Prostate cancer is currently the second highest diagnosed cancer in men worldwide. While the primary tumour is able to be removed and treated effectively, progression into advanced metastatic forms reduces the 5-year survival rate to less than 30%. This indicates the need to identify or understand biological phenomena and biomarkers that mediate the phenotype for treatments and diagnostic reasons.

An important biomarker in cancer progression is caveolin-1. In healthy human cells, this is usually co-localised and co-expressed with tumour suppressor, cavin-1. However, in many cancer types, caveolin-1 is expressed without cavin-1, which has been attributed to most of the hallmarks of cancer progression. Yet, adding cavin-1 to a cell line that contains this activity, such as the advanced prostate cancer cell line PC3, is able to reduce the metastatic phenotype. This establishes a system that can be used to assess and understand prostate cancer processes.

This system has been utilized by our lab recently to assess the role of extracellular vesicles in prostate cancer. Cancer-derived EVs are particularly interesting as they have been implemented in modifying the tumour microenvironment and establishing a pre-metastatic niche by transferring cytoplasmic material from the host tumour cell to a distant recipient. Primarily this is due believed to be due to the protein content of the EVs. While the introduction of cavin-1 did modulate protein content, the more interesting finding was the change in microrna content.

Now microRNAs in EVs is also an interesting new process that has been linked to cancer. MicroRNAs a small non-coding RNAs that regulate post transcriptional gene silencing to mediate cellular activity where secretion and absorption of these microRNAs can also mediate activity. Here we found that oncogenic mir-148a was secreted, where this secretion was reduced in cavin-1 positive cells lines. Mir-148a was found to induce osteoclastogensis so its secretion is believed to be a contributor to bone metastasis. This miR was differentially secreted but did not modify the cellular content which indicates an export mechanism to modify the content in the EVs, independent of the cellular concentration.

So what we believe to be happening, is that there is an RNA-binding protein mediating the export of mir-148a to EVs, similar to an export mechanism for nuclear export or the escrt protein complex for EV protein export, where this export is reduced upon expression of cavin-1. Ive set out three aims to address this export mechanism; investigate the micrornas that are being manipulate by this system, identify proteins that could be involved in this system, and lastly, look at their interaction and activity to get a better understanding of the mechanism itself.

First, we’ve attempted to use computational techniques to determine which microRNAs are being modified in the EVs, and whether this modification can be described by cellular changes or export mechanism. This was completed by RNA-seq analysis and subsequent differential expression analysis. Three replicates were taken from PC3 and PC3-cavin-1 cell lines and corresponding EVs to determine microRNA content by illumina sequencing for small RNAs. I received the raw counts for each microrna species, in PC3 EV and cells and pc3cavin1 evs and cells. DESeq2 is an R package that analyses RNA-seq data for differential expression. Here we used it to observe the difference in EV content between cell lines, and then a separate analysis to determine difference in cell expression upon cavin-1 expression. ……

From this analysis, we found 95 micrornas modulated by cavin-1 expression, where 12 of these were significantly modified in the EVs. However, as described before, EV modification can occur due to cellular changes or through selective export mechanism. Thereby cellular expression changed upon cavin-1 expression was also completed. This revealed that certain micrornas are indeed being manipulated by the export mechanism, including the aforementioned mir-148a, whereas some are a product of sampling.