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C1: Two Pathways for Cannabinoid Receptor 2 Cell Surface Delivery: Motif-driven versus Pharmacological Chaperoning

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G Protein-Coupled Receptor (GPCR) function is reliant on expression at the plasma membrane where extracellular ligands bind to initiate intracellular signalling responses. Few studies have investigated the control of GPCR cell surface delivery via the biosynthetic pathway, encompassing trafficking through the endoplasmic reticulum (ER) and Golgi. Cannabinoid Receptor 2 (CB2) mediates cannabis-induced immunosuppression and is a promising therapeutic target in immune-related diseases.

HA-tagged human CB2 stably expressed in HEK-293 cells was detected by fluorescent immunocytochemistry and quantified by automated imaging and analysis with an ImageXpress Micro XL widefield microscope. Confocal microscopy for colocalisation of CB2 with subcellular organelle markers was also carried out. We have made two novel observations regarding CB2 cell surface delivery. Firstly, mutation of a di-lysine ('KK') motif in the CB2 cytoplasmic tail near-abolishes basal delivery of CB2 to the plasma membrane. Overall expression levels are not reduced, indicating the mutant is likely inhibited from exiting the ER or Golgi. Secondly, although wild-type CB2 is expressed at the cell surface, a sizeable proportion is retained in a cytoplasmic compartment. These receptors can be re-distributed to the cell surface by CB2 ligands which, by virtue of their lipophilic nature, can enter the cell and likely act as pharmacological chaperones. Intriguingly, this chaperone effect is still evident in the 'KK' mutant suggesting that ligand-driven and basal surface CB2 delivery are distinct processes. In addition, this chaperone effect is unique to human CB2 and is not present in rat CB2.

This study of the molecular mechanisms of CB2 cell surface delivery reveals fundamental information about this therapeutically relevant receptor. Further research into cell surface delivery pathway mechanisms may reveal previously untapped approaches to and targets for CB2 therapeutic design.

C2: Histone deacetylase 1 is an important component of host innate antiviral response against influenza A virus

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Regular emergence of new and drug-resistant influenza A virus (IAV) strains emphasises the need to develop new anti-IAV strategies. Our lab has been working to discover novel host factors involved in IAV infection to identify new antiviral drugs targets. We have discovered the histone deacetylases 1 (HDAC1) as a novel host factor that is involved in IAV infection. HDACs are a family of host enzymes that are involved in multiple cellular functions. We have found that silencing of HDAC1 expression augmented IAV infection by more than 6-fold and conversely, the ectopic expression of HDAC1 decreased it by more than half. This indicated that HDAC1 has an anti-IAV function and is potentially a part of host innate antiviral response against IAV. To efficiently replicate, IAV has evolved multiple strategies to circumvent the host innate antiviral response. One possible strategy could be the downregulation of deacetylase activity, which is important for innate antiviral response. Indeed, treatment of infected cells with trichostatin A, a HDAC inhibitor resulted in the downregulation of STAT1 phosphorylation and interferon-stimulated genes (ISGs), viz. IFITM3, ISG15, viperin, expression and consequently, enhancement in IAV infection by more than 5-fold. Further, HDAC1 depletion resulted in about 58% decrease and overexpression resulted in about 55% increase in viperin expression. Furthermore, interferon regulatory factor 3 signaling and subsequently, interferon-α production was downregulated in HDAC1 depleted cells. These observations suggested a potential role of HDAC1 in regulating the host innate anti-IAV response by acting upstream of ISG production. Studies are underway to further understand HDAC1 role in IAV-induced host antiviral response. In summary, our data show that HDAC1 provides a cellular refractory state to IAV infection by acting as an antiviral host factor, and may open new avenues for developing a new anti-influenza strategy.

C3: Responses to vesiculin identify the existence of a signaling pathway that can bypass insulin resistance

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Pancreatic islet-derived peptide hormones play key roles in the maintenance of systemic energy homeostasis and glucose balance, and defects in their regulation are strongly implicated in the pathogenesis of obesity and diabetes, both of which have emerged as global pandemics in recent times. It is therefore important to understand the biological roles of islet hormones in both their target tissues and the whole organism. Insulin-like growth factor II (IGF-II) is an insulin homolog secreted by the islet β-cells. Vesiculin is a newly discovered peptide hormone, processed from IGF-II and secreted from islet β-cells in response to glucose, whose biological role is poorly understood [1-3]. Like insulin, vesiculin is a two-chain hormone and so has structural similarities to insulin although it has the amino acid sequence of IGF-II. Based on these observations, we postulated that vesiculin might act to regulate systemic glucose metabolism.

Here we report our original investigations of vesiculin's activity in glucoregulation. Insulin tolerance tests (ITTs) in mice were used to compare the capacity of vesiculin and IGF-II for lowering blood glucose. ITTs were also performed in two different mouse models of inslin resistance.

Vesiculin and IGF-II displayed similar dose-response relationships for lowering blood glucose in insulin-responsive mice. By contrast, the ability of IGF-II to lower blood glucose was blunted in insulin-resistant triprolyl human-amylin transgenic mice, whereas vesiculin's ability to lower blood glucose remained largely unaffected. Analysis of signaling by vesiculin and IGF-II in islet β-cells indicated that vesiculin does not signal through the type-1 IGF receptor (IGF1R), the main receptor for IGF-II, indicating that removal of only four amino acids has generated a new peptide hormone with distinct bioactivity relevant to blood-glucose regulation.

Investigating the differences among vesiculin, IGF-II and insulin signaling may provide new insights into the development of insulin resistance, a usual feature of progression to type-2 diabetes.

