University of Victoria | Graduate Studies

Notice of the Final Oral Examination
for the Degree of Master of Science

of

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BSc Hons (University of Essex, 2012)

"miRNA-7 Inhibition Restores *Pax6* Levels in Murine Haploinsufficient Islets"

Department of Biochemistry and Microbiology

Monday, December 5, 2016
2:00 P.M.
Harry Hickman Building
Room 120

Supervisory Committee:
Dr. Perry Howard, Department of Biochemistry and Microbiology, University of Victoria (Supervisor)
Dr. Caren Helbing, Department of Biochemistry and Microbiology, UVic (Member)
Dr. Bob Chow, Department of Biology, UVic (Outside Member)

External Examiner:
Dr. Craig Brown, Division of Medical Sciences, University of Victoria

Chair of Oral Examination:
Dr. Vera Pospelova, School of Earth and Ocean Sciences, UVic

Dr. David Capson, Dean, Faculty of Graduate Studies

Abstract

Aniridia is a rare genetic disorder that affects the development of the eye and is caused in most cases by mutations in the *PAX6* gene. Patients with a heterozygous mutation in their *PAX6* gene are born without irises. Aniridia patients are also prone to other eye diseases over their lifetimes such as cataracts and glaucoma. Aniridia's progressive nature suggests that therapeutic intervention aimed at restoring *PAX6* expression may be effective at ameliorating the progression of this disease.

PAX6 is necessary for the development and maintenance not only of the eye, but also the pancreas. Patients with aniridia have an increased likelihood of developing glucose intolerance and diabetes. Indeed, genetic studies in rodents have confirmed that haploinsufficient animals for *Pax6* develop glucose intolerance due to an ongoing requirement for *Pax6* expression in the pancreas and gut.

This thesis is a proof-of-concept study designed to determine the effects of repressing miRNA regulation of murine *Pax6*. *Pax6* is regulated by miRNA-7 and miRNA-375. I hypothesized that repression of miRNA-7 and miRNA-375 would restore *Pax6* expression and that this strategy might be useful in treating some of the progressive symptoms that emerge in aniridia patients in adulthood. As a first step towards evaluating miRNA inhibition as a therapeutic strategy for the treatment of aniridia, my first objective was to confirm whether miRNA-7 and miRNA-375 regulate *Pax6* expression in pancreatic cells and tissue. My second objective was to determine whether these miRNAs could be efficiently inhibited. My third objective was to determine whether repression of miRNA-7 or miRNA-375 alters endogenous PAX6 protein levels in pancreatic cell lines. My final objective was to determine whether target protectors, delivered to explants of pancreatic islets through an adeno-associated virus (AAV) vector, could be used to restore *Pax6* expression in murine haploinsufficient islets. From this study, I have confirmed that miRNA-7 and miRNA-375 regulate *Pax6* in pancreatic cells, and that these miRNAs can be specifically inhibited, and that inhibition leads to an increase in *Pax6* on both the reporter and protein levels. I have shown that target protectors against the miRNA-7 and miRNA-375 binding sites within the *Pax6* 3'UTR are effective at increasing the levels of PAX6 protein in pancreatic cell lines. Finally, I have also shown that a target protector against the miRNA-7 binding site can increase PAX6 protein levels in islets from murine haploinsufficient islets to near wild-type levels. My thesis lays the groundwork for the development of anti-miRNA-based therapies aimed at restoring *PAX6* expression in the eye and pancreas.