University of Victoria | Graduate Studies

Notice of the Final Oral Examination
for the Degree of Doctor of Philosophy

of

GILLIAN LEIGH DORNAN

MSc (University of Leicester, 2011)
BSc Honours (University of Leicester, 2009)

"Defining the Molecular Mechanisms Mediating Class IA Phosphoinositide 3-kinase (PI3K) Regulation and their Role in Human Disease"

Department of Biochemistry and Microbiology

Thursday, June 6, 2019
12:00 P.M.
Clearihue Building
Room B007

Supervisory Committee:
Dr. John Burke, Department of Biochemistry and Microbiology, University of Victoria (Supervisor)
Dr. Martin Boulanger, Department of Biochemistry and Microbiology, UVic (Member)
Dr. Perry Howard, Department of Biochemistry and Microbiology, UVic (Member)
Dr. Leigh Anne Swayne, Division of Medical Sciences, UVic (Outside Member)

External Examiner:
Dr. Raymond Blind, Department of Medicine, Vanderbilt University

Chair of Oral Examination:
Dr. Sara Ellison, Department of Physics and Astronomy, UVic

Dr. David Capson, Dean, Faculty of Graduate Studies

Abstract

The phosphoinositide species phosphatidylinositol 3,4,5, trisphosphate (PIP_3) is an essential mediator of many vital cellular processes involved in cell growth, survival, and metabolism. The class I PI3Ks are responsible for production of PIP_3, and their activity is tightly controlled through interactions with regulatory proteins and activating stimuli. The class IA PI3Ks are composed of three distinct p110 catalytic subunits (p110α, p110β, p110δ) and they play different roles in specific tissues due to disparities in both expression and engagement downstream of cell surface receptors. Disruption of PI3K regulation is a frequent driver of numerous human diseases. Growth of all cell types is dependent on PI3K signalling, and development of immune cells relies on a precise balance of PIP_3 production. Activating mutations in the genes encoding the catalytic and regulatory subunits of PI3K lead to cancer and immunodeficiencies. The *PIK3CA* gene encoding the p110α catalytic subunit of class IA PI3K is one of the most frequently mutated genes in cancer, and mutations in the *PIK3CD* gene encoding the p110δ catalytic subunit lead to primary immunodeficiency. All class IA p110 subunits interact with p85 regulatory subunits, and mutations/deletions in different p85 regulatory subunits (PIK3R1, PIK3R2, PIK3R3) have been identified in both cancer and primary immunodeficiencies. By asking how these mutations mediate activation and disease phenotypes, we can identify the natural regulatory molecular mechanisms of class IA PI3Ks. Fundamentally understanding how mutations in PI3K subunits mediate human disease will expand our knowledge of PI3K biology and is essential to the development of novel therapeutics.

To identify the molecular mechanisms of class IA PI3K activating mutations, I employed a sophisticated combination of hydrogen-deuterium eXchange mass spectrometry (HDX-MS) with biochemical activity assays to probe the regulatory mechanisms of PI3Ks. HDX-MS measures the exchange rate of amide hydrogens in solution, which in turn can provide information on protein conformation and conformational changes between different states. By comparing PI3K mutants identified in primary immunodeficiency and cancer patients to wild-type enzymes, I have identified dynamic, conformational changes induced by activating mutations. Biochemical and biophysical analysis of these mutants led us to generate a panel of engineered mutations to further characterise molecular mechanisms by which class IA PI3Ks are regulated. This thesis will consist of an introduction to class IA PI3K signalling and

an introduction to the method of HDX-MS, followed by two data chapters wherein I investigate the mechanisms of activating mutations in PIK3CD followed by an investigation into activating mutations in PIK3R1. A conclusion and discussion of future directions will be presented in the final chapter. This work provides novel insight into the complex regulatory mechanisms of the class IA PI3Ks, which may lead to better understanding of human diseases that activate these enzymes.