References

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C4: Hypoxic Exosome Signature Predicts Disease Progression in Non-Small Cell Lung Cancer Patients

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Lung cancer has one of the lowest survival outcomes of any cancer, and is the most common cause of cancer related mortality worldwide. Most lung cancer cases are categorized as Non-Small Cell Lung Cancer (NSCLC), and the prognosis for advanced stage NSCLC is poor. However, early stage NSCLC patients have an approximate 50% five year survival rate, largely depending on the development of metastasis. Currently, there is a significant unmet clinical need to identify early stage NSCLC patients at risk of developing metastasis, allowing more aggressive interventions.

Our previous work showed that hypoxia in the primary tumour is directly capable of promoting metastatic spread by secretion of factors into the blood circulation, causing systemic, pro-metastatic effects. Based on this knowledge, we postulated that hypoxia would modify the protein content of exosomes, thereby generating a protein signature capable of identifying patients at risk of developing metastasis. Using quantitative mass spectrometry to determine the content of exosomes secreted by hypoxic NSCLC cells, we found a number of proteins involved in metastatic progression of NSCLC to be at a higher abundance in hypoxic exosomes.

To determine if this exosome-based signature is capable of predicting disease progression in early stage NSCLC patients, exosomes were isolated form a discovery chemoradiation patient cohort. Comparing patients that relapsed before or after 18 months, the receiver operating characteristic (ROC) curves revealed that our hypoxic exosome signature was a perfect classifier, demonstrating a sensitivity and specificity of 100%. Exosomes were then isolated from an independent, surgically resected NSCLC patient cohort. In complete agreement with our discovery cohort, our protein signature was capable of accurately identifying patients with early relapse and those with no relapse within 5 years of initial therapy. Overall, this work has generated a novel, urgently needed predictive tool to identify NSCLC patients at risk of developing metastatic disease, allowing implementation of precise treatment decisions for early stage NSCLC

C5: Inhibitor of APoptosis proteins (IAPs) limit inflammation in the skin

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Inhibitor of APoptosis proteins (IAPs) play a crucial role in innate immunity by regulating TNF and Pattern recognition receptors families. Loss of IAPs, whether genetic or induced by IAP antagonist (smac-mimetic) drugs, reduces prosurvival signalling and can sensitise cells to apoptotic and necroptotic cell death induced by these receptors. As the skin is an important player in innate immunity we investigated the role of IAPs in skin development and homeostasis. We found that genetic deletion of cIAP1 in keratinocytes (*cIap1*$^{EKO/EKO}$) combined with ubiquitous cIAP2 deletion was lethal by post-partum day 10 and correlated with profound skin inflammation. To genetically investigate the role of other pathways regulated by IAPs in the skin is dauntingly expensive and prohibitively time consuming, therefore, I developed a novel method to explore the effects of IAP loss in the skin. I injected smac-mimetic subcutaneously into wt mice and followed the ensuing localised lesions as they developed and resolved. Subcutaneous injection of smac-mimetic induced a localised inflammation that mirrored the global inflammation observed in *cIap2*/*cIap1*$^{EKO/EKO}$ mice. We found that injection of smac-mimetic induced epidermal hyperplasia, cell death and increased production of pro-inflammatory cytokines. Interestingly loss of key effectors of the necroptotic cell death pathway such as RIPK1, RIPK3 and MLKL had different effects on lesion formation and resolution induced by smac-mimetic injection. For example, loss of MLKL did not prevent inflammation or cell death, but *Mlkl* KO mice resolved their lesions faster than WT mice suggesting a role for necroptosis in limiting healing. Consistent with our pharmacological approach, loss of one allele of *Ripk1* delayed onset of disease and significantly extended the lifespan of the *cIap1*$^{EKO/EKO}$*cIap2*$^{-/-}$*Ripk1*$^{+/-}$ mice. This result validates our method, and with drugs in development targeting these proteins, this work is defining new pathways for investigation into treating skin injury and inflammation.

C6: Cross-organism communication: stoichiometry and compatibility considerations

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Cross-organism effects of RNA have been well established in the model organism *Caenorhabditis elegans* and to a lesser extent in other species. For example, certain crop pests may be controlled when they feed on plant-incorporated or topically applied RNAi effectors. The possibility that dietary RNA might also function in mammals has led to a surge of renewed experimental activity despite previous negative results with oral siRNA delivery in the pharmaceutical industry. These studies have focused almost exclusively on microRNAs and other short RNA molecules. Several groups have indeed reported indirect evidence of dietary RNA uptake, while one lab has reported both uptake and function of dietary miRNAs. On the other hand, negative studies have been published. We examined uptake of dietary RNAs into blood of nonhuman primates and humans. Quantitative PCR and droplet digital PCR alike revealed no appreciable uptake of plant or mammalian miRNAs from the diet. We found further support for these findings through analysis of public sequencing datasets. Nevertheless, interest in cross-organism communication by RNA is likely to continue, especially in non-dietary situations. It may thus be helpful to identify aspects of hypothetical cross-organism relationships that are necessary for communication to occur. These include stoichiometry, packaging, and evolutionary considerations.

C7: Intercellular Mitochondrial Transfer: Genetic Approaches

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The transfer of mitochondria between mammalian cells is a physiologically relevant phenomenon, however the signals driving this process are not yet clear. Cellular stress during disease may alter the rate of mitochondrial transfer between cells and surrounding tissues. We hypothesise this form of intercellular communication to be fundamental to cell survival in disease, given the critical role of mitochondria within cell biology. Literature published in the field of intercellular mitochondrial transfer relies heavily on confocal microscopy to understand this process, however it is challenging to evaluate the frequency of transfer events by microscopy alone. In order to understand how cellular injury may alter the rate of mitochondrial transfer, two genetic tools have been developed. Application of these tools to trace and quantify the transfer of mitochondrial genomes among *in vitro* co-cultures provides quantitative data to support microscopic observations. These tools are highly sensitive, cost-effective and provide the throughput necessary to accurately evaluate how cellular injury alters mitochondrial transfer within a population of cells.

