**Introduction: 1257words**

**Advanced Prostate Cancer and Caveolin-1:**

Prostate cancer is the most commonly diagnosed cancer in men. While the primary tumour can be treated and removed efficiently resulting in almost 99% survival, patients inflicted with metastatic prostate cancer possess a reduced 5-year survival rate of 29.3% (SEER 2016). Bone metastasis is the most common complication derived from advanced prostate cancer formation which severely limits the survival outcome ([Bubendorf *et al.* 2000](#_ENREF_1)). This highlights the necessity to identify therapeutic targets and underlying biological phenomena that induce the metastatic phenotype.

Caveolin-1 has been linked to prostate cancer metastasis and has been a speculated biomarker for cancer progression ([Gumulec *et al.* 2012](#_ENREF_10); [Moon *et al.* 2014](#_ENREF_22); [Hayashi *et al.* 2015](#_ENREF_12)). This protein usually functions as a cholesterol transporter where its interaction with cytoplasmic protein Cavin-1 initiates the formation of specific lipid microdomains on the plasma membrane, called Caveolae ([Hill *et al.* 2008](#_ENREF_14); [Moon et al. 2014](#_ENREF_22)). These proteins are co-expressed and co-localised in healthy human tissue, however in the case of many cancer types only caveolin-1 is expressed ([Wu *et al.* 2011](#_ENREF_31); [Moumita *et al.* 2015](#_ENREF_23)). Increased proliferation, migration and differentiation are a result of the aberrant caveolin-1 expression, yet, the mechanism that links caveolin to these phenotypes is still actively being investigated ([Grande-García *et al.* 2007](#_ENREF_9); [Chatterjee *et al.* 2015](#_ENREF_2)).

Recent studies have shown that cavin-1 is capable of reversing the pro-metastatic action of caveolin-1 ([Moon et al. 2014](#_ENREF_22)). Cavin-1 expression inhibited prostate cancer PC3 cell proliferation, migration and anchorage-independent growth *in vitro*, and tumour growth, metastasis and angiogenesis *in vivo*. Mechanistically, cavin-1 expression altered the tumour microenvironment, including reduction of fibroblasts and secretion of IL-6 (Moon 2012). This reduced IL-6 secretion was determined to be through extracellular vesicle (EV) release as opposed to the classical secretion methods (Inder). Other proteins were also found to be differentially secreted via EVs, yet their role was not extensively studied. Hereby, in addition to the previous well-known methods of cancer progression, caveolin-1 appears to modulate extracellular vesicle mediated metastasis. Intriguingly, cavin-1 expression also attenuated the EV-mediated release of microRNA-148a, which was previously reported to mediate bone metastasis through osteoclastogenesis. These studies suggest that cavin-1 attenuates the pro-metastatic action of caveolin-1 by modulating EV microRNA content.

