Background: for amandas honours proposal it came to 2,450words.

Caveolae, Caveolin and Cavins; Basic molecular biology intro:

Caveolae, flask-like invaginations of the plasma membrane, exosome and microvesicle biogenesis and regulation have been linked to Caveolin family activity and expression. These vesicles allow for intercellular transport of proteins, Ribonucleic acids (RNA) and lipids which previously been attributed to a range of biological processes. Hereby, finding that disrupting this regulation has been attributed to multiple diseases is not unexpected. Namely, prostate cancer (PC) cells exemplify the range of negative effects that occurs when Caveolins are abnormally expressed. These Caveolins, particularly caveolin-1 (CAV1), facilitate vesicle formation by associating with cholesterol-enriched membrane microdomains on the plasma membrane. However, in the case of prostate cells where no Caveolin is usually expressed, CAV1 is upregulated to stimulate cancer-like properties, due to stimulating signalling processes attributed to tumour progression, invasion and metastasis. In contrast, typical function of CAV1 requires the introduction of coat proteins called Cavins (named 1-4) that stabilise Caveolae.

Exosomes and microvesicles:

Long range intercellular communication takes advantage of membrane bound vesicles, exosomes and microvesicles, secreted from a cell to allow for cell-specific homing of cargo and enhanced stability in interstitial fluid. These extracellular vesicles, which only differ by route of release, require cholesterol, sphingolipid and ceramide rich lipid microdomains to recruit a two main families of proteins to mediate release. Once released, the cytoplasmic contents, which also contains selectively exported ribonucleic acids (RNA), proteins and lipids, can be reabsorbed by other cells to facilitate biological function.

You’re floundering into other sections. Start again.

Significance and broad field: 117w excl. references.

Prostate cancer currently rates as the second most diagnosed cancer, with its progression resulting in a high incidence of death in males. Once an advanced stage is reached, these tumours begin to exhibit androgen independence, uncontrolled proliferation, angiogenesis and general metastasis to adjacent bone and lymph nodes. Such negative affects occur due to dysregulation of molecular entities, namely proteins and ribonucleic acids (RNA). Some prostate cancers exhibit abnormal expression of proteins related to caveolae and exosome formation, which have been implicated in the progression of prostate cancer. Furthermore, this occurrence reveals gaps in knowledge regarding caveolae, caveolae-associated proteins and their molecular consequence. Understanding this mechanism benefits clinical research and furthers the current knowledge regarding exosome cargo export.

Exosomes and microvesicles: 200w currently 158w, add more about caveolae, lipid rafts and exosome production.

Exosomes are defined as 40-100nm diameter extracellular vesicles formed by exocytosis of multivesicular bodies. Whilst similar in size and biochemical markers, microvesicles differ from exosomes by being released directly from budding off the plasma membrane. Despite being two different vesicle subtypes, their similarities make these difficult to distinguish experimentally. Multivesicular body biogenesis require membrane budding proceeding the formation of small invaginations of the membrane, deemed caveolae. This process is where exosomal cargo is loaded, either by ESCRT-independent or dependent methods. Cargo consists of cytoplasmic material with selectively exported ribonucleic acids (RNA), proteins and lipids due loading mechanisms with integral surface proteins. As such, this secretion facilitates long range intercellular communication, benefiting from homing mechanisms by surface proteins and enhanced stability of the contents due to being membrane bound. The membrane composition, being lipid raft like and cholesterol rich, recruits the caveolin family proteins to mediate caveolae formation. Additionally, cytoplasmic coat proteins, from the Cavin family, regulate the caveolin interaction.

Caveolin: 120w, currently 140w

The caveolin protein family are integral membrane proteins that dictate the formation of 50-nanometer-sized invaginations of the plasma membrane, called caveolae. The three isoforms of caveolin, named CAV1-3, are typically expressed in different types of tissues. CAV1-2 are expressed in epithelial, endothelial and smooth muscle cells, whereas CAV3 is predominately express 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, causes a loss of caveolae formation, unlike CAV2. Furthermore, *de novo* caveolae formation in lymphocytes occur following ectopic expression of CAV1. Due to a high prevalence of overexpressed CAV1 in cancers, expression pattern and detriment on caveolae formation, it will be the focus of this report.

