Synopsis of Approved Projects that are Available for Commencing in Semester 1 2021:

PROJECT DETAILS

Project Title: Characterising asthma and airway remodelling using a humanised 'lung-on-a-chip' platform.

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Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

Background: Asthma is the most common chronic respiratory disease worldwide and afflicts more than 300 million people. The key pathologic hallmark feature of asthma is airway wall remodelling involving structural changes in epithelial, endothelial, and smooth muscle cells. A major cause of airway remodelling is air pollution, exposure to dust particulates (diesel, coal, silicon etc) and viral exacerbation that lead to ER stress. In order to determine how these factors impact airway remodelling, ER stress and then develop targeted therapies beyond just targeting immune cells we need better humanised systems. Current mono-culture cell systems cannot recapitulate this important phenotype or model tissue level interaction. What is required to address this gap in the field is a human tissue model or 'lung-on-a-chip'.

Hypothesis: Current cellular model systems are not sufficient to re-create one of the most important features of asthma, the chronic airway remodelling induced by ER stress. We hypothesise that using a 'lung-on-a-chip' platform will better model the interactions of tissue remodelling, including the impact of airflow and multi-cell type interaction.

Aim 1. Use 3-dimensional lung organoids grown from stem cells to generate 4 airway cell types (airway epithelial cells, blood vessels, fibroblasts, and smooth muscle cells) then add these cell types to the 'lung-on-a-chip' micro-physiological device. **Aim 2.** Use the 'lung-on-a-chip' to model the impact of specific inhaled asthma triggers on ER stress, multi-cell type interaction and tissue remodelling.

Experimental approach: Completion of the aims will involve using our in-house established methods for lung organoid growth and adapting them into the 'lung-on-a-chip' platform using Mimetas micro-physiological devices. This will involve 3-dimensional cell culture, immunofluorescent staining with live cell imaging, tissue clearing techniques and flow cytometry. The second phase of the project will involve utilising the in-built chip channels for airflow capacity to assess how inhaled pollutants and virus can impact tissue remodelling physiology including oedema, smooth muscle hypertrophy, and fibrosis.

Laboratory Location: HMRI Level 2 East

PROJECT DETAILS

Project Title: Characterising the role of airway chemosensory cells in lung cancer using 3D lung organoids and 'lung on a chip' technology

Supervisory Details:
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Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

Background: Lung cancer is the most common cause of cancer mortality in Australia and the fifth most common cancer diagnosis. Advancements in therapy and early diagnosis that have led to significantly improved outcomes for other solid tumours have been lagging in lung cancer. Although smoking is a strong risk factor for lung cancer up to a third of new cases are not smoking-related. There are at least five known subtypes of lung cancer but there are potentially more as there is a strong unmet clinical need to improve diagnostic markers for molecular phenotyping to tailor individual patient therapies. We have identified a new subset of airway epithelial cells (airway chemosensory cells) both *in vitro* and *in vivo* and characterised their molecular signature by single cell RNA-seq (scRNA-seq) from patient airway tissue. Computational data mining shows that this airway chemosensory cell has an RNA transcriptional signature that strongly overlaps with a subtype of lung cancers.
Hypothesis: Airway chemosensory cells are a cell or origin for a subtype of lung cancer and their molecular signature may offer new diagnostic biomarkers and potential therapeutic targets.
Aim 1. Biomarker analysis: To confirm at the protein level whether our signature markers of airway chemosensory cells are specifically enriched in lung biopsies taken from multiple subtypes of lung cancer. **Aim 2.** 3-dimsensional lung organoids and lung-on-a-chip: To determine whether enrichment of airway chemosensory cells in 3D lung organoids and lung-on-a-chip can be used as an *in vitro* model system to study the origin of a lung cancer subtype, and function of our scRNA-seq signature genes.
Experimental approach: Completion of Aim 1 will require immunohistochemical and immunofluorescent antibody analysis of lung tumour sections supplied by the Hunter Cancer Biobank/NSW Health. This will involve staining and quantification of the protein expression of our scRNA-seq signature chemosensory markers in samples from lung squamous cell carcinoma, adenocarcinoma, large cell carcinoma, small cell lung cancer, and neuroendocrine lung carcinoid tumours. Completion of Aim 2 will involve using our in-house established methods for lung organoid growth and chemosensory cell differentiation in 3D cultures and in the lung-on-a-chip platform. We will test whether genetic inhibition (shRNA) of our specific scRNA-seq signature genes can inhibit cell proliferation and lead to cell death and thus identify new targets for the treatment of specific lung cancer subtypes from Aim 1.