**Horizontal Transfer of microRNAs via Extracellular Vesicles:**

Secreted membrane-bound vesicles, called extracellular vesicles, are important mediators of intercellular communication ([Pegtel](#_ENREF_25" \o "Pegtel, 2014 #174) *[et al.](#_ENREF_25" \o "Pegtel, 2014 #174)* [2014](#_ENREF_25" \o "Pegtel, 2014 #174)). 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 uptake of this content had been reported to influence a range of biological processes, such as the selective export of cytokines in immunological responses, mediating homeostasis and stress response ([McKechnie](#_ENREF_19" \o "McKechnie, 2006 #169) *[et al.](#_ENREF_19" \o "McKechnie, 2006 #169)* [2006](#_ENREF_19" \o "McKechnie, 2006 #169); [Wysoczynski and Ratajczak 2009](#_ENREF_32" \o "Wysoczynski, 2009 #176); [Hedlund](#_ENREF_13" \o "Hedlund, 2011 #175) *[et al.](#_ENREF_13" \o "Hedlund, 2011 #175)* [2011](#_ENREF_13" \o "Hedlund, 2011 #175)). However, recent studies have emerged that determined cancer-derived EVs absorbed into recipient cells are able to induce the establishment of the pre-metastatic niche in cancer progression ([Costa-Silva](#_ENREF_4" \o "Costa-Silva, 2015 #177) *[et al.](#_ENREF_4" \o "Costa-Silva, 2015 #177)* [2015](#_ENREF_4" \o "Costa-Silva, 2015 #177); [Ramteke](#_ENREF_26" \o "Ramteke, 2015 #178) *[et al.](#_ENREF_26" \o "Ramteke, 2015 #178)* [2015](#_ENREF_26" \o "Ramteke, 2015 #178)). Primarily this is attributed to the proteomic EV content being introduced into the endogenous population of the target cell, such as introduction of beta-catenin, epidermal growth factor receptor and major elements of the MAPK pathway ([Dovrat](#_ENREF_6" \o "Dovrat, 2014 #180) *[et al.](#_ENREF_6" \o "Dovrat, 2014 #180)* [2014](#_ENREF_6" \o "Dovrat, 2014 #180); [Kharmate](#_ENREF_16" \o "Kharmate, 2016 #181) *[et al.](#_ENREF_16" \o "Kharmate, 2016 #181)* [2016](#_ENREF_16" \o "Kharmate, 2016 #181); [Song](#_ENREF_28" \o "Song, 2016 #179) *[et al.](#_ENREF_28" \o "Song, 2016 #179)* [2016](#_ENREF_28" \o "Song, 2016 #179)). Yet, more intriguing is the discovery that microRNA export may be associated with this function.

MicroRNAs (miRNAs) are small non-coding RNAs 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](#_ENREF_11)). 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](#_ENREF_5)). A 2009 estimate predicted that approximately 60% of the mammalian genome are able to be directly mediated by the microRNA RISC mechanism where a single miRNA can target hundreds of transcripts ([Friedman *et al.* 2009](#_ENREF_8)). This indicates the necessity of tight temporal and spatial control over miRs to prevent dysregulation of vital pathways. This is thought to be maintained by the high content of RNases in the extracellular serum which would rapidly degrade any miRNAs that attempt translocation across extracellular space ([Reddi and Holland 1976](#_ENREF_27); [Tsui *et al.* 2002](#_ENREF_29)). However, EV-bound miRNAs were found to bypass this degradation which allows for the absorption of these molecules into recipient cells, thus evoking their canonical function in a potentially diverse cell type ([Kosaka *et al.* 2010](#_ENREF_17); [Montecalvo *et al.* 2012](#_ENREF_21)).

Earlier work from our lab utilizes the caveolin-1/cavin-1 system to investigate the role of caveolin-1 in prostate cancer ([Inder *et al.* 2014](#_ENREF_15)). Interestingly, the cellular modification inflicted by comparing between PC3 and PC3 cavin-1+ cells modified extracellular vesicle (EV) content, a pathway unrelated to the function of caveolin or cavin-1. In addition to limiting adhesion independent growth, hyper-proliferation and EV protein content of PC3 cells, the ectopic expression of putative tumour suppressor, cavin-1, modified miRNAs found within EVs; miR-148a and miR-125a ([Inder et al. 2014](#_ENREF_15)). Expression of miR-148a in bone marrow was reported to induce osteoclastogenesis by targeting an inhibitory transcription factor, MAFB, of the RANKL-induced osteoclastogenesis pathway, where the inverse was observed upon miR-148a inhibition ([Cheng *et al.* 2013](#_ENREF_3)). Bone fracture, pain and fragility are common co-morbidities associated with the bone metastasis-mediated prostate cancer due to increased bone resorption ([Luz and Aprikian 2010](#_ENREF_18)). Therefore the export of miR-148a from pro-metastatic prostate cancer cell line is consistent with clinical findings and may be one of the main regulators of metastatic progression. However upon closer investigation, the addition of cavin-1 does not modify the cellular expression levels of miR-148a, only the EV content. This suggests that there may be selectivity over the EV exported miRNAs, truncated by cavin-1 expression. Selective EV export of miRNAs had been observed in other studies, some of which links these miRNAs with disease states, particularly cancer metastasis ([Palma *et al.* 2012](#_ENREF_24); [Zhou *et al.* 2014](#_ENREF_33)). Yet, the mechanism that governs this selectively is mostly unknown.