C8: Characterization of Mycobacterial Membrane Vesicles

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Tuberculous and non-tuberculous Mycobacteria release membrane vesicles (MVs), which are reported to range between 60-300nm in diameter and predominantly contain lipoproteins and polar lipids. It is hypothesized that MVs facilitate the delivery of virulence factors and function as "immune decoys" modulating host immune responses and contributing to more severe disease pathogenesis.

To better understand the biology of mycobacterial MVs (MMVs) we have undertaken the analysis of three mycobacterial species: *Mycobacterium smegmatis* (non-pathogenic, fast-grower), *Mycobacterium abscessus* (human pathogen, fast-grower) and *Mycobacterium marinum* (fish and opportunistic human pathogen, slow-grower). The *M. marinum*-zebrafish model has been proposed to be one of the best models to mimic and study human Tuberculosis.

MMVs were isolated by sequential centrifugation and purified by density gradient centrifugation. Composition (protein, DNA and RNA) in MMV subpopulations was assessed by commercial kits, size and concentration and release with respect to cell growth and viability were investigated with Nanoparticle tracking analysis (NTA) and Live/Dead fluorescence staining using a commercial kit. Electron microscopy was also used to image MMVs.

We isolated MMVs with diameters ranging between 80-180nm. MMVs were found predominantly in the least dense fraction for *M. marinum* and were found to be associated with DNA but not with RNA. *M. smegmatis* MVs were found in a denser fraction and were found to be associated with both DNA and RNA. MMVs were produced throughout growth, but with most produced at the transition between exponential and stationary phase. Viability experiments demonstrated that vesicles were produced by populations with a high percentage of live cells, suggesting vesicle production is an active biological process. Further investigation is required to identify other macromolecular components and to explore the *in vitro* effects of the vesicles and their individual components on host cells.

C9: Investigation of maternal natural killer cell intracellular signalling cascades during pregnancy

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CD56 natural killer (NK) cells are significant in maternal immune response during early pregnancy. In other parts of human body, CD56 NK cells kill aberrant cells by secretion of cytotoxins and certain cytokines. Maternal NK cells react differently to feta cells. In normal pregnancy, decidual NK cells do not kill the incoming fetal cells during implantation. Upon interaction with target cells, multiple signals from CD56 NK cell surface receptors regulate an intracellular signalling cascade. If there are sufficient activation signals, cytokines like IFN-γ, TNF-α, TGFβ2 and TGFβ3 secretion promote pre-eclampsia.

NK cell regulation relies on a dynamic interplay of signals from activation and inhibition receptors that are expressed on the cell surface. But, the mechanisms of interaction between NK cell activation and inhibition pathways are not well defined. This study uses computational modelling to investigate the interactions of NK cells' activation and inhibition receptors (NKG2D-DAP10 and CD94-NKG2A, respectively) with major histocompatibility complex class I polypeptide-related sequence A (MICA) ligand and human leukocyte antigen HLA-E. Ordinary differential equations are used to describe the relevant intracellular pathways.

Guanine nucleotide exchange factor (Vav1) is a key determinant of NK cell activation. Activation signals in NK cells induce Vav1 phosphorylation (pVav1), which facilitates immunological synapse formation between NK cells and target cells. Inhibition signals, on the other hand, mediate de-phosphorylation of pVav1 which blocks the activation signals. The formation rate of pVav1 is expected to increase with the accumulation of MICA and decrease with the present of HLA-E. The preliminary results of our model match qualitatively to the functional hypotheses. We are parameterizing this model to experimental data and it will be used to investigate the interactions between NK cells and their target cells.

C10: VEGF-A Release is Higher in Melanoma Cells Harbouring V600E BRaf Mutations

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Background VEGF-A is an essential mediator in tumour microenvironment for cancer progression and metastasis. Therefore, targeting its signalling pathway has been an important approach in cancers. However, the link between VEGF-A biology and the highly diverse genotypes of melanomas remains unclear.
Aims To study the link between VEGF-A release and melanoma mutations and how this information might be used to improve the current treatment.
Methods Melanoma cells were extracted from patients' biopsies and cultured under a physiologic 5% O_2. Genetic analysis was performed in 102 melanoma cell lines. VEGF secretion was measured by Milliplex kits. VEGF-A and VEGFR2 genes were knockout using CRISPR/Cas9 and inhibitors. Antitumorigenicity was studied in vitro and in vivo using immunodeficient mice.
Results Our melanoma line panel is representative of the spectrum of human melanoma as it has percentages of mutations similar to those previously described in melanoma tumours. Our most important observation was that VEGF-A secretion levels were significantly higher in melanoma cells harbouring BRAF/V600E mutation than wild type BRAF cells. Furthermore, vemurafenib upregulated VEGF-A secretion in RAS-mutant cell lines. This lead us to investigate the effects of vemurafenib in wild type BRAF xenografts. Strikingly, we observed that the RAS-mutant NZM40 xenograft grows faster with vemurafenib and that the VEGFR2 inhibitor axitinib has very little effect alone; but when vemurafenib and axitinib are combined, the combination has a very strong synergistic effect in inhibiting tumour growth.
Discussion/Conclusion Our study reports for the first time that melanoma cells containing V600E mutation have higher levels of VEGF-A secretion than other cells. In addition, our data also shows evidence about the link between VEGF-A secretion and vemurafenib-induced paradoxical growth of wild type BRAF tumours. Importantly, our data suggest that the efficacy and therapeutic utility for BRAF inhibitors might be significantly expanded by combination therapy with a VEGFR2 inhibitor.

