Introduction:

Extracellular vesicles are cell-derived lipid bound vesicles that house proteins and RNAs, including messenger and microRNAs, originating from the host cell. These vesicles perform cell-cell communication vital to cellular biology by regulating pathways in recipient cells. Cargo sorting is mediated by changes in lipid raft composition, which has been somewhat documented in terms of protein sorting. However, microRNA sorting has not been elucidated. Functional microRNAs that are reabsorbed into recipient cells down regulate their target proteins and therefore pathways, commonly exploited by advanced staged cancers. Similarly, abnormalities in lipid raft and caveolae composition had been linked to multiple pathologies, including cardiac hypertrophy, Alzheimer’s disease and diabetes(Simons and Simons 2002; Cohen *et al.* 2003). Caveolae are membrane invaginations that form a domain of lipid rafts, formed by presence of the structural protein, caveolin-1, and expression of cavins. To understand the miRNA sorting mechanisms, an advanced prostate cancer cell model, PC3, will be employed due to exhibiting abnormal caveolae activity as a result of abnormal caveolin-1 expression. The PC3 model lacks cavin expression while still expressing caveolin-1. Introduction of cavin-1 to this model re-establishes caveolae formation, modifies lipid raft composition and correlates to a change in miRNA secretion, which may unlock the mechanism that regulates miRNA sorting. Understanding this mechanism furthers the current knowledge regarding exosome cargo export and may translate to clinical significance due to the role of caveolae in disease.

Background:

Exosomes and microvesicles: Extracellular vesicles detrimental to biological processes.

Exosomes are defined as 40-100nm diameter extracellular vesicles formed by exocytosis of multivesicular bodies(Gu *et al.* 2014). Multivesicular body biogenesis require membrane budding proceeding the formation of small invaginations of the membrane. Whilst similar in size and biochemical markers, microvesicles differ from exosomes by being released directly from budding off the plasma membrane(Minciacchi *et al.* 2015). These extracellular vesicles (ECVs) are typically enriched in particular lipid domains, known as lipid rafts, which also integrate embedded proteins or peripheral membrane proteins. This composition mediates formation and cargo loading. ECV cargo consists of cytoplasmic material with selectively exported ribonucleic acids (RNA), proteins and lipids due loading mechanisms with integral surface proteins within the lipid rafts. This method of secretion facilitates long range intercellular communication, benefiting from homing mechanisms by surface proteins and enhanced stability of the contents due to being membrane bound. Secretion and reabsorption of the extracellular vesicles has been attributed to a range of biological processes. This includes the secretion of selectively exported cytokines in immunological responses and establishing a pre-metastatic niche in cancer progression by sequestering growth factors to exosomes(De Toro *et al.* 2015). Hereby, understanding the cargo loading mechanisms can reveal how certain transcellular communication is mediated which plays a role in multiple cellular processes.

Caveolae: enriched lipid domain with potential.

In particular tissue types and/or circumstance, such as disease state, these ECVs become enriched in caveolin-1, the structural protein involved in caveolae formation. Caveolae are 50-100nm diameter invaginations of the plasma membrane, enriched in cholesterol, ceramides, sphingolipids and the caveolin family proteins (Parton *et al.* 2006). Additionally, cytoplasmic coat proteins, from the recently discovered Cavin family, regulate the caveolae formation and morphology (Nabi 2009). The enrichment of caveolin-1 domains had been correlated to a change in lipid raft proteins, linked to a flux in the cargo within ECVs. Adding the cavin family members to these domains also modifies this observed flux.

Caveolin-1: Mediating caveolae formation.

The caveolin protein family are integral membrane proteins that dictate the formation of caveolae by facilitating structural change of membrane curvature(Ariotti *et al.* 2015). The three isoforms of caveolin, named CAV1-3, are typically expressed in different types of tissues. CAV1 and 2 are expressed in epithelial, endothelial and smooth muscle cells, whereas CAV3 is predominately expressed in cytoskeletal muscle cells. These proteins oligomerise and bind to cholesterol when in proximity within the lipid raft domain. Here, they promote a variety of signalling activities, including the mediation of growth, secretion and adhesion. Lack of CAV1 and 3, through genetic ablation, yields a loss of caveolae formation, unlike loss of CAV2(Drab *et al.* 2001; Galbiati *et al.* 2001; Razani *et al.* 2001). Furthermore, *de novo* caveolae formation in lymphocytes occur following ectopic expression of CAV1(Fra *et al.* 1995). This exemplifies the necessity for CAV1 production in caveolae formation and therefore the importance in ECVs. However, it should be noted that these knockdown/over-expression studies were performed in a cell model that still contains other associated proteins required to facilitate the formation of caveolae. As such, the findings that non-caveolar caveolae exists demonstrates that, while caveolin is present, it is not sufficient for caveolae production on its own(Hill *et al.* 2008). Additionally, non-caveolar caveolae has been implicated in additional pathways and pathologies(Bosch *et al.* 2011; Low and Nicholson 2015).

Cavins.

In addition to CAV1, cavins are required in caveolae production by acting as caveolar coat proteins that stabilise caveolin interaction. The cavin family consists of 4 cavins, named Polymerase I and Transcript Release Factor (PTRF or cavin-1), Serum Deprivation Response (SDPR or cavin-2), Sdr-Related gene product that Binds to C-kinase (SRBC or cavin-3) and Muscle Related Coiled-Coil protein(MURC or cavin-4). These proteins are co-expressed and co-distributed with caveolin and interact with each other as oligomeric cavin complexes in healthy cells. Interaction with caveolin initiates caveolae formation, morphology and other properties.

Maybe include a figure detailing the above overall process, including caveolae formation, caveolin and cavins prior to formation of exosomes.

Cavin roles in caveolae formation and function.

Cavin-1 plays a major role in the formation of caveolae. Expression of cavin-1 in cells with functional caveolin dramatically increases the caveolae density. In contrast, cavin-1 knockdown in mice yielded a significant decrease in caveolae formation. Hereby, cavin-1 must be required for formation of caveolae with the presence of CAV1. Co-immunoprecipitation studies with the cavin members and CAV1 reveal that cavin form distinct complexes. These complexes require the presence of cavin-1 and either cavin-2 or cavin-3 to form and initiate its function, however these cavins have additional functions without being involved in the complexes. Once cavin-1 or cavin-1 containing complexes associate to CAV1, caveolae formation initiates. Overexpressing cavin-2 in HeLa cell lines, which includes natural CAV-1, was found to increase membrane tubule formation from the caveolae. So, while cavin-2 presence may not be mandatory, its addition to these complexes affects size and tabulation of caveolae. Additionally, cavin-3 has been associated with internalisation and trafficking by further knockdown and ectopic expression studies. Cavin-4 is only present in cardiac and skeletal muscle and will associate with Caveolin-3, where its specific action in this system had not been as extensively studied.

**Caveolae and cargo export**

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