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.

Background.

Prostate cancer

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, including mechanisms hypothesised to be involved with cargo sorting, which have been implicated in the progression and metastatic potential of prostate cancer. Furthermore, this occurrence reveals gaps in knowledge regarding caveolae, caveolae-associated proteins and their molecular consequence. Understanding this mechanism benefits cancer research and furthers the current knowledge regarding exosome cargo export.

Exosomes and microvesicles: Extracellular vesicles implicated in prostate cancer.

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. The membrane composition, containing cholesterol rich lipid raft-like domains, recruits the caveolin family proteins to mediate caveolae formation. Additionally, cytoplasmic coat proteins, from the recently discovered Cavin family, regulate the caveolae formation and morphology. Furthermore, recent studies investigating this interaction had revealed a significant change in the exosomal contents depending on the cavin presence in caveolin containing cells. This may indicate a cargo sorting role for this system. 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. 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. Additionally, the selective export of proteins has been well documented as a function in exosomes that allow for a certain level of control over intracellular protein concentration. Hereby, understanding the cargo loading mechanisms in metastatic cancers can reveal how certain processes are being mediated and exploited to aid in progression.

Caveolin

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 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, causes a loss of caveolae formation, unlike CAV2. Furthermore, *de novo* caveolae formation in lymphocytes occur following ectopic expression of CAV1. This exemplifies the necessity for CAV1 production in caveolae formation, involved in exosome production. 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. Additionally, non-caveolar caveolae has been implicated in additional pathways and pathologies.

Caveolin in tumour activity.

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. This formation of non-caveolar caveolin was shown to facilitate anchorage-independent growth and metastasis. 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 and introducing its potential as a biomarker for aggressive cancer types. The mechanism in which this occurs is said to be due to the CAV1 direct interaction with G-proteins involved with cellular replication and differentiation. 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 or case specific. Due to a high prevalence of overexpressed CAV1 in metastatic cancers, expression pattern and detriment on caveolae formation, it will be the focus of this report.

^^Total of 819 words thus far (no references yet). doi:10.1038/nrurol.2013.168

Cavins.

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 in healthy cells. Interaction with caveolin initiates caveolae formation.

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 caveloae with the presence of CAV1. Co-immunoprecipation 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 compared to cavin-1 homomeric complexes due to this change in morphology. Additionally, cavin-3 has been associated with internalisation and trafficking by further knockdown and ectopic expression studies. Again, due to the secondary function of cavin-3 in cavin complexes, it is likely that its activity facilitates a currently uncharacterised pathway. Cavin-4 is only present in cardiac and skeletal muscle, where its specific action in this system had not been as extensively studied.

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

Cavins in cancer: 282w Convience me that the cavin interacting with the caveolin causes the attenuated TP function.

As cavins are typically co-expressed with caveolin, the unusual lack of this complete system in cancer has been researched as a potential target for therapy. It was found that in some cancers, cavin expression had been reduced by hypermethylation, allowing for the establishment a caveolin-positive/cavin-negative cell type, thus permitting for the aforementioned phenotype. It was found that the addition of cavin to non-caveolar caveolin re-establishes the normal system and truncates the tumour promoting role of the caveolin. Reestablishment of functional cavin-1 reinstates the caveolae formation in these cells and limits the aggressive behaviour of the caveolin. Similarly, cavin-2 expression in PC3 cells (caveolin expressive) had reduced the aggressive behaviour despite not producing caveolae. Cavin-3 overexpression, too, limited tumourgenesis. Several hypotheses are proposed to explain this occurrence. One suggests that the presence of the cavins truncate the secondary tumour promoting function of the caveolin by physically hindering the interaction by trapping caveolin in caveolae. While studies had confirmed that cavin-1 does indeed sequester caveolin, this hypothesis is no longer supported when considering cavin-2 and 3 do not exhibit this function. Another hypothesis implicates that protein export by selective sequestering into extracellular vesicles are mediated by the cavin-caveolin interaction to change phenotypic response. EVIDENCE (look for rob patons paper). Earlier work from our lab revealed that, while proteomic changes were true, additional selective transport of microRNAs had been observed following the cavin/caveolin interaction. This change in microRNA, although linked to cavin presence, did not appear to be the direct mechanism of sorting due to some reason. Hereby, prior evidence strongly implements that cavin/caveolin interaction is having an impact on exosomal cargo export by selecting for particular protein and, as recently suggested, microRNAs, though not being in direct contact.

^^1422w currently.

microRNAs in Cancer: 500w

The importance of microRNAs (miRNAs) had only been recently suggested as a functional member in biological processes. These RNAs form complementary base paring to the untranslated region of a gene, recruit complexes to either degrade the transcript or inhibit its translation, thus controlling protein function and processes. Further explain the RNA functionality. Link into cancer. Evidence that it facilitates cancer progression.

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. 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.)

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 which 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.
3. Observe the candidate miRNA escort proteins by observation of localisation with Cavins and exosomes.

Methods:

Cell type: 200wds, Explain basic set up, why we use PC3 cells and HEK. And expected outcoes.

Aim 1: 300wds, Which miRNAs are selectively exported? Mention that this is completely bioinformatics. Go through set up for miRNA-seq data. Which packages will be using and what they do and what youre hoping to get out of it. Validation of resuts by rt-qpcr.

Aim 2: 300wds, bioinformatics again. Using pickle prediction file to make lists of interaction partners incding rationale. Run that with expression data collected to find if predcted values are there. Suggest candidates bsed on criteria (membrane associated or integrated, rna binding or poly-a tail binding) Analyse the validity with rtqpcr.

Aim 3: 400wds. laboratory based. Expain rationale behind co-ip study and that process. Microscopy ocationation study

Timeable of events.

PC3, advanced cancer cell line.