C11: Fungal cell walls are dynamic structures that can enhance cell survival in the presence of antifungal molecules

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Fungal cell walls are central to the battle between fungal pathogens and innate immunity systems in both plants and animals. Innate immunity molecules work by exploiting differences between the host and the pathogen. There are a variety of differences between prokaryotic bacteria and eukaryotic hosts that can exploited in defence. However, fungi are eukaryotes and as such the differences are minimal. One of the major differences between fungi and host organisms is the fungal cell wall. Over the course of evolution there has been a perpetual tug-of-war between pathogens and hosts. That is, specific recognition of fungal cell walls by the host stimulates the host's innate immunity response including the production of antifungal molecules. On the other hand the fungal cell wall acts as a defence barrier that can physically block entry of the host's innate immunity molecules and senses changes induced by these proteins that in turn stimulates a defence response from the fungus. We have examined the interaction between various AMPs and components of the fungal cell wall to examine how AMPs transit this barrier. We have also discovered that AMPs trigger changes in the cell wall that protect the cell against the deleterious effects of antifungal molecules.

C12: Learning the language of the chloroplast: Retrograde signals that regulate stomatal and ABA responses

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The chloroplast is an environmental sensor for the cell, communicating with the nucleus via retrograde signals to change the expression of up to thousands of genes. Recent advances have identified carotenoid derivatives, phosphoadenosines such as PAP, tetropyrroles and heme, together with reactive oxygen species and proteins that build a communication network. To date, retrograde signaling pathways have largely been viewed as a means for bi-lateral communication between organelles and nuclei, ignoring the potential for interaction with hormone signaling regulating physiological processes. Our new findings on how organelle communication is initiated, transmitted and perceived to regulate not just chloroplast processes, but intersect with hormonal signaling will be considered. Specifically, how oxidative and redox stress is perceived by the SAL1-PAP pathway and how the retrograde signal, PAP, enables ABA-mediated stomatal closure. This is likely via a distinct pathway to ABI1/OST1. Thus, SAL1-PAP retrograde signaling regulates stomata closure and germination implicating PAP as a secondary messenger.

C13: A genetic analysis of TOR in plants: Novel facets of an ancient form of growth control.

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The TOR signalling pathway is pivotal to growth control in all eukaryotic groups, but its specific roles in plants remain obscure. We have used genetic and pharmacological approaches in Arabidopsis to dissect the relative roles of protein subunits that make up TORC1, a protein complex whose kinase activity is central to TOR regulated growth responses. Similar to other eukaryotic groups, the kinase encoding subunit TOR is essential for viability and growth in plants; however, the requirement for LST8 and RAPTOR, two well conserved kinase-associated scaffold proteins, differs. In contrast to animals and fungi, plants that lack intact forms of either of these proteins are viable, but grow extremely slowly. More refined analyses reveal distinctions between LST8 and RAPTOR, as well as plant specific aspects. LST8 deficient plants display increased sensitivity to specific light regimes, suggesting a role in linking photosynthetic processes with growth outputs. Additional plant specific aspects are also evident for RAPTOR, with genetic and expression analyses supporting growth related roles in not only the sporophyte, but also gametophytes. These results offer a useful platform for further studies aimed at understanding how this ancient pathway contributes to regulated patterns of growth in plants and how its activity might be optimised to enhance agricultural productivity.

C14: The catenins as a major component of the glucose sensitive insulin secretion system

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C15: Insulin secretion systems

Weiping Han,
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C16: Amylin agonists in diabetes and obesity

Debbie Hay
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C17: Understanding biased antagonism at the GLP-1 receptor

Patrick Sexton,
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C18: Mitochondrial genome transfer from the microenvironment to tumour cells without mitochondrial DNA enables tumour growth and metastasis in metastatic melanoma, in breast cancer and in a brain tumour model

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The view that genes are constrained within somatic cells is challenged by *in vitro* evidence, and by *in vivo* studies which show that mitochondria containing hundreds of copies of mitochondrial DNA (mtDNA) not only can, but do move between cells. Using metastatic melanoma (B16), breast carcinoma (4T1) and brain tumour (GL261) models lacking mtDNA (□^{o} cells) we investigated whether these glycolytic cells form tumours in syngeneic mice. Tumour growth was measured, and cell lines developed from primary tumours, circulating tumour cells (CTCs) and lung metastases used to investigate mitochondrial respiration, the presence of mtDNA polymorphisms and molecular and biochemical properties of the cells [1]. In each model, tumour growth was delayed and was shown to be associated with the presence of mitochondrially-encoded Cytochrome b. To exclude the possibility that latent mtDNA in □^{o} cells could explain the presence of mitochondrial genes, we showed that tumour cell lines contained mtDNA polymorphisms of the host mouse, distinct from those of the parental tumour. With the 4T1 model, respiration recovery was shown to be dependent on the local microenvironment from which the cell line was derived, but with the B16 model, full respiration recovery was seen in cell lines from all sites. Mitochondria from □^{o} cells that lack cristae were replaced by mitochondria with well-ordered cristae and biochemical and molecular markers of oxidative phosphorylation, including respirasome assembly, were restored following mtDNA acquisition. Mitochondrial transfer to □^{o} cells was demonstrated using cell co-culture and mice transgenic for mitochondrially-imported *dsRed*. We conclude that mitochondria move from cells in the local microenvironment to tumour cells lacking mtDNA and that this transfer is essential for tumour growth and metastasis. We propose that mitochondrial transfer between somatic cells is an evolutionarily-conserved physiological phenomenon used to address mtDNA damage.