Laboratory Location
HMRI Level 2 East

PROJECT DETAILS

Project Title: Uncovering the mechanisms by which (P)RR promotes placental development

Primary Supervisor Name: A/Prof. Kirsty Pringle
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Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

Preeclampsia affects around 3-5% of all pregnancies and is associated with multiple adverse maternal and fetal outcomes, including preterm birth and fetal growth restriction. It is widely accepted that the pathophysiology of preeclampsia can be attributed to inadequate placental development. Based on our extensive published and pilot data, we propose the (pro)renin receptor ((P)RR) is essential for appropriate placental development. The (P)RR is a multi-functioning receptor that stimulates tissue growth, migration and angiogenesis, and is found in high levels in the developing placenta. The (P)RR can activate the renin-angiotensin system pathway, which predominately acts through the angiotensin II type 1 receptor (AT_1R), and stimulate the Wnt/B-catenin signalling pathway. Furthermore, the (P)RR forms part of the vacuolar-H^+-ATPase, which is critical for cell survival.

In preliminary studies, we have demonstrated that knocking down the (P)RR using an siRNA inhibits placental trophoblast proliferation, migration and invasion, suggesting that the (P)RR is critical for appropriate placental development. This honours project aims to identify the mechanisms through which the (P)RR is acting to promote trophoblast proliferation, invasion and angiogenesis using *in vitro* models.

This project will utilise both the HTR-8/SVneo cells and first trimester human primary extravillous trophoblast cells (EVTs). Cells will be treated for 48h, in the presence or absence of either: a (P)RR targeting siRNA, a specific AT_1R antagonist, losartan, an inhibitor of Wnt Production (IWP-2) or Bafilomycin, a V-ATPase inhibitor.

The effects of the (P)RR siRNA, AT_1R antagonist, Wnt and V-ATPase inhibitors on (P)RR induced trophoblast proliferation, migration and invasion will be measured in both HTR-8/SVneo cells and first trimester primary EVTs using the xCELLigence RTCA DP System. To determine if these treatments affect placental angiogenesis, conditioned media will be collected from primary EVTs and HTR-8/SVneo cells and cultured with human umbilical vein endothelial cells (HUVECs). The effects of each treatment on the ability of trophoblasts to regulate angiogenesis in HUVECs will be assessed using an endothelial tube formation assay and analysed by an Angiogenesis Analyzer.

Laboratory Location

HMRI Level 3 East

PROJECT DETAILS

Project Title: The role of beta adrenergic receptors in breast cancer

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Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

Nerves and associated neurosignalling are emerging promoters of cancer growth and metastasis. Our laboratory has obtained preliminary data showing that beta adrenergic receptors are expressed in breast cancer. The hypothesis of this project is that beta adrenergic receptors are involved in breast cancer cell growth and dissemination, and that they could be targeted to treat breast cancer. The aims will be: i) Clarify the expression of beta adrenergic receptors in breast cancer. This will be done by using IHC and tumour microarrays of human breast tumours of various histological subtypes. ii) Define the impact of targeting beta adrenergic receptors on breast cancer cell growth and invasion. This will be done with breast cancer cell lines in vitro by using pharmacological inhibitors and siRNA. All methodologies used in this project are routinely used in our laboratory. We anticipate that the expression of beta adrenergic receptors will be associated with breast cancer aggressiveness and that targeting them will decrease growth and survival of breast cancer cells.

Laboratory Location Life Science Building

PROJECT DETAILS

Project Title: The role of cholinergic receptors in breast cancer

Supervisory Details:
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Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

Nerves and associated neurosignalling are emerging promoters of cancer growth and metastasis. Our laboratory has obtained preliminary data showing that cholinergic receptors are expressed in breast cancer. The hypothesis of this project is that cholinergic receptors are involved in breast cancer cell growth and dissemination, and that they could be targeted to treat breast cancer. The aims will be: i) Clarify the expression of cholinergic receptors in breast cancer. This will be done by using IHC and tumour microarrays of human breast tumours of various histological subtypes. ii) Define the impact of targeting cholinergic receptors on breast cancer cell growth and invasion. This will be done with breast cancer cell lines in vitro by using pharmacological inhibitors and siRNA. All methodologies used in this project are routinely used in our laboratory. We anticipate that the expression of cholinergic receptors will be associated with breast cancer aggressiveness and that targeting them will decrease growth and survival of breast cancer cells.

