopy and super-resolution microscopy as needed.

INTERACTIONS WITH OTHER RESEARCHERS- TS will collaborate and work with both supervisors and their respective lab members, which will include postdoctoral fellows, graduate students, medical students, undergraduate students, research associates and technical staff. TS will attend weekly lab meetings to discuss and network with other lab members, participate in journal club presentations. TS will give progress reports on their data every two weeks.

AVAILABLE RESOURCES- TS will work in an established basic science laboratory fully equipped for proposed studies using protein assays, tissue culture, paraffin histology, bright and dark-field microscopes, confocal and super-resolution microscopes. TS will work with team members in the in vivo imaging lab situated in the Jack Bell animal facility, equipped with both commercial ophthalmic imaging and custom-designed imaging devices for mice. TS will have opportunities to present their research results at departmental research days, and in virtual or in person scientific meetings held in Canada and USA.

TIMELINE- In order to meet the 16 week timeline, TS will work together and obtain assistance when needed from the lab members of the two faculty supervisors. In Months 1 and 2, TS will work together to complete protein assays (WB and Immunofluorescence) using previously collected eye tissue samples. In Month 3, TS will continue assays and begin to learn and analyze in vivo retinal images of mouse models obtained from Micron IV and custom hardware systems. In Month 4 TS will correlate data from 1) protein assays and confocal images of RPE cells and 2) in vivo images towards defining the in vivo "signature" of RPE degeneration and blood-eye barrier dysfunction.