Phospoinositides and Lipid Kinases in Oxidative Stress Signalling and Cancer
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Summary

Phosphoinositides are implicated in virtually all aspects of cellular welfare. They help to maintain the structure of biomembranes, but are also essential signal transduction molecules. This is illustrated by PtdIns(4,5)P_2, a versatile and important phosphoinositide that regulates many cellular processes including cell survival, calcium homeostasis, vesicular trafficking, cytokinesis, stress responses and cell adhesion. Cells use various enzymes to regulate PtdIns(4,5)P_2 levels, including lipid kinases, lipid phosphatases and lipases. PtdIns(4,5)P_2 can be synthesized by two closely related families of lipid kinases. Phosphatidylinositol-4-phosphate (PtdIns4*P*) 5-kinase (PIP5K) phosphorylates PtdIns4*P* and phosphatidylinositol-5-phosphate (PtdIns5*P*) 4-kinase (PIP4K) phosphorylates PtdIns5*P* to make PtdIns(4,5)P_2. Cellular PtdIns4*P* is approximately ten times more abundant than PtdIns5*P* and therefore PtdIns(4,5)P_2 is likely generated by PIP5K mainly. *In vivo* studies in animals and in mammalian cells suggest that PIP4Ks primarily regulate the level of PtdIns5*P*, although they may also regulate a specific pool of PtdIns(4,5)P_2. PtdIns5*P* is the latest addition to the 'palet' of phosphoinositides and it is the least understood one.

In this thesis, we have examined biochemical and functional aspects of PtdIns5*P* and the PIP4Ks that determine its levels in the cell. We have investigated how PtdIns5*P* levels change in response to redox signaling, or in response to the activity of the isoforms PIP4K2A and PIP4K2B and we have evaluated PIP4K2B expression in breast tumours.

In **Chapter 1,** we introduce the phosphoinositides PtdIns5*P* and PIP4Ks as a preface to the rest of the thesis. In **Chapter 2,** we review oxidative stress signaling and modulation of PtdIns5*P* and the prolyl-isomerase Pin1. In **Chapter 3,** we implicate Pin1 in regulating PtdIns5*P* levels since mouse embryo fibroblasts from Pin1 knock-out mice had increased amounts of PtdIns5*P.* Upon exposure to oxidative stress, cells were protected by an increase in PtdIns5*P* levels and more prone to die upon reduction of PtdIns5*P* levels. A reduction in PtdIns5*P* signaling led to increased accumulation of cellular Reactive Oxygen Species, coinciding with a down regulation of genes that manage ROS. These data imply that a ROS induced increase in PtdIns5*P* can lead to changes in gene expression that enable cells to adapt to environmental stimuli.

Previous studies have suggested that proteins interacting with lipid kinases are often regulated by the same lipid. We observed that Pin1 interacted with a number of inositide kinases. In **Chapter 4**, we therefore evaluated binding of Pin1 to phosphoinositides using Surface Plasmon Resonance (SPR). We show that Pin1 interacts with phosphoinositides, showing the strongest association with PtdIns(4,5)P_2. The interaction requires the WW domain of Pin1 which mediates its interaction with phosphorylated proteins. Modulation of the abundance of PtdIns(4,5)P_2 by overexpressing PIP5K decreased Pin1-dependent transcriptional activity from the promoters of CDKN1B and CCND1. Our data suggest that PtdIns(4,5)P_2 is a negative regulator of Pin1. In **Chapter 6,** we used mass spectrometry to identify PIP4K2A as a PIP4K2B interacting protein. We employed several biochemical techniques to investigate differential activities of PIP4K2A and 2B isoforms. We found that PIP4K2A is 2000 times more active than PIP4K2B. Additionally, we showed that PIP4K2B can target PIP4K2A to the nucleus. Intracellular pairing of the two isoforms may be important for their physiological and pathological roles. In **Chapter 7,** we characterized a PIP4K2B-specific antibody for its use to interrogate tumour derived tissue samples. In a Tissue Micro Array,

containing samples from 489 advanced breast cancer tumours with associated clinical outcomes, we observed a correlation between PIP4K2B expression and several clinical-pathological parameters. We demonstrated that decreased expression of PIP4K2B correlated with worse prognosis for breast cancer patients. Using breast tumour (MCF7) and normal (MCF10A) breast epithelial cell lines, we discovered that silencing of PIP4K2B expression decreased transcription and expression of the cell adhesion protein E-cadherin.

Silencing of PIP4K2B in MCF10A cells primed them to undergo an epithelial to mesenchymal transition (EMT) when stimulated with TGFβ. Analysis of expression data sets from three thousand breast tumour samples (Curtis breast array analysed using Oncomine) confirmed a correlation between low E-cadherin levels and low PIP4K2B levels. Highly aggressive forms of breast tumours have decreased E-cadherin expression and the regulation of E-cadherin expression by PIP4K2B may in part explain why patients with low expression levels of PIP4K2B in their breast tumor cells have a poor prognosis.