Discussion: 1,272 words

This project attempted to investigate a mechanism that mediates the selective export of microRNAs to extracellular vesicles in prostate cancer cell lines. Ectopic expression of cavin-1 in PC3 cell lines was found to mediate the selective EV export of 19 miRNAs via reduction of their export. These miRNAs were found to share similar sub-sequences presumed to be the conserved RNA-binding motifs to mediate this selectivity. Proteomic content of EVs derived from these cell lines revealed that RNA-binding protein, heterogeneous nuclear ribonucleoprotein K (hnRNPK), possesses reduced export upon cavin-1 expression and is predicated to bind to the RNA-binding motif. When investigated, hnRNPK seems to change subcellular localization from multivesicular bodies (MVBs) to endoplasmic reticulum (ER) due to the expression of cavin-1. In the PC3 cell line, hnRNPK co-localizes with selectively exported miRNA, miR-148a, in cytoplasmic puncta where this co-localization was not observed for PC3-cavin-1 cell lines or with non-selectively exported miRNA, miR-589, in either cell line. Altogether, these results indicate that cavin-1 in PC3 cell lines modulate the export of a subset of miRNAs to EVs by modulating the subcellular localization of miRNA export protein, hnRNPK.

The selective export of 19 miRNAs reduced by the expression of tumour suppressor, cavin-1, in PC3 cell lines indicates that the selective miRNA export mechanism may be a major mediator in cancer metastasis. As 20% (19 of the 95 miRNAs) of the miRNAs found in the EVs are manipulated by the selective export mechanism (Fig. XX), the truncation of the mechanism may provide dramatic changes to the function of the EVs. Amongst the selectively exported miRNAs is oncomiR-148a consistent with previous findings (Inder *et al*. 2014). While the function of these miRNAs were not assessed in this report, surveying the literature reveals that many of the miRNAs selectively exported may possess roles associated with cancer and cancer progression. Huang *et al* (2014) determined that miR-98, 148b, 30e, 30a, 148a, 3615 and 20b contribute to immune response regulation in papillary thyroid carcinoma. Additionally, miR-22, 200a and 429 were found to be involved with epithelial to mesenchyme transition in various cancers. This suggests that these miRNAs play roles in modifying the tumour microenvironment and establishment of the pre-metastatic niche. This is consistent with past research that linked cancer derived EVs with these roles. Therefore this miRNA export mechanism may be key in modulating the pro-metastatic phenotype associated with EV secretion.

As the selectively exported miRNAs contain enriched motifs, this suggests these similarities mediate their export. RNA-binding proteins are known to bind to conserved RNA regions to mediate selectively over their targets, therefore indicating that this motif could be the conserved region. Considering that one of these motifs is found in the majority (12/19) of the miRNAs would suggest potentially a single protein may be mediating most of this export, later identified as hnRNPK. The identification of two enriched motifs sustains the hypothesis that selectively exported miRNAs, including miR-148a, share similar sequence motifs that permits export. Given that motif discovery is a method to identify binding sites based on probabilistic models, experimental validation will be required. CRISPR gene editing may be a useful tool to mutagenize this motif contained within these exported miRNAs, similar to a method conducted by Ebina *et al.* (2013), to ultimately confirm its role in the export mechanism.

As hnRNPK demonstrated reduced export upon cavin-1 expression and is predicted to bind one of the enriched selective export motifs, it was identified as a candidate export protein that mediates the selective export of miRNAs. Interestingly, hnRNPK has been consistently associated with cancer progression. A review by Lu & Gao (2016) summarized its role in metastasis, where multiple reports link overexpression of hnRNPK with various mechanisms mediating metastasis such as invasion, reduction of apoptosis, angiogenesis and cell motility ([Revil *et al.* 2009](#_ENREF_24); [Gao *et al.* 2013](#_ENREF_7); [Brown *et al.* 2015](#_ENREF_1)). Some these phenotypes are believed to be induced by transcriptional control mediated by mRNA-hnRNPK interactions or kinase activity, however further work is required to elucidate the roles of hnRNPK in metastasis. Additionally, hnRNPK is commonly found in EVs secreted from various cancer types, including late stage bladder, advanced prostate, metastatic colorectal and advanced brain cancers ([Welton *et al.* 2010](#_ENREF_30); [Ji *et al.* 2013](#_ENREF_12); [Ramteke *et al.* 2015](#_ENREF_23); [Zhang, Peng *et al.* 2015](#_ENREF_33)). Yet, the significance and function of EV hnRNPK in these cancers is unknown. Hereby, identifying hnRNPK as a major mediator of EV miRNA content perhaps establishes a novel role and function of EV hnRNPK in advanced cancers.