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C19: Evasive manoeuvres: diversion and divergence in signalling pathways

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Pathogens are engaged in an arms race with their hosts, leading to an ever-expanding evolution of mechanisms by which they can subvert detection and clearance by their hosts. Recent work has uncovered such as strategy utilised by enteropathogenic bacteria to evade clearance by their mammalian hosts. Here, I will describe the role of a fascinating, conserved bacterial protease, which has evolved a function in cleaving and disabling the host proteins that promote cell death by the necroptosis (programmed necrosis) signalling pathway to avoid clearance by altruistic cell death. Moreover, these data support a role for necroptosis signalling in host response to pathogens, and raise interesting questions about selective pressures that have led to the loss of this pathway from some animals, including *Carnivora* such as the Tasmanian devil.

While pathogens are known to have evolved strategies to evade clearance by their hosts, recent studies indicate the same is true of rare, but devastating, transmissible cancers, such as that decimating the Tasmanian devil population. We have devised immunotherapeutic strategies to promote immune recognition of Tasmanian devil facial tumour disease cells as non-self. By capitalising on basic knowledge of immune signalling in the devil, we have developed a promising strategy to vaccinate and protect the devil population from further decline.

C20: The role of extracellular vesicles in neurodegenerative diseases

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Neurodegenerative disorders such as Alzheimer's (AD), Parkinson's (PD) and prion diseases are associated with proteins that misfold and deposit in the brain. Many cell types, including neurons, release extracellular vesicles (EVs) which include microvesicles and exosomes. EVs have been shown to be involved in processing of proteins such as APP, α-synuclein, and PrP which are those involved in AD, PD and prion diseases respectively. Roles for these vesicles include cell-cell signalling, removal of unwanted proteins, and transfer of pathogens (including prion-like misfolded proteins) between cells. Our group has shown that EV's contain distinct processed forms of these proteins and that, in the case of prion disease, they contain the transmissible form of the misfolded protein. In addition to their protein content these vesicles have recently been shown to contain genetic material in the form of protein coding (mRNA) and noncoding RNA species. We have analysed the protein and genetic cargo of EVs from a number of cell types and using deep sequencing, characterised the RNA cargo of these vesicles. As exosomes can be isolated from circulating fluids such as serum, urine, and cerebrospinal fluid (CSF), they provide a potential source of biomarkers for neurological conditions. This talk will review the roles these vesicles play in neurodegenerative disease and highlight their potential in diagnosing these disorders through analysis of their RNA content.

C21: Procoagulant and immunogenic properties of tumour exosomes, microvesicles and apoptotic vesicles

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Extracellular vesicles (EV) are lipid particles released from eukaryotic cells into the extracellular fluid. Depending on the cell type or mechanism of release, vesicles vary in form and function and exert distinct functions in coagulation and immunity. Tumor cells may constitutively shed vesicles known as exosomes or microvesicles (MV). Alternatively, apoptosis induces the release of apoptotic vesicles (ApoV) from the plasma membrane.
EV have been implicated in thrombotic events (the second highest cause of death in cancer patients) and tumour progression, but tumor vesicles may also contribute to the anti-cancer immune response. In this study we focused on the well characterized B16 melanoma model to determine the molecular composition, and the procoagulant and immunogenic potential of exosomes, MV and ApoV. Coagulation results obtained for melanoma ApoV were confirmed using an additional human or murine lung, prostate, colon, breast, and B and T lymphoma tumour cell lines.
Distinct patterns of surface and cytoplasmic molecules (tetraspanins, integrins, heat shock proteins and histones) were expressed between the vesicle types. Moreover, *in vitro* coagulation assays revealed that membrane-derived vesicles, namely MV and ApoV, were more procoagulant than exosomes. Tumour vesicles displayed a higher procoagulant activity compared to protein content normalised preparations of living or dying parental tumour cells. ApoV procoagulant activity required phosphatidylserine, tissue factor, Factor VII, and the prothrombinase complex, but Factor VIII and Factor IX were dispensable. Interestingly, Factor V, an integral component of the prothrombinase complex, was detected in the proteome of tumor-derived extracellular vesicles and determined to be functional in a sensitive thrombin generation assay. However, ApoV associated Factor V was non-essential for ApoV procoagulant activity and could not support fibrin generation in the absence of exogenous FV.
To determine the immunogenic activity of B16 extracellular vesicles, mice were immunized with antigen-pulsed extracellular vesicles and challenged with B16 melanoma. ApoV immunised mice challenged with B16 tumors were protected out to 60 days, while lower protection rates were afforded by MV and exosomes. Together the results demonstrate distinct phenotypic and functional differences between vesicle types, with important procoagulant and immunogenic functions emerging for membrane-derived MV and ApoV versus endosome-derived exosomes. This study highlights the potential of EV to contribute to the prothrombotic state, as well as to anti-cancer immunity[1].

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C22: Integrated methodology for purification, detailed biophysical characterization and phenotyping of extracellular vesicles from biological fluids

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Extracellular Vesicles (EVs) have ben shown to play important role in broad range of physiological and pathological processes. The size, number, membrane composition and contents of EVs are highly heterogeneous, dynamic and depend on the cellular source, state and environmental conditions. Thus, understanding the biophysical diversity in EV population is paramount for linking the impact of EV properties to its biological role and function.

Izon has developed an integrated methodology that utilizes simple Size Exclusion chromatography (SEC) based qEV columns for rapid EV purification from variety of biological fluids (urine, serum, plasma, CSF, cell culture etc.) followed by accurate, high-resolution particle-by-particle EV biophysical characterization (size, size range, concentration and surface charge) through qNano that utilizes Tunable Resistive Pulse Sensing (TRPS) technology. The SEC columns provide a convenient, reproducible and highly effective means of eliminating >99% of non-vesicular protein from biological fluid samples.