Caveolin in Cancer: 100w currently 131.

Caveolin 1 expression has been associated with aggressive late stage prostate cancer. This was unveiled by observing its abnormal expression in prostate epithelial cells, where CAV-1 expression does not occur in previously healthy cells. Additionally, the absence of CAV-1 in a prostate cancer model had resulted in hindered progression into a highly invasive and metastatic form. Hence, this demonstrates the role of CAV1 in prostate cancer as a tumour promotor. Similarly, some oesophageal, breast, renal, brain and lung cancers had also revealed CAV1 to correlate with angiogenesis, cancer recurrence and elevated metastasis, solidifying its tumour promotor function. In contrast, some breast and pancreatic cancers revealed a tumour suppressor function where CAV1 deficiency promotes MAPK and PI3K signalling to induce growth. Hereby, the function of CAV1 in cancers appears to be tissue specific.

^^Total of 517 words thus far (excl. titles and no references yet).

Cavins: 100w #Need to explain abbreviations, currently 60w.

In addition to CAV1, cavins are required in exosome production by acting as caveolar coat proteins that stabilise caveolae. The cavin family consists of 4 cavins, named cavin 1-4 or PTRF, SDPR, SRBC and MURC respectively. These proteins are co-expressed and co-distributed with caveolin and interact with each other as oligomeric cavin complexes. Interaction with caveolin causes caveolae to form.

Cavin-1, 2 and 3. 114w

Cavin-1 plays a major role in the formation of caveolae. Expression of cavin-1 in cells with caveolae expression 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 caveloae with the presence of CAV1. Co-immuprecipation 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. Once associated to CAV1, caveolae formation initiates. While cavin-2 presence is not mandatory, its addition to these complexes play roles in size and tabulation of caveolae. PUT IN EVIDENCE. This may indicate that cavin-2 recruit differential signalling to cavin-1 only due to this change in morphology. Additionally, cavin-3 in the cavin-1/3 complex has been associated with Cavin-4 is only present in cardiac and skeletal muscle, so, whilst still fulfilling its function in caveolae, its role is not germane to this report.

Use PLOS ONE paper with Mhill in it for localisation data and migration data.

Cavins in cancer: 200w

Reiterate that cavins are involed in the stabilisation of interactions. Explain that when cavins are introduced it attenuated the cancer progression. Introduce that recent evidence reveals that cavin differential expression in regards to cancer caused a flux of miRNAs (miR148a). add unpublished info about the pellet changes, and that cavins are strongly linked to this flux. Follow up with info about why we study the exosomes in respects to mirnas and cavins. (eg. Hereby this may indicate that Cavins may play a role in selectivity of miRNA export.)

microRNAs in Cancer: 500w

State what miRNAs are. Provide evidence that miRNAs appear to be playing part in cancer progression.

Cavins and miRNAs: 200w

Include evidence that miRNA148 supports this and that it is selectively exported. That cavins may be recruit or taking part in selection of exported material.

INCLUDE CRITICAL REVIEW OF RELAVNT LITERATURE (60% of the report so ~2400words)

Hypothesis:

This project will assess the hypothesis that miRNAs are selectively exported via exosomes, and that cavin family members are somewhat responsible for this in a PC3 model. will also require the assessment of any newly found binding partners of the exported miRNAs.

Aims: No methods are to be used. But at this point, its good enough.

1. Establish if miRNAs are selectively exported by exosomes and whether this relationship is robust across cell types (HEK293 and PC3 cells).
2. Identify potential interaction partners involved with miRNA sorting by bioinformatic and network analysis.
3. Verify miRNA candidate escort proteins by observation of localisation with Cavins and exosomes.