Script.

So prostate cancer currently rates as the second most diagnosed cancer in men worldwide. While the primary tumour is fairly innocuous, patients with the more advanced prostate cancer face limited treatment options and high mortality rates due to additional comorbidities. For these reasons we need understand and identify biological phenomena and biomarkers that induce the metastatic phenotype to hopefully lower mortalities. (23s)

Caveolin-1 is an important and commonly researched biomarker for cancer progression. In healthy human cells, caveolin-1 is co-expressed and co-localized with putative tumour suppressor, cavin-1, to evoke their canonical function. However, in the case of many cancer types, caveolin-1 is overexpression without corresponding binding partner, which has been linked to many of the hall marks of cancer and cancer progression, though the mechanisms that link caveolin to cancer are still being investigated. Interestingly, knocking down caveolin-1 or adding cavin-1 to a cancer cell line that exerts this activity reduces the oncogenic phenotype induced by the caveolin. (34s)

We’ve used this information previously on the advanced prostate cancer cell line, PC3. This cell line lacks cavin-1 expression, but over expression on caveolin is thought to contribute to many of the aggressive processes. When transfected with cavin-1, our lab determined that this transforms the usually aggressive PC3 cell line to a more placid form by nutralising caveolin-1. BY using this model, we can compare between the aggressive prostate cancer cells and less aggressive cell lines to identify the pathways that caveolin-1 is involved with but also the processes that lead to the increase mortalities associated with advanced prostate cancer. (40s)

Using this model, we focused on the role of extracellular vesicles in these cancer. These vesicles transfer material from the host cell to the recipient cell, where this content can then evoke their canonical function. This is an import mode of intracellular communication that has been of recent focus in cancer research. Here, the EVs contain content from the host tumour cell that initiate modifications of the microenvironment and establishment of pre-metastatic niche when absorbed into the recipient. Hereby, indicating a role in cancer progression. Primarily this was believed to be due to proteomic content, however our work found that the expression of cavin-1 modulates EV specific concentrations of microRNAs as well as protein. () \*\*Fix up the diagram.

Recently, vesicle contained microRNAs had been implemented in cancer progression. Alike protein, the EV transported microRNAs have been reported to modulate microenvironments and pre-metastatic niche formation, but through their canonical function. MicroRNAs are small non-coding RNAs that mediate post-transcriptional gene silencing through complementary base pairing to target protein transcripts or messenger RNA and recruiting the RNA-induced silencing complex. This is particularly important when you consider that a single microRNA can target hundreds of protein transcripts and therefore modulates many vital cellular pathways. Hereby, being transported between cells could be viable from of intercellular communication, or a major source of disarray in disease states. Previous work using the PC3-cavin-1 cell model found that the prostate cancer cells did secrete oncogenic material, namely mir-148a, whereas the less tumorigenic cell did not. Interesting, this modification was not reflected by a change in cellular expression. This indicates that there is some form of export mechanism that populates the microRNA content of the EVs in prostate cancer.

So, this project basically set out to understand this mechanism. We hypothesize that cavin-1 is attenuating the export of an RNA-binding protein which modulates the miRNA content. This was investigated by looking at the microRNA manipulated in the system, identification of candidates and further though investigation of their activity.

Firstly, we wanted to take a comprehensive look into all of the microRNAs that are manipulated by the proposed export mechanism where previously only a select few microRNAs were investigated. To do this, we wanted to observe microRNAs that change EV levels between cell lines, where this change is not proportional to the cellular change, similar to what was observed for the miR-148a data. In contrast, the null hypothesis for this system would suggest that the cavin-1 cell lines only modify the overall expression of certain microRNAs, thus causing the changes in EV population. To do this, I collected RNA-seq data of small RNAs in the EVs for cavin-1 and GFP cell lines, and the cells that those EVs originated from and analysed via DESEq2 R package.

This analysis compared the miRNAs found in the EVs between the cell lines. In total, there were 95 microRNAs detected in the EVs, where 12 of these were significantly modified between cell lines, as shown in this graph as the black bars. As discussed before, this change can be from selective export or a change in cellular expression, so we compared this to the cellular changes, shown in grey. What we observe here is a mix of microRNAs undergoing selective export including miR-148a as consistent with past research, shown by large EV changes and little cellular changes, and sampling. This ultimately demonstrates that the selective export of microRNAs can cause quite dramatic changes for some microRNAs however some sampling will occur to populate the EVs. Recorded- 6minutes.

Some of those discovered microRNAs were later validated by RT-qPCR. This used a standard pipeline. Here I used delta delta ct to determine changes in microRNA levels between the EVs and cells. While this did confirm the trend first identified from the RNA-seq data, the detection of microRNAs in the EVs were quite variable. Unfortunately, this occurs due to the limited consistency in extraction, conversion and detection used by this pipeline. In the future, we plan on recompleting this analysis using more specific detection techniques, such as digital droplet PCR which has been noted as a better detection method for low abundance microRNAs to hopefully improve resolution.

Next, we attempted to look for candidate RNA-binding proteins that could be mediating the selective export. RNA-binding proteins are known to associate to their targets based on conserved RNA-binding sequences of the target RNAs. For that reason, I first analysed the selectively exported microRNAs to identify any similarities that are occurring between the sequences. Unfortunately, the 5 identified selectively exported microRNAs from the previous analysis would not return a significant motif, merely for the fact that the sample number was to0 low. So for this particular reason we had to extend the data set. I did this by reanalysing all of the detected microRNAs in the EVs, and comparing their EV change to the cellular change to result in a single metric to describe relative changes. When we observe this in the form of a frequency distribution graph, we can determine the prevalence of each from of export. Here, we two populations, sampling which approximated 0 and a small population of miRNAs that change disproportionally to their cellular change and are thereby considered the selective microRNA. Using this analysis, we’ve now defined 19microRNas considered to be under the action of the selectively export mechanism.

Next, I analysed these 19miRNAs to identify motifs by using the MEME algorithm. This looks for sequences that are similar across all of the inputted microRNAs. Theoretically, for the RNA-binding protein to be a reliable export protein, it would need to be specific to the selectively exported microRNAs and not be able to bind to microRNAs in the sampling group. For this reason, I analysed microRNAs in the selective export group to identify shared regions and then matched the resulting motif to all microRNAs expressed to determine to determine selectivity and specify. This analysis returned two enriched motifs, displayed here, which only matched to the microRNAs found in the selectively exported group. Hereby identifying potential binding motifs that could be mediating the selective export of these microRNAs. While this is an excellent prediction for our further analysis, further analysis is required to validate this motif.

Subsequently, we looked for a protein that is likely to be mediating this activity. For the protein to adhere to the hypothesis, it would need to be differentially exported due to cavin-1 expression, bind to RNAs and be able to bind to the selectively exported microRNAs. To identify these proteins, I re-analysed previously published mass spectrometry data that analysed the change in EV proteomic content between these cell lines. Using gene ontology analysis, I then analysed that differentially exported proteins to determine if any of these are known to possess RNA-binding abilities. This resulted in these 5 proteins as viable candidates. Further assessment into the binding interaction of these proteins limited the candidate selection further.

One of these candidates, hnRNPK binding sites were found to match significantly to the known selectively exported microRNA motif, previously determined. hnRNPK was found to strongly associate to a specific sequence in micro-122 as found by mutagenesis, which just so happens to match to the motif using the FIMO motif scanning algorithm. This establishes that hnRNPK may be the export protein to populate the EVs in this cell line.