Following the identification of hnRNPK as a candidate, we decided to investigate the activity of this protein. While hnRNPK is reported to be predominately nuclear based, due to the nuclear localization signal, with cytoplasmic shuttling ability, several studies had observed that hnRNPK in tumour cells possess aberrant localization (ref). hnRNPK in advanced colorectal cancer possesses increased cytoplasmic accumulation and limited nuclear localization whereas the healthy counterpart is predominately nuclear ([Hope *et al.* 2011](#_ENREF_8)). This is consistent with the observed localization in the PC3-GFP cells (fig. XX), however the cytoplasmic accumulation includes compartmentalization into forming EVs, particularly the multivesicular bodies. As the proteomic data detected hnRNPK in EVs and past research consistently demonstrates hnRNPK presence in cancer-derived exosomes, its presence in the MVBs was somewhat expected. However, this is the first study to demonstrate MVB localization of hnRNPK. Hereby, confirming hnRNPK is present in the forming EVs to allow for its activity as an EV export protein. Interestingly, the PC3-cavin-1 cell lines depict hnRNPK predominately in the endoplasmic reticulum (ER). While this indicates that cavin-1 expression inflicts a change in subcellular localization, hnRNPK has not been detected in the ER previously. Cavin-1 may be inducing hnRNPK localization dissimilar to both healthy and cancer cells or a newly found localization of hnRNPK. As the ER is primarily the site of protein maturation and folding, perhaps cavin-1 is preventing hnRNPK transport to its usual localization following translation thus retaining hnRNPK in the ER. Retention to the ER would explain the reduction of EV hnRNPK upon cavin-1 expression, though how cavin-1 induces this phenotype is unknown. Observations of hnRNPK in healthy prostate cells, such as the RWPE-1 cell line which expresses caveolin-1 and cavin-1, would determine whether this ER retention is a common function of cavin-1 or an abnormality found in only PC3-cavin-1 cell lines. Nonetheless, this supports the hypothesis that cavin-1 reduces the export of candidate miRNA export proteins to the extracellular vesicles, seemingly through localization modifications.

Consistent with the motif discovery predictions and correlative evidence, hnRNPK was found to co-localize with miR-148a in the PC3 cell line at punctate cytoplasmic structures. This supports the predicted interaction between hnRNPK and selectively exported miRNAs to facilitate the export mechanism. However, while hnRNPK changed localization in the cavin-1 positive cells, the miRNAs didn’t co-localize with it. Additionally, miR-148a did not form puncta in these cells. The lack of miR-148a in MVBs is consistent with the observed limited export of miR-148a in cavin-1 positive cells from the RNA-seq, RT-qPCR results and previous findings (Inder). Though it does not seem like the ER hnRNPK is retaining miR-148a, shown by lack of co-localization. This may suggest that hnRNPK is inducing miRNA export in the PC3 cell line, where lack of inducive export reduces the miR-148a EV content from PC3-cavin-1 cell lines. Furthermore, hnRNPK was not observed mediating the export of non-selectively exported miRNA, miR-589. miR-589 was analysed as its export did not change upon cavin-1 expression and does not contain the export motif and therefore should not bind to hnRNPK or be found in punctate structures. As the results confirmed our expectations, it is believed that hnRNPK confers selective export of miR-148a to EVs where expression of cavin-1 prevents this activity.

However, the link between cavin-1 expression and hnRNPK activity is unknown. Yet, the major finding seems to highlight a change in subcellular localization of hnRNPK induced by cavin-1. The change in hnRNPK location could suggest a modification in localisation signal, commonly achieved through post translational modification. When investigated, hnRNPK and many of the members of the hnRNP family undergo SUMOylation where this modification dictates nuclear localization. However, the site of SUMOylation, Lys422, overlaps with the third K-homology domain (387-451, one of four RNA-binding sites (3 KH domains and 1 RGG-box). This may potentially serve as the reason that both subcellular localization and reduced interaction is occurring between hnRNPK and miR-148a in the cavin-1 PC3 cells. Though, to our knowledge no link is found between cavin-1 and sumolyation. Therefore, identifying hnRNPK post translational modifications may be beneficial in understanding the regulation of hnRNPK to the EVs and the miRNA export.