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](#_ENREF_30)). 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 ([Mili *et al.* 2001](#_ENREF_20); [Dreyfuss *et al.* 2002](#_ENREF_7)). Further questions arise due to this finding, such as the use of other hnRNP proteins for miRNA subcellular localization, 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.

**Hypothesis and Aims:**

This project investigated the hypothesis that miRNAs are selectively exported via extracellular vesicles moderated by the expression of cavin-1 to the PC3 model. Specifically, it is likely that cavin-1 indirectly modulates miRNA escort proteins to the EVs, thereby mediating selective miRNA export similar to the mechanism identified by Villarroya-Beltri *et at* (2014). 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.

References:

Bubendorf, L., A. Schöpfer, U. Wagner, G. Sauter, H. Moch, N. Willi, T. C. Gasser and M. J. Mihatsch (2000). "Metastatic patterns of prostate cancer: An autopsy study of 1,589 patients." Human Pathology **31**(5): 578-583.

Chatterjee, M., E. Ben-Josef, D. G. Thomas, M. A. Morgan, M. M. Zalupski, G. Khan, C. Andrew Robinson, K. A. Griffith, C.-S. Chen, T. Ludwig, T. Bekaii-Saab, A. Chakravarti and T. M. Williams (2015). "Caveolin-1 is Associated with Tumor Progression and Confers a Multi-Modality Resistance Phenotype in Pancreatic Cancer." Scientific Reports **5**: 10867.

Cheng, P., C. Chen, H. B. He, R. Hu, H. D. Zhou, H. Xie, W. Zhu, R. C. Dai, X. P. Wu, E. Y. Liao and X. H. Luo (2013). "miR-148a regulates osteoclastogenesis by targeting V-maf musculoaponeurotic fibrosarcoma oncogene homolog B." J Bone Miner Res **28**(5): 1180-1190.

Costa-Silva, B., N. M. Aiello, A. J. Ocean, S. Singh, H. Zhang, B. K. Thakur, A. Becker, A. Hoshino, M. T. Mark, H. Molina, J. Xiang, T. Zhang, T.-M. Theilen, G. Garcia-Santos, C. Williams, Y. Ararso, Y. Huang, G. Rodrigues, T.-L. Shen, K. J. Labori, I. M. B. Lothe, E. H. Kure, J. Hernandez, A. Doussot, S. H. Ebbesen, P. M. Grandgenett, M. A. Hollingsworth, M. Jain, K. Mallya, S. K. Batra, W. R. Jarnagin, R. E. Schwartz, I. Matei, H. Peinado, B. Z. Stanger, J. Bromberg and D. Lyden (2015). "Pancreatic cancer exosomes initiate pre-metastatic niche formation in the liver." Nat Cell Biol **17**(6): 816-826.

Djuranovic, S., A. Nahvi and R. Green (2012). "miRNA-Mediated Gene Silencing by Translational Repression Followed by mRNA Deadenylation and Decay." Science **336**(6078): 237-240.

Dovrat, S., M. Caspi, A. Zilberberg, L. Lahav, A. Firsow, H. Gur and R. Rosin-Arbesfeld (2014). "14-3-3 and β-catenin are secreted on extracellular vesicles to activate the oncogenic Wnt pathway." Molecular Oncology **8**(5): 894-911.

Dreyfuss, G., V. N. Kim and N. Kataoka (2002). "Messenger-RNA-binding proteins and the messages they carry." Nat Rev Mol Cell Biol **3**(3): 195-205.

Friedman, R. C., K. K. Farh, C. B. Burge and D. P. Bartel (2009). "Most mammalian mRNAs are conserved targets of microRNAs." Genome Res **19**(1): 92-105.

Grande-García, A., A. Echarri, J. de Rooij, N. B. Alderson, C. M. Waterman-Storer, J. M. Valdivielso and M. A. del Pozo (2007). "Caveolin-1 regulates cell polarization and directional migration through Src kinase and Rho GTPases." The Journal of Cell Biology **177**(4): 683-694.