Laboratory Location Life Science Building

PROJECT DETAILS (FOR CIRCULATION TO STUDENTS – all fields must be completed)

Project Title: Regulation of s(P)RR release from the placenta

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Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

Preeclampsia affects around 3-5% of all pregnancies and is associated with multiple adverse maternal and fetal outcomes. It is widely accepted that the pathophysiology of preeclampsia can be attributed to inadequate placental development. In women with preeclampsia, there is shallow implantation of the placenta into the uterus resulting in inadequate invasion and remodelling of the maternal uterine spiral arteries. This compromises placental perfusion and leads to fluctuations in oxygen delivery that cause oxidative stress. What is seen to be the disease is in fact the maternal response to placental stress.

We have recently shown that the human placenta releases the soluble form of the (pro)renin receptor (s(P)RR). Soluble (P)RR is found in the circulation, and its levels are increased in women with preeclampsia. Recent studies have demonstrated that there is a strong positive correlation between maternal s(P)RR levels and maternal blood pressure and urinary protein during pregnancy, and a negative association between maternal s(P)RR and estimated glomerular filtration rate (eGFR), implicating s(P)RR in the pathophysiology of preeclampsia. We propose these high levels of circulating s(P)RR in pregnancy disorders such as preeclampsia result from increased placental secretion due to the increased levels of oxidative stress. Other factors have been shown to regulate s(P)RR levels in the kidney, including arginine vasopressin (AVP). AVP levels are significantly higher in women who have preeclampsia. Therefore, we propose that AVP may also increase levels of s(P)RR from the placenta in women with preeclampsia.

This study will test the hypothesis that oxidative stress and/or AVP stimulates the release of s(P)RR from the placenta. Term primary trophoblast cells from uncomplicated pregnancies will be isolated and cultured with xanthine/xanthine oxidase (X/XO) to induce oxidative stress or cultured with AVP. Levels of (P)RR (both intact and soluble) will be measured by qPCR and western blotting. We will then determine whether oxidative stress or AVP increase the levels of proteases known to cleave s(P)RR within the placenta (furin and site 1 protease) as a mechanism of its increased release in women with preeclampsia.

Laboratory Location

HMRI Level 3 East

PROJECT DETAILS

Project Title: Development of Dried Blood Spot (DBS) Assays for Tyrosine Kinase Inhibitors

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Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

Tyrosine kinase inhibitors (TKIs) are anti-cancer drugs that target enzymes involved in signal transduction processes that drive tumour cell proliferation, invasion and metastasis. Currently, all TKIs are administered to cancer patients in a fixed dose, despite observed large interindividual variability in their pharmacokinetics. For several TKIs, the minimum effective drug concentration for efficacy and a maximum concentration to reduce toxicity have been defined. Therapeutic drug monitoring (TDM) (where blood concentrations are measured to personalise dosing) is currently not used in Australia for TKIs. TDM involves development of analytical methods capable of measuring drug concentrations in venous blood/plasma. Recently, the use of fingerprick blood samples (dried blood spot) techniques has emerged as an alternative to collection of venous blood samples for drug monitoring. This approach enables patients to collect blood samples at home and post these to the laboratory.

Hypothesis: Developing Dried blood spot drug analysis for TKIs would support implementation of TDM for these drugs in Australia

Aims:

- To develop HPLC-MS/MS analysis techniques suitable for use in TDM of TKIs
- To develop and evaluate DBS techniques for monitoring TKI blood concentrations

This project involves developing analytical techniques for TKIs using liquid chromatography and tandem mass spectrometry. It would also develop different DBS techniques that could be used by patients or health professionals to monitor drug concentrations and provide the most effective treatment of their cancer.

Laboratory Location HMRI Level 3 West wing

PROJECT DETAILS

Project Title:

Supervisory Details:
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Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

Patients with Inflammatory bowel disease (IBD) are at increased risk of infectious complications and this suggested to be a side-effect of immunosuppressive drugs, such as anti-TNF therapy or corticosteroids, used to manage intestinal inflammation. In particular, respiratory infections in IBD patients are more likely to develop into severe pneumonia often requiring hospitalisation for treatment and discontinuation of IBD medications. However, recent studies have shown that over one third of IBD patients have subclinical pulmonary inflammation not detected in routine clinical investigation and our work in animal models suggests that this is directly related to intestinal inflammation as part of the gut-lung axis. This occurs independently of treatment and increases expression of microbial receptors in the lung which may in turn increase the risk of secondary infections. Importantly, our work has shown that this process can be inhibited by targeting microbial receptors in the lung with small molecule inhibitors.