Moreover, the integrated methodology has been further developed and utilized for quantification of EV surface markers (CD9, CD63) and phenotyping of specific EVs retrieved from biological samples. Here, specific EVs are retrieved post SEC purification through antibody conjugated to magnetic beads. The resulting EV-Magnetic complex (Immunoprecipitate) is then directly analyzed and the extent of aggregation monitored through TRPS. Our results showed a proportional increase in size, volume and surface charge of the EV-Magnetic bead complex over a defined dose-range. Changes in physical properties indicated the positive selection of specific sub-population.

Thus, the proposed integrated methodology and TRPS based real-time monitoring of immunoreactions provides a simple, rapid, reliable, and cost efficient approach that has tremendous utility for biophysical characterization and EV based diagnostics.

C23: Extracellular vesicles in the transgenerational transmission of environmental effects

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The ability of environmental exposures to induce phenotypic change across multiple generations of offspring has gathered an enormous amount of interest in recent years. There are by now many examples of non-genetic transgenerational effects of environmental exposures, covering a broad range of stressors. Available evidence indicates that epigenetic inheritance may mediate at least some of these transgenerational effects, but how environmental exposures induce changes to the epigenome of the germline is unknown. One possibility is that exposed somatic cells can communicate their exposures to the germline to induce a stable change. We propose that extracellular vesicles shed by somatic cells represent a credible means by which environmental experience could effect a transmissible epigenetic change in the germline, leading to the inheritance of acquired traits. This idea will be discussed.

C24: Microparticles: immunomodulators, active players and potential biomarkers in immunopathology

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A hint for a role of membrane microparticles (MP) in immunopathology was adduced from experiments in which we attempted to understand the effector mechanisms of TNF in microvascular damage. Specifically, we investigate the significance of MP release in diseases characterised by pro-inflammatory cytokine overproduction and endothelial activation (*JAMA* 291:2542, 2004, *Nat Rev Immunol* 9:722, 2005). MPs are submicron elements which bud from the plasma membrane of most cells, in a process called vesiculation.

An effector role for MP is supported by the following evidence: 1./ MPs can alter endothelial cell phenotype and function (*FASEB J* 10:3449, 2009), 2./ MPs are strongly pro-inflammatory elements (*PLoS Pathog* 6:e1000744, 2010), 3./ blocking MP production is beneficial for endothelial integrity (*PLoS Med* 2:e245, 2005) and can prevent mortality due to experimental cerebral malaria (*PNAS* 105:1321, 2008), 4./ interfering with MP binding to target cells reduces their activation (*PLoS ONE* 5:e11869, 2010).

On this basis, using as vesiculation models human brain microvascular endothelial cells and monocytes, we set out to investigate the consequences of MP release (*Prog Neurobiol* 91:140, 2010). Using LPS activation of monocytes to model some aspects of sepsis, we found that LPS-induced monocytic MPs display a dual potential: enhanced pro-inflammatory and procoagulant properties, while protecting endothelial integrity. Our data obtained in other experimental models, notably of bacterial and viral infections, will also be discussed.

We then evaluated the immunomodulating potential of endothelial MPs themselves, which are found in high circulating numbers in inflammatory diseases. We showed that MPs can enhance T cell activation and potentially ensuing antigen presentation, thereby pointing toward a novel role for MPs in neuro-immunological complications of infectious diseases.

Our results illustrate the importance of MPs in cell-cell communication and indicate that they can be viewed as active players and biomarkers in a number of inflammatory pathologies.

C25: Exosomal signaling during pregnancy and ovarian cancer progression: small packages but big players

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The past decade has observed an extraordinary explosion of research in the field of extracellular vesicles (EVs), especially in a particular type of EVs originating from endosomal compartments, called exosomes. Exosomes are a distinct subtype of secreted vesicles which are defined as small (~30-120 nm) but very stable membrane vesicles that are released from a wide range of cells, including placental and cancer cells. Exosomes are correctly package with signaling molecules (including: protein, mRNA, micro RNA, and non-coding RNA) and are released by exocytosis into biofluid compartments. Exosomes regulate the activity of both proximal and distal target cells, including: translational activity, angiogenesis, proliferation, metabolism, and apoptosis. As such, exosomal signaling represents an integral pathway mediating intercellular communication. In the last five years, we have mainly focused on elucidating the role of exosomes during gestation and ovarian cancer progression. During pregnancy, the placenta releases exosomes into the maternal circulation from as early as six weeks of gestation. The release is regulated by factors that include both oxygen tension and glucose concentration and correlates with placental mass and perfusion. On the other hand, ovarian cancer is the most lethal gynecological cancer, and an effective early diagnosis has the potential to improve patient survival. The aim of this talk, thus, is to review the biogenesis, isolation, and role of nanovesicles; and their release from the placenta cells and tumour cells and their potential role during complication of pregnancy and ovarian cancer progression.

