While primary prostate tumours are usually successfully treated, advanced prostate cancer patients are limited in treatment options often resulting in mortalities. Hereby, efforts to investigate biomarkers or therapeutic targets is required to reduce the number of prostate cancer deaths. Caveolin-1 is a common marker in aggressive prostate cancer where its overexpression has been linked to increased proliferation, migration and differentiation. However, these phenotypes can be truncated by ectopic expression of caveolin binding partner and putative tumour suppressor, cavin-1, to these cells. Previous studies from our lab assessed the role of extracellular vesicles (EVs) in prostate cancer by utilising this caveolin-1/cavin-1 switch. This revealed that cavin-1 truncates the export of oncomiR miR-148a via EVs without corresponding cellular expression changes, indicating a novel microRNA specific export mechanism that is modulated by cavin-1.

This project investigated the proposed microRNA export mechanism and miR targets by combining bioinformatics and wet lab techniques. Specifically, analysis of RNA-seq data revealed a subset of microRNAs, including miR-148a, are selectively exported from PC3 cell lines that are truncated by cavin-1. Additionally, motif discovery showed similarities in miR sequences which may explain selectivity of these targets for export. Heterogeneous nuclear ribonuclear protein K (hnRNPK) was determined to be a viable candidate in the export mechanism as MS/MS analysis revealed differential export of this protein upon cavin-1 expression. Furthermore, motif scanning indicated that hnRNPK binds similar miR regions to the shared motif thus establishing potential interactions. Immunofluorescence and microRNA *in situ* hybridization confirmed that hnRNPK is compartmentalised with miR-148a in multivesicular bodies in pro-tumourigenic PC3-GFP cell lines that is not occurring in PC3-cavin-1 cells. hnRNPK in PC3-cavin-1 cells localise to endoplasmic reticulum, which suggests a change in subcellular localisation may contribute to differential export of hnRNPK and its binding partners. While immunoprecipitation confirmed that hnRNPK binds RNA, identifying specific miR species requires further work.

Ultimately, these results suggests that many microRNAs are being modified by hnRNPK differential export in PC3, where expression of cavin-1 reduces hnRNPK and miR export. However, the direct interaction between hnRNPK and miRs is yet to be seen and how cavin-1 mediates the export of hnRNPK is unknown. While a previous study determined that hnRNPA2B1 mediates EV export of miRs in T-lymphocytes, this is the first study to identify a link between secreted hnRNPK and microRNA export in cancer.

While localised prostate tumours are almost always successfully treated, treatment options for late stage metastatic prostate tumours are limited. Therapeutic targets specific for advanced prostate cancer are therefore required to decrease the number of prostate cancer related deaths. Cavin protein family members, cavin-1, cavin-2 and cavin-3, have been implicated as tumour suppressors in prostate cancer. Along with caveolin-1 (CAV1), cavin-1 expression is required to form caveolae, a cholesterol rich membrane microdomain (lipid raft). Furthermore, cavins -2 and -3 are thought to stabilise caveolae in a cavin-1 dependent manner. Intriguingly, caveolin-1 is a known prostate tumour promoter and is associated with advanced, metastatic prostate cancer. Introduction of cavin-1 expression into CAV1-positive prostate cancer cells suppresses tumourigenicity, partly by a reduction in migration. Additionally, cavin-1 expression recruits protein kinase C alpha (PKCα), a known regulator of cell migration, to cholesterol rich membrane fractions known as lipid rafts. Cavin-2 and cavin-3 have also been identified as binding partners of PKCα and PKCδ respectively.

Therefore, the current project aimed to connect the potential link between cavin-mediated PKCα recruitment to lipid rafts and prostate cancer cell migration. To do this, cavin family members were expressed individually and in combination in the aggressive, CAV1 positive prostate cancer model, PC3 cells. Using functional assays it was shown that migration is reduced upon expression of cavins -1, -2 and -3 compared to control cells. Furthermore, adhesion of cavin-1 cells to fibronectin and collagen was increased, consistent with an increase in integrin α6β4 phosphorylation in the lipid rafts based on analysis of unpublished proteomics results. Bioinformatics analysis of potential protein protein interaction networks showed a potential role for other proteins involved in linking the cytoskeleton to the membrane and extracellular matrix. Due to its connection with cavins, the localisation of PKCα was investigated in each cell line. Using a proximity ligation assay, co-localisation between PKCα and CAV1 only in cavin-1 expressing PC3 cells was demonstrated. It was previously established that cavin-1 expression induced PKCα recruitment to lipid rafts but this is the first study to demonstrate that this recruitment is caveolae specific. The same analysis of PKCδ, which binds to cavin-3, showed the first reported co-localisation between PKCδ and CAV1. This co-localisation occurred in all cell lines and was therefore independent of cavin expression.

Together, these results suggest that PKCα co-localisation with CAV1 may play a part in adhesion and migration in cavin-1 expressing cells. Furthermore, a role for cavin-2 and cavin-3 in reducing migration of PC3 cells was established in further support of a role in prostate tumour suppression.