Additionally, another link between cavin-1 expression and hnRNPK function may lie in lipid raft composition. Lipid rafts are microdomains that are enriched in specific lipid types, such as cholesterol, ceramide and sphingolipid, and protein. These rafts can be found in EVs, the membrane of the ER, Golgi bodies and in the plasma membrane. Interestingly, expression of cavin-1 in PC3 cell lines was found to reduce the amount of cholesterol found in lipid rafts, hereby changing their composition. hnRNPK is known to interact with lipid rafts in PC3 cell lines, where there is no expression of cavin-1. Perhaps, this includes recruitment of hnRNPK to these lipid rafts in extracellular vesicles in PC3 cells. Modification of the lipid raft composition is known to modulate the proteins that interact with the rafts. Cavin-1 expression was found to therefore modify the proteins associating with the lipid rafts in the PC3 cells, most of which decrease association including hnRNPK. Hereby, lipid raft modifications inflicted by cavin-1 expression could be controlling the subcellular localization of hnRNPK and therefore its export function.

It should also be noted that while this study analysed the mixed population of EVs, the localization of hnRNPK seems to indicate a preference for MVBs which precede the formation of exosomes. The recruitment to the MVB and not dramatic localization with the plasma membrane suggests that hnRNPK export may be through exosome release rather than microvesicles. This distinction is important in vesicle research to define differences between EV subpopulations where some studies state subpopulations may have varying roles ([Evans-Osses *et al.* 2015](#_ENREF_6)). Needs more I think.

Next paragraph not properly fixed up: An analysis of previously published proteomic data from our lab revealed 5 candidate RNA-binding proteins that may be involved in this export mechanism where we only focused on one. Villarroya-Beltri *et al* (2014) is to date the only published study that has identified proteins associated with miRNA EV export. In this study they revealed that members of the hnRNP family modulate this activity. For this reason, we focused further into the roles of FUS and hnRNPK. Subsequently, hnRNPK was chosen as the candidate for further investigation due to matching to the selective export motif. FUS was not further analysed in this study merely for the fact that current RNA binding information is inadequate to determine accurate binding predictions ([Lerga *et al.* 2001](#_ENREF_14)). However, considering that members of the hnRNP family usually interact in complexes, it is plausible to suggest that these proteins are working together to mediate the export ([Krecic *et al.* 1999](#_ENREF_13)). Wang *et al* (2014) confirmed that hnRNPK and FUS interact directly. Furthermore, as the selective export motif identified did not correspond to all of the selectively exported miRNAs, there could be multiple proteins mediating their export. This suggests that multiple proteins could be working collaboratively to populate the EVs. Therefore, analysing the interaction between hnRNPK, FUS and miRNAs in future research may assist in further understanding the mechanism.

Technical difficulties: I don’t know how to introduce RT-qpcr data.

Attempts to validate some of these miRNAs was completed using RT-qPCR. While the trends from the RNA-seq data was maintained for these validated miRNAs, high variation of the EV data was observed. Unfortunately, this appears to be an issue with the low quantities of miRNAs extracted from the EVs in combination with the RT-qPCR sensitivity. Ideally, repeating this experiment using more sensitive count based methods, such as the digital droplet PCR (ddPCR) could limit this variation.

Furthermore, we’d like to confirm the direct interaction between hnRNPK and miR-148a. However, from our initial pull down assays, it appears the yield from this process is incredibly low. This interaction is important because why? Confirms motifs, confirms selectivity, confirms its role in this mechanism. What we plan on doing to achieve this, ddPCR, scaling up experiments.

In conclusion, this study has identified a viable export protein that can mediate the selective export of miRNAs to EVs, specifically exosomes, in prostate cancer cell lines. hnRNPK was found to modulate the selective export of miR-148a, and is predicted to mediate additional miRNAs, where expression of cavin-1 prevents its appropriate MVB localization to fulfil this function. However, the underlying link between cavin-1 and hnRNPK function/localization is currently unknown. Future efforts to identify this link and validate some of the interactions determined here is required. Ultimately, identifying this mechanism assists in understanding how pro-oncogenic miRNAs are regulated in the EVs to facilitate their role in cancer progression.

**Information from here on out is removed bits, notes or references.**

Paragraph on Pull down stuff. 250w

Different techniques, what I need to do now. Past research. Also talk about the validation of miRNA bound by ddPCR an how that could also help with the rt-qPCR woes.