C26: Exosomes and tumorigenesis – regulating the tumour microenvironment

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The metastatic cascade describes the process by which tumour cells escape their primary site and colonize secondary locations. Tumour angiogenesis facilitates passage, and cells at the leading edge of the primary tumour are thought to undergo epithelial-mesenchymal transition (EMT) to acquire increased motility and invasiveness. Whether leading edge EMT cells directly promote endothelial cell recruitment and angiogenesis remains largely unknown, and the role of exosomes (30-150 nm diameter extracellular vesicles) in this process has not yet been explored. Using Ras-transformed epithelial cells (21D1 cells) that exhibit a complete EMT phenotype, and MDCK cells stably expressing YBX1 ($MDCK^{YBX1}$) that display an intermediate EMT phenotype, we examined the effects of exosomes from these cells on recipient endothelial cells. Firstly, we monitored in vitro cell motility and tube formation of 2F-2B cells supplemented with exosomes. MDCK exosomes had no effect, while $MDCK^{YBX1}$and 21D1 exosomes significantly enhanced 2F-2B cell motility, and increased the tube length and tube branch points. Next, exosome-supplemented 2F-2B cells were subcutaneously injected as Matrigel™ plugs into NOD/SCID mice. After 21 days, tail vein injection of FITC-dextran revealed that only 2F-2B cell plugs treated with $MDCK^{YBX1}$and 21D1 exosomes were perfused systemically. Comparative proteomic analysis highlighted that 21D1 exosomes contained VEGF-associated proteins (NRP1, NRP2, and TNFRSF12A), while $MDCK^{YBX1}$ exosomes were enriched with activated Rac1 and PAK2. To validate this, 2F-2B cells and HUVECs were pre-treated with PAK inhibitors then supplemented with exosomes. While PAK inhibitor treatment did not significantly impede tube formation promoted by 21D1 exosomes, tube length and branching was reduced to baseline levels despite treatment with $MDCK^{YBX1}$ exosomes. Our results demonstrate for the first time that oncogenic cells undergoing EMT can communicate with endothelial cells via exosomes, and establish exosomal Rac1/PAK2 as angiogenic promoters that may function from very early stages of the metastatic cascade.

C27: Serum exosomal RNA as a biomarker in multiple sclerosis

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Multiple Sclerosis (MS) is an idiopathic chronic inflammatory demyelinating disease of the central nervous system that leads to progressive motor and cognitive disability. It is the most common nervous system disease of young adults, with diagnosis usually between the ages of 20-40 years, and there are currently over 26,000 people living with MS in Australia and New Zealand. Disease activity monitoring is crucial to ensure effective disease control with the aim of avoiding transition to the secondary progressive form which is not amenable to treatment. Currently, disease activity monitoring is via expensive MRI scans performed every 6-12 months. There is no blood-based assay to detect the disease, to monitor its progression, or to judge therapeutic response. We are studying small noncoding RNAs derived from exosomes in patient serum to assess their potential as biomarkers in MS. At present we have recruited over 160 MS patients from one of Australia's largest MS multidisciplinary clinics. Next generation sequencing of a subset of patients has identified multiple microRNAs and other small noncoding RNAs as potential disease biomarkers. Currently efforts are concentrated on validating the panel of changes in a larger number of patients of varying disease progression, with the goal of producing specific and sensitive assays of MS disease activity in blood samples.

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C28: Placental trophoblastic debris mediates feto-maternal communication via small RNA delivery: implications for preeclampsia

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Introduction: Preeclampsia is a pregnancy complication characterized by maternal systemic endothelial cell dysfunction. The surface of human placenta is covered by a single multinucleated cell, the syncytiotrophoblast, which extrudes debris into the maternal blood. The small RNA contents of this trophoblastic debris (TD) are thought to play an important role in the pathology of preeclampsia[1,2]. The aims of this study were 1) to identify small RNAs that were differentially expressed between preeclamptic and normal TD and 2) to investigate how these RNAs could affect endothelial cell function.
Methods: Small RNA in TD from four preeclamptic and control placentae was sequenced and differentially expressed miRNAs were validated by qRT-PCR. To investigate the function of individual miRNAs on HMEC-1 endothelial cells, HMEC-1 cells were treated with TD that had been transfected with miRNA mimics. The expression of these miRNAs and their predicted target mRNAs were quantified by qRT-PCR in the treated cells.
Results: The levels of 86 miRNAs were significantly different between preeclamptic and control TD. Eight miRNAs were further validated by qRT-PCR, including miR-145 which was significantly up-regulated in preeclamptic TD. Quantitative RT-PCR also confirmed that miRNA mimics were transfected into placental explants and present in the TD released from those explants. Trophoblastic debris could also directly deliver the mimics to HMEC-1 cells. Following exposure to miR-145 transfected TD, HMEC-1 cells expressed significantly lower levels of angiogenic gene, *ANGPT2* and the anti-apoptosis gene, *BIRC3* compared to control TD exposed HMEC-1 cells. This mimicked the transcriptomic changes we observed in preeclamptic TD treated HMEC-1 cells. The effect on the angiogenesis pathway was also validated at the protein level.
Discussion Trophoblastic debris can directly deliver its small RNA contents to maternal endothelial cells. Our data suggest that dysregulated miRNAs in preeclamptic debris such as miR-145 could directly contribute to maternal endothelial cell dysfunction.

References:

1. Redman CW, Tannetta DS, Dragovic RA, et al. Review: *Does size matter? placental debris and the pathophysiology of pre-eclampsia*. . 2012;33 Suppl:S48-54.
2. Escudero CA, Herlitz K, Troncoso F, et al. *Role of extracellular vesicles and microRNAs on dysfunctional angiogenesis during preeclamptic pregnancies*. *Front Physiol*. 2016;7:98.

C29: Extracellular vesicles – the next small thing in epigenetic inheritance

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Epigenetics is the study of mitotically heritable changes in gene expression that are not caused by changes in DNA sequence. Environment effects such as diet and stress are able to affect an individual's epigenotype and phenotype. These effects can persist beyond the initial exposed generation, suggesting that a signal is passed through the germline. However it is not known what the signal is, or its mechanism of transmission. We propose that the signal is somatic-derived non-coding RNA that is packaged inside extracellular vesicles (EVs)[1]. We have started to test this hypothesis *in vitro* using Sertoli cells, a germline-associated somatic cell. We aimed to determine if Sertoli EVs interact with germ cells, and investigate whether environment stress alters Sertoli EV small RNA cargo.

