Good morning everyone. This project will hopefully reveal mechanisms in fundamental cellular biology, particularly that relating to miRNAs.

These microRNAs are small non-coding RNAs that mediate cellular activity by gene regulation. Traditionally, this gene regulation occurs by complementary binding to protein transcripts, where through recruitment of RNA induced silencing complex, argonates and dicer proteins degrade the transcript. Thus limiting that proteins activity and potentially impacting on entire pathways. Hereby, tight spatial and temporal regulation is required to prevent dysregulation of vital cellular processes. Recently, an exciting finding had shown that miRNAs are able to be exported from the host cell, via extracellular vesicles, and integrated into recipient cells to perform this regulatory function.

These extracellular vesicles are composed of microvesicles and exosomes, which encompass various protein, RNAs and DNA. Typically the extracellular space is rife with RNase activity that would rapidly degrade miRNAs, however, packaging into extracellular vesicles increases its stability. This allows the miRNAs to move between cells. Hereby detailing a novel and recently discovered mechanism for intracellular communication.

However, the mechanisms surrounding the miRNA sorting into the extracellular vesicles is still mostly unknown. Originally, miRNAs were previously considered to be packaged into vesicles non-selectively, where the miRNAs contained in EVs were merely representative of their cellular concentration. Yet now there is a stream of evidence emerging that reveals that certain miRNAs can be over or underrepresented in extracellular vesicles given a change in condition. Hereby indicating a selective export mechanism for some the miRNAs into EVs. In attempts to elucidate the mechanism, a single protein was found. This was found to bind a subset of 30 miRNAs to mediate the subcellular localisation and exosomal export. However, how this is regulated is still unknown.

Fortunately, recent studies had revealed that lipid rafts may regulate cargo export. Lipid rafts are small microdomains of membrane enriched in specific lipids and proteins that act as signalling hubs. In particular, cholesterol, sphingolipid and ceramide enriched lipid rafts are known to be present on the surface of extracellular vesicles. In order to investigate their function in extracellular vesicles a series of deletion studies were completed. Individually depleting ceramide, cholesterol and sphingolipid had a dramatic effect on protein sorting into extracellular vesicles.

Furthermore, an experimental system utilised by our lab had further verified the role of rafts in sorting for both protein and microRNAs. This system uses an Advanced Prostate cancer line, PC3, which exhibits abnormal caveolin-1 expression without its usual functional partners, Cavins. Together, these produce a specialised lipid microdomain called caveolae. However, caveolin-1 on its own possesses cholesterol transporting roles. Interestingly, caveolin-1 is particularly enriched upon the surface of Extracellular vesicles.

By introducing cavin-1 to PC3 cells, we attempt to investigate the role of the caveolin-1 on extracellular vesicles and lipid rafts. Here, they found a decrease in lipid raft specific cholesterol, modulation of a third of lipid raft and vesicle contained proteins, and most interestingly, miRNAs. Hereby verifying that lipid raft composition are having an effect on protein and miRNA cargo sorting into EVs, and establishes a system to assess this occurrence.

Hereby, we hypothesise that miRNA content is mediated RNA-binding proteins that are modified by lipid raft composition changes. This is will be tested by completing these three aims on the aforementioned Cavin-1 PC3 experimental system. I will attempt to identify the full list of selectively exported miRNAs from this system, find candidate RNA-binding proteins and lastly confirm binding ability and export function of the candidate *in situ.*

So first, I’d attempt to determine the full repertoire of miRNAs selectively exported from this system. This is will use both bioinformatics and experimental approaches. RNA-seq data was compiled for PC3 GFP cell lines and PC3 GFP-tagged cavin-1 cell lines for both the cellular and vesicle content. Using R packages, DESeq2 and EdgeR, I can compile a list of miRNAs that are regulated by a change in lipid raft composition mediated by cavin-1 introduction. Further comparing between cell and extracellular vesicle will reveal which of those miRNAs are selectively exported in this system. Following this, RT-qPCR will be completed to verify this list of selectively exported miRNAs. Ultimately, this should produce a combination of selectively and non-selectively exported miRNAs from this system, where the non-selective miRNAs can be used as negative controls.

Subsequently, we’ll attempt to identify potential RNA-binding proteins that could be regulating the selective export of those particular miRNAs. It is expected that this protein will be modified by the rafts and extracellular vesicles upon cavin-1 expression. Furthermore, performing Gene Ontology analysis will reveal proteins with RNA-binding capacity. Proteins that fulfil this criteria will be considered the candidate miRNA escort proteins.

However, even if they possess binding ability, we need to know if it’s likely that they’ll bind the selectively exported miRNAs. Most known RNA-binding proteins will have some information regarding the region of RNA they bind. Usually this is expressed as a position weight matrix or sequence logo, as shown. Comparing this information back to the selectively exported miRNA sequences should produce a match, whereas no matches to the non-selective miRNAs. Hereby, this aim should reveal several candidates modified by lipid rafts, integrated into the extracellular vesicles known to bind the miRNAs selectively exported from this system.

Aim 3 will verify the binding capacity and interaction between the found selectively exported miRNAs and candidate miRNA escort proteins utilising two experimental methods. Firstly a pull down of RNA-binding protein will confirm binding ability. This will use the streptavidin/ biotin system as shown. Western blot using the candidate specific antibody should hopefully confirm the miRNA binding ability. Performing the same methods on non-selectively exported miRNAs will not pull down this same candidate.

Secondly, co-localisation will confirm their interaction in situ. Here, we’d be using PC3 cells transfected with biotinylated miRNAs. Incubation with the GFP tagged streptavidin will fluoresce miRNAs green, and the use of the candidate specific antibody and secondary antibody will highlight the RNA-binding protein Red. Therefore, their overlap visualised by a change in colour though confocal microscopy will determine co-localisation and interaction.

Ultimately, elucidating the selective export mechanism for miRNAs in this system which hadn’t previously been investigated before. As miRNAs can regulate almost every pathway, their regulation is of utmost importance. Selecting for the miRNAs to be shuttled between cells indicates a method of control over intercellular communication of this system. Some elements exported via extracellular vesicles had been linked with cancer metastasis. For instance, miR-148a exported from PC3 cells had been implemented in increased proliferation of bone when integrated into recipient cells, mediated by the selective export mechanism. Hereby, understanding this the export mechanism of miRNAs can reveal its impact on intercellular communication and diseases related to its dysregulation.

Thank you for listening.