Conclusions and reiterations of the aims, hypothesis and future directions: 200w.

Hypothesis supported: Yes that similar micrornas are exported and yes cavin-1 is modulating an RNA binding protein, through definitive evidence that these interact is still required. I need to do X, Y and Z to confirm this. This data adds to the current knowledge of EVs in cancer, Micrornas in EVs and the general mechanisms that mediates micrornas.

As past research detailed a change in proteomic EV content upon cavin-1 expression in PC3 cell lines and many the selectively exported miRNAs were decreased upon cavin-1 expression ([Inder *et al.* 2014](#_ENREF_10)), it was had hypothesised that proteins involved with the miRNA export mechanism would be decreased in the EVs as well.

([Ebina *et al.* 2013](#_ENREF_5); [Wang *et al.* 2015](#_ENREF_29); [Lu *et al.* 2016](#_ENREF_17))

This project attempted to investigate a mechanism that mediates the selective export of microRNAs to extracellular vesicles in prostate cancer cell lines. Previous studies found that the addition of cavin-1 to the PC3 cell line reduced the export of proteins found in the EVs and reduction of oncomiR, miR-148, without modifying its cellular content. This resulted in the hypothesis that cavin-1 expression is linked to miRNA EV export by modifying RNA-binding export proteins in the EVs.

The findings presented here indicate that the expression of cavin-1 in PC3 cells modifies the extracellular vesicle export of a subset of microRNAs by modulating the export of hnRNPK. While previous research indicated that miR-148a is a target of this selective export, a comprehensive independent analysis of all miRNAs contained within in the EVs was conducted to potentially find additional targets. miR-148a

While this demonstrates that selective export of oncomiRs to the EVs is fairly common in the PC3 cell line, the mechanism that drives this selectivity was still unknown, hereby prompting an investigation into the mechanism itself. Here, I assumed that the export mechanism would be predominately protein based, where differential export of an RNA-binding protein would be the driving factor for miRNA export.

We found in our analysis that the selectively exported miRNAs share similar motifs, suggesting that these miRNAs could all be controlled by/ these sub-sequences.

While previous research suggested that selectively of miRNA export to cancer-derived EVs must be mediated by an export mechanism, this is the first study to assess proteins involved in the mechanism and their targets.

mediation of the miRNA EV content modulated by hnRNPK may be a novel hnRNPK function attributing to metastasis in these advanced cancer types.

This report attempted to investigate the underlying mechanism that facilitates the selective export of certain microRNAs in prostate cancer derived extracellular vesicles (EVs). Earlier research found that the ectopic expression of cavin-1 in PC3 cell lines was able to reduce selective miRNA export and was hereby used as a tool to investigate this proposed mechanism. Initially, the findings determined that selective export of miR-148a and a further subset of miRNAs were indeed selectively exported from the PC3 cell lines, where this was reduced by cavin-1 expression.

While previous research suggest that selective export of miRNAs to EVs exist, the mechanism that facilitates this is unknown.

As cavin-1-PC3 cell lines only act as a less tumorigenic cell line as opposed to healthy phenotype,

Outline:

* Reiterations of the overall findings. And reinstate the hypothesis and aims or maybe past research.
* Paragraph 1: hnRNPK. Why it was found and how. Information about hnRNPK and how it relates to this stuff.

