### MicroRNAs:

MicroRNAs (miRNAs) have been found to be involved in most developmental and pathological processes due to its ubiquitous gene regulatory function. The functional miRNA sequences (~19-24 nt) are derived from longer transcripts that undergo processing and shuttling events to give rise to functional mature sequences, known to induce RNA degradation (Ha and Kim 2014). Typically, the mature miRNA sequence interact with the 3’ untranslated region (3’-UTR) of its target transcripts and guides a multi-protein RNA induced silencing complex (RISC) to destine these molecules for degradation or translational inhibition (Djuranovic et al. 2012). As of 2014, 2,588 mature miRNA sequences had been discovered, where each miRNA can target hundreds of transcripts for degradation using the RISC mechanism (miRBase 2014). In total, approximately 60% of mammalian protein transcripts are directly regulated by miRNA induced repression (Friedman *et al.* 2009). Hereby, tight spatial and temporal regulation of miRNAs are required to avoid dysregulation in many vital cellular pathways (Ha and Kim 2014). In particular, dysregulation of miRNAs that dictate differentiation, replication and adhesion had been implicated in cancer-like properties, thus highlighting major pathological involvement (Hashimoto *et al.* 2013). Furthermore, discovering that miRNAs can be integrated into extracellular vesicles reveals novel intercellular communication mediated from its gene regulatory role that adds to the complexity in disease and biological function.

### Extracellular vesicles:

Secreted membrane-bound vesicles, consisting of exosomes and microvesicles, collectively called extracellular vesicles (EV) are important mediators of intercellular communication (Figure 1). Exosomes are defined as 40-100nm diameter extracellular vesicles which are released upon fusion of the multivesicular bodies with the plasma membrane (Gu *et al.* 2014). Whilst similar in function and biochemical markers, microvesicles (≥100nm) differ from exosomes by being released from budding of the plasma membrane (Minciacchi *et al.* 2015). EV cargo consists of cytoplasmic material, functional RNA and proteins, where this content can differ between the subpopulations (Stoorvogel 2015). Secretion and uptake of the extracellular vesicles has been reported to influence a range of biological processes, such as the selective export of cytokines in immunological responses and establishing a pre-metastatic niche in cancer progression (Campos *et al.* 2015; De Toro *et al.* 2015). Therefore, understanding the cargo loading mechanisms can reveal how certain intercellular communications are mediated.

### Extracellular vesicle mediated horizontal transfer of microRNAs:

Recently, miRNAs were found to be secreted via EVs and transferred to other cells to promote the post-transcriptional regulatory function, thus providing as a novel mechanism for intercellular communication (Valadi et al. 2007; Hannafon and Ding 2013). Previously, miRNAs were considered unstable molecules that undergo rapid degradation in order to maintain temporal control of their gene regulatory function due to high abundances of RNases in extracellular space (Valencia-Sanchez *et al.* 2006; Reddi and Holland 1976). However, packaging of miRNAs into EVs increases the stability of miRNAs in circulation, due to being membrane bound (Köberle *et al.* 2013). Hereby, the extensive gene regulatory mechanisms evoked by miRNAs are able to be integrated into the endogenous miRNA population of the distant recipient cells, thus modifying pathway activity (Weilner *et al.* 2013). While this may provide as a beneficial source of intercellular communication required in cellular stress response and developmental processes, dysregulation can cause adverse differential activity uncharacteristic of the recipient cell (Kamhieh-Milz *et al.* 2014; Schober *et al.* 2015). For example, aberrant extracellular miRNAs have been linked to metastasising cancers due to inducing proliferation and adhesion-independent growth (Zhou *et al.* 2014).

Despite the pathological implication of exported miRNAs, the mechanisms that dictate transport through extracellular vesicle release are mostly unknown (Zhang *et al.* 2015). Previously, miRNA vesicular secretion had been considered a non-selective process, where the RNAs found within vesicles are merely representative of the total cellular miRNAs (Zhang et al. 2015). Yet, recent assessment of the intracellular miRNA levels compared to the EV contained miRNAs revealed that particular miRNAs are enriched or lacking in the vesicles (Collino *et al.* 2010; Inder et al. 2014). This indicates a selective mechanism in which RNAs are exported that is yet to be extensively researched.

A recent clue was provided by Villarroya-Beltri et at, who reported that sumoylated ribonucleoprotein, hnRNPA2B1 mediate the transport and subcellular localization of particular miRNAs in T-lymphocytes (Villarroya-Beltri *et al.* 2013). Typically, the hnRNP family are involved in mRNA processing within the nucleus for translational control, mRNA stability and subcellular localisation, yet this is the first reported case of EV localisation occurring from this mechanism and one of the first reports of its ability to bind to miRNAs. Further questions arise due to this finding, such as the use of other hnRNP proteins for this function, how hnRNPs are targeted to the EVs and whether this protein family could be responsible for miRNA EV export in other cell types and stimuli.

### Experimental Model and Hypothesis:

While the mechanism that mediates the selective transfer of miRNAs via extracellular vesicles is mostly unknown, recent experimentation of the prostate cancer cell line, PC3, had been suggested as a model for determining miRNA export. This experimental system takes advantage of the aberrant caveolin-cavin1 expression where human cells typically produce caveolin-1 and cavin-1 or lack both. Yet, the PC3 cell line expresses only caveolin which is attributed to an increased oncogenic behaviour. Interestingly, addition of cavin-1 to this cell line attenuates the oncogenic behaviour, cholesterol redistribution and EV protein and miRNA content. Furthering the interest in this cell line, is the finding that selective export of microRNA, miR-148a-3p, is able to be absorbed into the endogenous miR population of bone marrow cells, which facilitates increased osteoclastogensis. This is consistent with the advanced prostate cancer phenotype of aggressive bone metastasis resulting in weaken bones prone to fracture. Hereby, this demonstrates the importance of selectively exported miRNAs in a biological system where dysregulation can enhance pathogenic effects. Thus utilising this model, by comparing between PC3 and PC3-cavin-1 transfected, can be used to assess the evident cellular modifications that facilitate miRNA export that contribute to pro-oncogenic behaviour.

This project investigated the hypothesis that miRNAs are selectively exported via extracellular vesicles moderated by the expression of cavin-1 to the PC3 model. As cavin-1 cannot directly mediate the export of miRNAs as it is not present within EVs, it is hypothesised that cavin-1 indirectly modulates miRNA escort proteins to the EVs, thereby mediating selective miRNA export. The following aims were devised to address this hypothesis:

1. Assess the microRNA species that are modified by this model
2. Identify candidate export proteins that participate in microRNA EV export.
3. Verify the interaction between candidate protein and microRNA by *in situ* and *ex vivo* experimental methods.