Given that our models show pulmonary inflammation in the absence of IBD therapies, we hypothesise that intestinal inflammation and not IBD therapies increase the risk of lung infection but suggest that these therapies may affect the clearance of these infections. Using animal models of colitis, we will examine whether IBD therapies increase the severity of secondary infection with respiratory pathogens and whether inhibiting microbial receptors in the lung can improve outcomes in these models.
This study may clarify the cause of a serious clinical problem in IBD patient management and identify a new therapeutic approach to preventing severe respiratory infection in these patients

Laboratory Location: This project will be conducted in the Hunter Medical Research Institute as a collaboration between the Keely Lab and the Horvat Labs. The student will utilise the expertise, support and models of both labs.

Project Title: P53's little helpers and their role in regulating cellular stress responses.

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Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

Stress responses are crucial for normal cellular function. They enable cells to repair damage or initiate apoptosis if the damage is too severe. One protein that is central to cellular stress responses is p53. P53 is one of the most studied transcription factors. It is colloquially known as "guardian of the genome" due to its role in regulating cell cycle arrest and DNA repair in response to mild DNA damage or coordinating apoptosis following severe DNA damage. However, p53 orchestrates many other processes: P53 regulates more than 3,600 genes, regulating not only cell cycle arrest, DNA repair, and apoptosis, but also cell transformation, angiogenesis, cell differentiation and stemness, embryo implantation, inflammation, and metabolism. Clearly the roles of p53 are highly versatile. Until 2005 it was thought that p53 alone was responsible for determining how a cell would respond to an environmental stressor. However, in 2005 Dr Bourdon, a collaborator on this project, discovered that p53 is expressed as full-length p53 and as N- and C-terminally truncated isoforms. The isoforms have been found to regulate p53's function in both a positive and negative manner, contributing to cell fate decisions.

This project will focus on cell culture and functional assays to investigate the hypothesis that p53 isoforms are expressed in a stressor-specific fashion and guide cells towards a specific response in human cell lines.

Aim 1: Through this project, we will determine which isoforms are expressed in response to a range of stressors (hypoxia, nutrient deficiency, heat shock, UV radiation, oxidative stress, and DNA damage) using RNA (RT-qPCR) and protein (Western blot) analysis methods.

Aim 2: We will also determine which cellular response is associated with the expression of a particular isoform through an array of functional assays, including assays to measure apoptosis, proliferation, DNA damage, and reactive oxygen species generation.

The new knowledge gained through this project will contribute to a better understanding of p53 isoforms. This understanding will pave the way for the development of pharmaceuticals to target p53 isoforms and thereby favourably modify cellular responses to environmental stressors.

Laboratory Location: This work will be conducted at the Hunter Medical Research Institute (Level 3 West), which houses many successful molecular biologists and state-of-the-art equipment.

PROJECT DETAILS

Project Title: Investigation of hypothalamic control of binge eating behaviour

Supervisory Details:
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Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

Eating disorders are a group of mental health illnesses where by eating, exercise and body weight preoccupy an individual's thinking creating significant distress. It is estimated that more than a million Australian's suffer from an eating disorder, and eating disorders have the highest mortality rate of any mental illness. Binge eating refers to uncontrollable consumption of large quantities of food in a short period of time and is a cross-diagnostic feature of eating disorders. Stress is thought to play an important role in eating disorders, triggering bouts of binge eating. Importantly, the mechanisms in the brain that link stress and binge eating are poorly understood.

The hypothalamus is a brain region that contains distinct cell populations that express different signalling peptides that play an important role in control of feeding (orexin cells) and stress (corticotrophin releasing hormone cells). Recent studies suggest that these populations interact, which may play a role in the stress-induced binge eating. Using optical control and/or visualization of distinct hypothalamic populations during the evolution of pathological binge eating behaviour in a preclinical model, the goal of this project is to better understand the neural control of binge eating behaviour to guide the development of novel treatment approaches. Transgenic mouse lines established in the Dayas group allow selective targeting of hypothalamic populations involved in feeding and stress, and fiber photometry and optogenetics allow temporally precise visualization and control of neural activity in these populations during feeding and stress related behaviours. The successful applicant will receive training to complete behavioural testing and analysis necessary for these studies, as well as exposure to stereotaxic surgery and calcium imaging analysis related to the project. Our hypothesis is that dysregulation of these stress-relevant neuropeptide systems contribute to binge eating and that aberrant patterns of neural activity underpinning these behaviours can be detected and targeted using fibre photometry and optogenetics.