We exposed Sertoli cells to dimethyl sulfoxide (DMSO) and bisphenol A (BPA), a known reproductive toxin, for 12 days. EVs were isolated by differential centrifugation and filtration, and analysed by transmission electron microscopy, nanoparticle tracking analysis and protein mass spectrometry. EVs were labelled with fluorescent cytosolic and membrane dyes, cocultured with spermatogonial stem cells, and then visualised by confocal microscopy. RNA was extracted from cells and EVs, small RNA libraries were constructed, and then sequenced on an Illumina HiSeq 2500. Differential expression analysis was performed in R using the edgeR package.

Here we show that Sertoli EVs are able to bind and fuse with spermatogonial stem cells *in vitro*. Exposure to both BPA and DMSO changed the number of EVs released per Sertoli cell. Additionally, exposure altered the selective packaging of small RNA inside the EVs, with differentially expressed microRNAs implicated in defective germ cell renewal and maintenance. Taken together, this suggests that EVs may be transmitting information about the environment from the soma to the germline – that EVs are a vector for epigenetic inheritance.

1. Eaton, S. A., Jayasooriah, N., Buckland, M. E., Martin, D. I., Cropley, J. E., & Suter, C. M. (2015). *Roll over Weismann: extracellular vesicles in the transgenerational transmission of environmental effects*. Epigenomics, 7(7): 1165-1171.

C30: Elucidating the host-microbe interplay in chronic rhinosinusitis

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Chronic rhinosinusitis (CRS) is a group of chronic inflammatory sinonasal diseases which significantly reduce quality of life. CRS affects approx. 5% of the Western population and costs $8.6 billion p.a. in direct medical expenditures in the USA alone. Despite an acknowledged involvement of bacteria in this disease, the precise relationship between sinus bacteria and the resultant inflammation is not well understood. This lack of understanding is in part due to an historical reliance on the culture of mucosal swabs. We are taking a multi-pronged approach, incorporating analysis of the sinus microbiome, inflammatory markers and *in vitro* cell culture in order to better understand this complex condition.

Bacterial communities and local inflammatory response were assessed in 106 patients with CRS undergoing endoscopic sinus surgery (ESS) and 29 controls undergoing ESS for indications other than CRS. Controls and idiopathic CRS subjects tended to be dominated by members of the genera *Corynebacterium* and *Staphylococcus*, together with lower abundances of several other genera. Aberrant (dysbiotic) bacterial assemblages and increased inter- and intra-patient variability were more common in subjects with comorbidities such as asthma and cystic fibrosis, together with distinct associated immune profiles. Dysbiotic communities were variably dominated by genera such as *Staphylococcus*, *Haemophilus* or *Fusobacterium*.

To further explore the host response in CRS, we analysed inflammatory cytokines, bacterial community structure, and bacterial abundance in sinonasal mucus of patients with and without CRS. In addition, the activation of toll-like receptors (TLR) was measured *in vitro* using transfected human embryonic kidney cells. TLR activation was correlated both with overall bacterial abundance and the presence of specific members of the microbiota.

Taken together, our microbiological and immunological data are providing novel, clinically translatable insights into the interplay between host and microbe in CRS.

C31: Membrane vesicles as vectors for cross-kingdom communication

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Bacterium-to-host signalling during an infection manipulates host cell biology for pathogen survival. Bacteria release membrane vesicles (MV) that can carry a cargo of effector molecules directly into host cells. We hypothesise that these MVs associate with RNA, which may be directly involved in the modulation of the host response to infection.
Using uropathogenic *Escherichia coli* as a model we have isolated MVs and found they carry a range of RNA species. Density gradient centrifugation further fractionated and characterised the MV preparation and confirmed that the isolated RNA was associated with vesicle-rich fractions. RNA-sequencing of RNA populations (<50nt, 50-200nt and 200nt+) isolated from MVs shows carriage of rRNA, tRNAs, other small RNAs as well as full-length protein coding mRNAs. Confocal microscopy visualised the delivery of lipid labelled MVs into cultured bladder epithelial cells and showed their RNA cargo, labelled with 5-ethynyl uridine, was transported into the host cell. MV RNA uptake by the cells was confirmed by droplet digital RT-PCR of bacterial csrC. Microarray analysis has identified transcriptional changes induced by challenge with MV-RNA and has allowed us to hypothesise a role for MV-RNA in the subversion of antimicrobial responses from the host cell.
Using *Mycobacterium* species as a model we have expanded our knowledge of the range of pathogens that release RNA that is associated with MVs.

Summary of Abstracts for the Poster Session

No.	Title	Presenter	Institutions
C7	Intercellular Mitochondrial Transfer: Genetic Approaches	Matthew Rowe	Victoria University of Wellington, NZ
C8	Characterization of Mycobacterial Membrane Vesicles	Vanessa Chang	The University of Auckland, NZ
C9	Investigation of maternal natural killer cell intracellular signalling cascades during pregnancy	Nurul Izza Ismail	Auckland Bioengineering Institute, UoA, NZ
C10	VEGF-A Release is Higher in Melanoma Cells Harbouring V600E BRaf Mutations	Khahn Tran	The University of Auckland, NZ
Q32	Statins as novel therapeutic agents in melanoma	Khahn Tran	The University of Auckland, NZ
Q33	Immune cell-derived microparticles promote inflammatory phenotypes of endothelial cells lining the blood brain barrier	Angelica Panopoulos (Georges Grau)	The University of Sydney, AU
Q34	Characterisation of the microparticle miRNA cargo during experimental cerebral malaria: one more clue towards understanding the pathogenesis?	Amy Cohen (Georges Grau)	The University of Sydney, AU
Q35	Methods of Isolating exosomes and the impact on their functional analysis	S. Sharma (Carlos Salomon)	University of Queensland, AU
Q36	Exosomes isolated from ovarian cancer cultured under hypoxic conditions contribute to chemotherapy resistance	Mona Alharbi (Carlos Salomon)	University of Queensland, AU