Gumulec, J., J. Sochor, M. Hlavna, M. Sztalmachova, S. Krizkova, P. Babula, R. Hrabec, A. Rovny, V. Adam, T. Eckschlager, R. Kizek and M. Masarik (2012). "Caveolin-1 as a potential high-risk prostate cancer biomarker." Oncology Reports **27**(3): 831-841.

Ha, M. and V. N. Kim (2014). "Regulation of microRNA biogenesis." Nat Rev Mol Cell Biol **15**(8): 509-524.

Hayashi, T., T. Ichimura, N. Yaegashi, T. Shiozawa and I. Konishi (2015). "Expression of CAVEOLIN 1 in uterine mesenchymal tumors: No relationship between malignancy and CAVEOLIN 1 expression." Biochemical and Biophysical Research Communications **463**(4): 982-987.

Hedlund, M., O. Nagaeva, D. Kargl, V. Baranov and L. Mincheva-Nilsson (2011). "Thermal- and Oxidative Stress Causes Enhanced Release of NKG2D Ligand-Bearing Immunosuppressive Exosomes in Leukemia/Lymphoma T and B Cells." PLoS ONE **6**(2): e16899.

Hill, M. M., M. Bastiani, R. Luetterforst, M. Kirkham, A. Kirkham, S. J. Nixon, P. Walser, D. Abankwa, V. M. J. Oorschot, S. Martin, J. F. Hancock and R. G. Parton (2008). "PTRF-Cavin, a Conserved Cytoplasmic Protein Required for Caveola Formation and Function." Cell **132**(1): 113-124.

Inder, K. L., J. E. Ruelcke, L. Petelin, H. Moon, E. Choi, J. Rae, A. Blumenthal, D. Hutmacher, N. A. Saunders, J. L. Stow, R. G. Parton and M. M. Hill (2014). "Cavin-1/PTRF alters prostate cancer cell-derived extracellular vesicle content and internalization to attenuate extracellular vesicle-mediated osteoclastogenesis and osteoblast proliferation." J Extracell Vesicles **3**.

Kharmate, G., E. Hosseini-Beheshti, J. Caradec, M. Y. Chin and E. S. Tomlinson Guns (2016). "Epidermal Growth Factor Receptor in Prostate Cancer Derived Exosomes." PLoS ONE **11**(5): e0154967.

Kosaka, N., H. Iguchi, Y. Yoshioka, F. Takeshita, Y. Matsuki and T. Ochiya (2010). "Secretory mechanisms and intercellular transfer of microRNAs in living cells." J Biol Chem **285**(23): 17442-17452.

Luz, M. A. and A. G. Aprikian (2010). "Preventing bone complications in advanced prostate cancer." Current Oncology **17**(Suppl 2): S65-S71.

McKechnie, N. M., B. C. R. King, E. Fletcher and G. Braun (2006). "Fas-ligand is stored in secretory lysosomes of ocular barrier epithelia and released with microvesicles." Experimental Eye Research **83**(2): 304-314.

Mili, S., H. J. Shu, Y. Zhao and S. Pinol-Roma (2001). "Distinct RNP complexes of shuttling hnRNP proteins with pre-mRNA and mRNA: candidate intermediates in formation and export of mRNA." Mol Cell Biol **21**(21): 7307-7319.

Montecalvo, A., A. T. Larregina, W. J. Shufesky, D. B. Stolz, M. L. Sullivan, J. M. Karlsson, C. J. Baty, G. A. Gibson, G. Erdos, Z. Wang, J. Milosevic, O. A. Tkacheva, S. J. Divito, R. Jordan, J. Lyons-Weiler, S. C. Watkins and A. E. Morelli (2012). "Mechanism of transfer of functional microRNAs between mouse dendritic cells via exosomes." Blood **119**(3): 756-766.