|  |  |
| --- | --- |
| **microRNA** | **Role in cancer** |
| Mir-429 | Induction of epithelial to mesenchyme transition in ovarian cancer ([Chen *et al.* 2011](#_ENREF_3)) |
| miR-32-5p | Predicted biomarker for castration resistant prostate cancer ([Jalava *et al.* 2012](#_ENREF_11)) |
| miR-196a-5p | Role in proliferation in laryngeal cancer ([Saito *et al.* 2013](#_ENREF_25)) |
| miR-147b | Potential biomarker for colon cancer. May be through modifying inflammatory processes. ([Omrane *et al.* 2014](#_ENREF_18)) |
| miR-186 | Overexpression linked to invasive phenotype in pancreatic cancer. ([Zhang, Zheng-liang *et al.* 2015](#_ENREF_34)) |
| miR-98 | Tumour suppressor in melanoma ([Li *et al.* 2014](#_ENREF_15)). Modulation of immune response in thyroid carcinoma ([Huang *et al.* 2014](#_ENREF_9)) |
| miR-3615 | Modulation of immune responses in thyroid carcinoma ([Huang et al. 2014](#_ENREF_9)) |
| miR-148b | Modulation of immune responses in thyroid carcinoma ([Huang et al. 2014](#_ENREF_9)). Secreted from breast cancer and may be biomarker ([Shen *et al.* 2014](#_ENREF_26)) |
| miR-148a | Modulation of immune responses in thyroid carcinoma ([Huang et al. 2014](#_ENREF_9)) |
| miR-181d-5p | Cell cycle regulation in thyroid carcinoma ([Huang et al. 2014](#_ENREF_9)). Major role in inflammation and malignant transformation ([Liu *et al.* 2014](#_ENREF_16)) |
| miR-16-2-3p | Disruptive role in electron transport chain ([Huang et al. 2014](#_ENREF_9)) |
| miR-151a | Modulates migration and invasion in prostate cancer ([Chiyomaru *et al.* 2012](#_ENREF_4)) |
| Mir-19a | Promotes growth and tumourgenecity in gastric cancer by targeting SOCS1 ([Qin *et al.* 2013](#_ENREF_22)) |
| miR-10a-3p | Regulates EMT ([Yan *et al.* 2015](#_ENREF_31)). Targets PTEN to induce metastasis ([Zeng *et al.* 2014](#_ENREF_32)) |
| miR-22 | Roles in EMT, proliferation and ability for breast tumours to metastasize to lung ([Pola 2013](#_ENREF_21)) |
| miR-375 | Both tumour suppressor and promotor roles, reviewed by Yan et al 2014. |
| miR-30e | Modulates the tumour microenvironment and proliferation in gastrointestinal cancer ([Sugihara *et al.* 2013](#_ENREF_27)). Anchorage independent growth in breast cancer ([Ouzounova *et al.* 2013](#_ENREF_19)) |
| miR-30a | Anchorage independent growth in breast cancer ([Ouzounova et al. 2013](#_ENREF_19)) |
| miR-20b | Modulates angiogenesis, proliferation, migration ([Cascio *et al.* 2010](#_ENREF_2); [Wang *et al.* 2016](#_ENREF_28)) |
| miR-200a | Regulates EMT. Tumour suppressor roles in colorectal cancer ([Pichler *et al.* 2014](#_ENREF_20)) |

Brown, G. T. and G. I. Murray (2015). "Current mechanistic insights into the roles of matrix metalloproteinases in tumour invasion and metastasis." J Pathol **237**(3): 273-281.

Cascio, S., et al. (2010). "miR-20b modulates VEGF expression by targeting HIF-1 alpha and STAT3 in MCF-7 breast cancer cells." J Cell Physiol **224**(1): 242-249.

Chen, J., et al. (2011). "Overexpression of miR-429 induces mesenchymal-to-epithelial transition (MET) in metastatic ovarian cancer cells." Gynecol Oncol **121**(1): 200-205.

Chiyomaru, T., et al. (2012). "Genistein Suppresses Prostate Cancer Growth through Inhibition of Oncogenic MicroRNA-151." PLoS ONE **7**(8): e43812.

Ebina, H., et al. (2013). "Harnessing the CRISPR/Cas9 system to disrupt latent HIV-1 provirus." Scientific Reports **3**: 2510.

Evans-Osses, I., et al. (2015). "Exosomes or microvesicles? Two kinds of extracellular vesicles with different routes to modify protozoan-host cell interaction." Parasitol Res **114**(10): 3567-3575.

Gao, R., et al. (2013). "Heterogeneous nuclear ribonucleoprotein K (hnRNP-K) promotes tumor metastasis by induction of genes involved in extracellular matrix, cell movement, and angiogenesis." J Biol Chem **288**(21): 15046-15056.

Hope, N. R. and G. I. Murray (2011). "The expression profile of RNA-binding proteins in primary and metastatic colorectal cancer: relationship of heterogeneous nuclear ribonucleoproteins with prognosis." Human Pathology **42**(3): 393-402.

Huang, C.-T., et al. (2014). "MicroRNA-mediated networks underlie immune response regulation in papillary thyroid carcinoma." Scientific Reports **4**: 6495.

Inder, K. L., et al. (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**.

Jalava, S. E., et al. (2012). "Androgen-regulated miR-32 targets BTG2 and is overexpressed in castration-resistant prostate cancer." Oncogene **31**(41): 4460-4471.

