Results:

Expression analysis of miRNAs:

To understand the selective export mechanism of microRNAs, understanding which miRNAs are actually affected was required. By using previously complied RNA-seq data for small RNAs which measured miRNAs found in the cellular and extracellular vesicle content of the GFP-PC3 and the cavin-1::GFP-PC3 cell line, I can determine which of these miRNAs are in fact mediated by a change in lipid raft composition. The fold change between miRNA counts in the GFP and the cavin-1 cell lines was found for both cellular contents and the extracellular vesicles derived from these cells. By taking the difference between the fold change in the cell from the fold change in the extracellular vesicle, we can sort the miRNAs into three defined groups; miRNAs differentially down regulated upon lipid raft compositional change, miRNAs exported by chance due to proportional increase/decrease of the cell and exosome counts, and miRNAs differentially up regulated in the exosomes due to lipid raft composition.

|  |  |  |  |
| --- | --- | --- | --- |
| **Differential Up** | **Non-Diff** | | **Differential Down** |
| hsa-miR-140-3p | hsa-miR-222-3p | hsa-miR-181a-2-3p | hsa-miR-10b-5p \* |
| hsa-miR-6087 | hsa-miR-769-5p | hsa-miR-362-5p | hsa-miR-32-5p |
| hsa-miR-185-5p | hsa-let-7g-5p | hsa-miR-532-5p | hsa-miR-147b |
| hsa-miR-31-5p | hsa-miR-28-3p | hsa-miR-582-3p ψ | hsa-miR-186-5p |
| hsa-miR-877-5p | hsa-miR-15b-5p | hsa-miR-183-5p | hsa-miR-98-5p \* |
| hsa-let-7e-5p | hsa-let-7d-5p | hsa-miR-221-3p ψ | hsa-miR-148b-3p |
| hsa-miR-339-5p | hsa-miR-26b-5p | hsa-miR-25-3p | hsa-miR-3615 |
| hsa-miR-574-3p \*ψ | hsa-miR-182-5p | hsa-miR-125a-5p | hsa-miR-151a-3p |
| hsa-miR-205-5p | hsa-miR-93-5p | hsa-miR-629-5p | hsa-miR-19a-3p \*ψ |
| hsa-miR-361-5p | hsa-miR-92b-3p | hsa-miR-503-5p | hsa-miR-10a-3p |
| hsa-miR-27a-5p | hsa-miR-671-5p | hsa-miR-125b-2-3p | hsa-miR-22-3p |
| hsa-miR-1269a | hsa-miR-149-5p ψ | hsa-miR-148a-5p | hsa-miR-30e-5p |
| hsa-miR-4664-3p | hsa-miR-502-3p | hsa-miR-374a-3p | hsa-miR-30a-5p \* |
| hsa-miR-196b-5p | hsa-miR-1180 | hsa-miR-363-3p \*ψ | hsa-miR-20b-5p \*ψ |
| hsa-miR-106b-3p | hsa-let-7d-3p | hsa-miR-500a-3p | hsa-miR-16-2-3p \* |
| hsa-miR-484 | hsa-miR-191-5p | hsa-miR-99b-3p | hsa-miR-429 \* |
| hsa-miR-30e-3p | hsa-miR-589-5p | hsa-miR-542-3p | hsa-miR-148a-3p \* |
| hsa-let-7i-5p | hsa-miR-200b-3p | hsa-miR-450b-5p | hsa-miR-200a-3p \* |
| hsa-miR-30b-5p | hsa-miR-30a-3p | hsa-miR-1307-3p | hsa-miR-17-5p |
| hsa-miR-221-5p ψ | hsa-let-7b-5p | hsa-miR-146a-5p \*ψ | hsa-miR-125a-3p |
| hsa-miR-375 | hsa-miR-200a-5p | hsa-miR-340-5p |  |
|  | hsa-miR-27a-3p | hsa-miR-421 |  |
|  | hsa-miR-99a-5p |  |  |

\* indicates miRNAs with FCEXO p≤ 0.05 ψ indicates miRNAs with FCCELL p≤0.05

Proteomics data:

Previously, proteomics data (MS/MS) was compiled for the proteomic content of the total membrane, lipid rafts, extracellular vesicles, and total extracellular space for both the GFP and the cavin-1::GFP PC3 cell lines. The mean ratio was completed between GFP and cavin-1 cells to determine which proteins are modified upon lipid raft lipid composition, induced by cavin-1. Furthermore, a protein would be required to possess RNA-binding abilities to perform as a RNA-escort protein. By using Gene Ontology R package (BiomaRt), data was filtered for only proteins that can be a possible RNA escort protein by possessing RNA-binding domains. From this analysis, there were only 6 proteins found within the extracellular vesicles that possess RNA binding ability, where 5 of these are downregulated upon cavin-1 introduction. Within this data set, there is the FUS protein, that had been a previous point of interest, and hnRNPK, which is family members with a previously known RNA binding and potential miRNA escort protein. Furthermore, hnRNPK was found within the lipid raft fraction at a proportionate decrease, where FUS was not found at all. Lack of proteins in detected the fractions may occur due to low presence or due to crude extraction measures resulting in a higher threshold for detection. Other candidates either were incorrectly labelled at RNA binding, or only bind poly-(N) regions, by the ontology database or are not known to be found within lipid rafts, as per RaftProt.

Motif data:

Proteins will bind to certain miRNAs based on their sequence, where this sequence needs to be conserved to enable binding. Therefore, a set of RNAs would possess the same or similar motif in order to bind to the same protein domain.