Moon, H., C. S. Lee, K. L. Inder, S. Sharma, E. Choi, D. M. Black, K. A. Le Cao, C. Winterford, J. I. Coward, M. T. Ling, D. J. Craik, R. G. Parton, P. J. Russell and M. M. Hill (2014). "PTRF/cavin-1 neutralizes non-caveolar caveolin-1 microdomains in prostate cancer." Oncogene **33**(27): 3561-3570.

Moumita, C., B.-J. Edgar, G. T. Dafydd, A. M. Meredith, M. Z. Mark, K. Gazala, R. Charles Andrew, A. G. Kent, C. Ching-Shih, L. Thomas, B.-S. Tanios, C. Arnab and M. W. Terence (2015). "Caveolin-1 is Associated with Tumor Progression and Confers a Multi-Modality Resistance Phenotype in Pancreatic Cancer." Scientific Reports **5**.

Palma, J., S. C. Yaddanapudi, L. Pigati, M. A. Havens, S. Jeong, G. A. Weiner, K. M. E. Weimer, B. Stern, M. L. Hastings and D. M. Duelli (2012). "MicroRNAs are exported from malignant cells in customized particles." Nucleic Acids Research **40**(18): 9125-9138.

Pegtel, D. M., L. Peferoen and S. Amor (2014). "Extracellular vesicles as modulators of cell-to-cell communication in the healthy and diseased brain." Philosophical Transactions of the Royal Society B: Biological Sciences **369**(1652): 20130516.

Ramteke, A., H. Ting, C. Agarwal, S. Mateen, R. Somasagara, A. Hussain, M. Graner, B. Frederick, R. Agarwal and G. Deep (2015). "Exosomes secreted under hypoxia enhance invasiveness and stemness of prostate cancer cells by targeting adherens junction molecules." Mol Carcinog **54**(7): 554-565.

Reddi, K. K. and J. F. Holland (1976). "Elevated serum ribonuclease in patients with pancreatic cancer." Proceedings of the National Academy of Sciences **73**(7): 2308-2310.

Song, X., Y. Ding, G. Liu, X. Yang, R. Zhao, Y. Zhang, X. Zhao, G. J. Anderson and G. Nie (2016). "Cancer Cell-Derived Exosomes Induce Mitogen-Activated Protein Kinase-Dependent Monocyte Survival by Transport of Functional Receptor Tyrosine Kinases." Journal of Biological Chemistry.

Tsui, N. B., E. K. Ng and Y. M. Lo (2002). "Stability of endogenous and added RNA in blood specimens, serum, and plasma." Clin Chem **48**(10): 1647-1653.

Villarroya-Beltri, C., C. Gutiérrez-Vázquez, F. Sánchez-Cabo, D. Pérez-Hernández, J. Vázquez, N. Martin-Cofreces, D. J. Martinez-Herrera, A. Pascual-Montano, M. Mittelbrunn and F. Sánchez-Madrid (2013). "Sumoylated hnRNPA2B1 controls the sorting of miRNAs into exosomes through binding to specific motifs." Nat Commun **4**.

Wu, H.-C., C.-H. Chang, Y.-A. Tsou, C.-W. Tsai, C.-C. Lin and D.-T. Bau (2011). "Significant Association of Caveolin-1 (CAV1) Genotypes with Prostate Cancer Susceptibility in Taiwan." Anticancer Research **31**(2): 745-749.

Wysoczynski, M. and M. Z. Ratajczak (2009). "LUNG CANCER SECRETED MICROVESCILES: UNDERAPPRECIATED MODULATORS OF MICROENVIRONMENT IN EXPANDING TUMORS." International journal of cancer. Journal international du cancer **125**(7): 1595-1603.

Zhou, W., M. Y. Fong, Y. Min, G. Somlo, L. Liu, M. R. Palomares, Y. Yu, A. Chow, S. T. F. O’Connor, A. R. Chin, Y. Yen, Y. Wang, E. G. Marcusson, P. Chu, J. Wu, X. Wu, A. X. Li, Z. Li, H. Gao, X. Ren, M. P. Boldin, P. C. Lin and S. E. Wang (2014). "Cancer-secreted miR-105 destroys vascular endothelial barriers to promote metastasis." Cancer Cell **25**(4): 501-515.