Ji, H., et al. (2013). "Proteome profiling of exosomes derived from human primary and metastatic colorectal cancer cells reveal differential expression of key metastatic factors and signal transduction components." PROTEOMICS **13**(10-11): 1672-1686.

Krecic, A. M. and M. S. Swanson (1999). "hnRNP complexes: composition, structure, and function." Curr Opin Cell Biol **11**(3): 363-371.

Lerga, A., et al. (2001). "Identification of an RNA Binding Specificity for the Potential Splicing Factor TLS." Journal of Biological Chemistry **276**(9): 6807-6816.

Li, F., et al. (2014). "miR-98 suppresses melanoma metastasis through a negative feedback loop with its target gene IL-6." Exp Mol Med **46**: e116.

Liu, J., et al. (2014). "miR-181b as a key regulator of the oncogenic process and its clinical implications in cancer (Review)." Biomedical Reports **2**(1): 7-11.

Lu, J. and F. H. Gao (2016). "Role and molecular mechanism of heterogeneous nuclear ribonucleoprotein K in tumor development and progression." Biomed Rep **4**(6): 657-663.

Omrane, I., et al. (2014). "MicroRNAs 146a and 147b Biomarkers for Colorectal Tumor&#x2019;s Localization." BioMed Research International **2014**: 9.

Ouzounova, M., et al. (2013). "MicroRNA miR-30 family regulates non-attachment growth of breast cancer cells." BMC Genomics **14**(1): 1-15.

Pichler, M., et al. (2014). "MiR-200a regulates epithelial to mesenchymal transition-related gene expression and determines prognosis in colorectal cancer patients." Br J Cancer **110**(6): 1614-1621.

Pola, C. (2013). "Cancer: miR-22 attacks on several fronts." Nat Med **19**(8): 980-980.

Qin, S., et al. (2013). "miR-19a promotes cell growth and tumorigenesis through targeting SOCS1 in gastric cancer." Asian Pac J Cancer Prev **14**(2): 835-840.

Ramteke, A., et al. (2015). "Exosomes secreted under hypoxia enhance invasiveness and stemness of prostate cancer cells by targeting adherens junction molecules." Mol Carcinog **54**(7): 554-565.

Revil, T., et al. (2009). "Heterogeneous Nuclear Ribonucleoprotein K Represses the Production of Pro-apoptotic Bcl-x(S) Splice Isoform." J Biol Chem **284**(32): 21458-21467.

Saito, K., et al. (2013). "MicroRNA-196a Is a Putative Diagnostic Biomarker and Therapeutic Target for Laryngeal Cancer." PLoS ONE **8**(8): e71480.

Shen, J., et al. (2014). "Circulating miR-148b and miR-133a as biomarkers for breast cancer detection." Oncotarget **5**(14): 5284-5294.

Sugihara, H., et al. (2013). "Identification of miR-30e\* Regulation of Bmi1 Expression Mediated by Tumor-Associated Macrophages in Gastrointestinal Cancer." PLoS ONE **8**(11): e81839.

Wang, B., et al. (2016). "MicroRNA-20b (miR-20b) Promotes the Proliferation, Migration, Invasion, and Tumorigenicity in Esophageal Cancer Cells via the Regulation of Phosphatase and Tensin Homologue Expression." PLoS ONE **11**(10): e0164105.

Wang, T., et al. (2015). "Interaction of amyotrophic lateral sclerosis/frontotemporal lobar degeneration-associated fused-in-sarcoma with proteins involved in metabolic and protein degradation pathways." Neurobiol Aging **36**(1): 527-535.

Welton, J. L., et al. (2010). "Proteomics analysis of bladder cancer exosomes." Mol Cell Proteomics **9**(6): 1324-1338.

Yan, Y., et al. (2015). "miR‐10a controls glioma migration and invasion through regulating epithelial–mesenchymal transition via EphA8." FEBS letters **589**(6): 756-765.

Zeng, T. and G. Li (2014). "MicroRNA10a enhances the metastatic potential of cervical cancer cells by targeting phosphatase and tensin homologue." Mol Med Rep **10**(3): 1377-1382.

Zhang, P., et al. (2015). "A preliminary quantitative proteomic analysis of glioblastoma pseudoprogression." Proteome science **13**(1): 12.

Zhang, Z.-l., et al. (2015). "miR-186 and 326 Predict the Prognosis of Pancreatic Ductal Adenocarcinoma and Affect the Proliferation and Migration of Cancer Cells." PLoS ONE **10**(3): e0118814.