Laboratory Location

MSB (Callaghan campus)

PROJECT DETAILS

Project Title: Understanding the link between Chlamydia, Pelvic Inflammatory Disease and Chronic Pelvic Pain

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Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

Pelvic pain is a common and debilitating condition in women. While an important subset of women have endometriosis, the majority of women sufferers have chronic pelvic pain of unknown aetiology. One of the high risk factors for chronic pelvic pain is inflammation of the female reproductive tract (FRT), commonly in the form of pelvic inflammatory disease (PID). PID is prevalent in young women and is most strongly associated with sexually transmitted infections with Chlamydia and/or gonorrhoea. How this acute phase of pelvic inflammation progresses to intractable chronic pain remains a mystery. We have developed a mouse model of Chlamydia-induced PID to characterise and investigate the inflammatory and neurological processes involved in the transition from PID to chronic pelvic pain.

Hypothesis: Chlamydia-induced PID alters spinal cord sensory neural circuits, resulting in allodynia and hyperalgesia which persist after infection and inflammation resolves.

We will explore this hypotheses through the following specific ***Aims***:

1. Profile the immunological responses in the FRT and spinal cord at all stages of PID.
2. Quantify behavioural changes associated with the progression of PID.
3. Measure neuroanatomical and functional changes in the sensory pathways that convey noxious and non-noxious information from the FRT to the dorsal horn.

Experimental approach: We will infect mice with Chlamydia and monitor immune activation, FRT pathology, pain behaviour and dorsal horn neurophysiology at different time points. We will use home cage video monitoring to assess behavioural changes associated with pain. We will assess immune and neurobiological (activation of neurons and microglia) responses in both the FRT and spinal cord using qPCR and immunofluorescence. Hyperalgesia will be assessed by measuring reflex responses to non-noxious stimuli in anaesthetised mice. The induction of immune factors throughout infection will then be correlated with the development of pain responses in our model.

Laboratory Location: Level 2 East Wing HMRI Building

PROJECT DETAILS

A) Project Title:

Do environmental pollutants trigger premature development of brain neurodegenerative changes through iron-related mechanisms and is this reversible?

B) Supervisory Details:

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C) Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach: *(MAX HALF-PAGE).*

Fine/ultrafine particulate matter (e.g. $PM_{2.5}$) in coal dust and combustion emissions from vehicles, industry and bushfires can penetrate and damage tissues.[1,2] The 2018 Lancet Commission on pollution and health[1] has identified potential effects of $PM_{2.5}$ in neurological conditions such as Alzheimer's disease as the top pollution-related health research priority.[1] A US Environmental Protection Agency report suggests pollutant $PM_{2.5}$ toxicity involves interactions with tissue iron.[2]

Research from Mexico City[3] suggests high $PM_{2.5}$ levels in air pollution trigger Alzheimer's-related changes during childhood, leading to memory problems as early as the teens and early twenties. Exposure to extreme levels of $PM_{2.5}$ from bushfire smoke may have similar effects. It is thus urgently importance to investigate the effects of air pollutants on the brain and the mechanisms involved.

The project will investigate the Hypothesis that exposure to high levels of fine/ultrafine particulate matter in coal dust or bushfire smoke and other combustion-derived emissions increases Alzheimer's-like changes in the brain by mechanisms involving iron which may be partly reversible initially but lead ultimately to irreversible changes.

The project aims to investigate this hypothesis by studying Alzheimer's-related changes in the brains of wildtype mice or genetic mouse models of Alzheimer's disease amyloidogenesis with normal or increased brain iron loading exposed to vehicle alone or to fine/ultrafine particulate matter from coal dust, bushfire smoke and other combustion-derived emissions.

1. Landrigan P.J. *et al*. The Lancet Commission on pollution and health. Lancet 391, 462-512 (2019).
2. Ghio A.J. *et al*. Air pollution particles and iron homeostasis. Biochimica et Biophysica Acta 1860, 2816–2825 (2016).
3. Calderón-Garcidueñas L.M. *et al*. Alzheimer's disease starts in childhood in polluted Metropolitan Mexico City. A major health crisis in progress. Environmental Research 183:109187 (2020).

Laboratory Location Research will mainly be in MS516, MS307C and MS609 and may also involve visits to collaborator A/Prof. Jay Horvat's lab Level 2, East Wing HMRI Building and the laboratory of co-supervisor Dr Dan Johnstone at the University of Sydney.

PROJECT DETAILS

Project Title:
Investigating mir137 involvement in adolescent vulnerability to schizophrenia-like behaviours

Supervisory Details:

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Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

Schizophrenia is a severe mental illness affecting ~1-2% of the population that is characterized by positive symptoms (behaviours not observed in healthy subjects, e.g. psychosis and hallucinations), negative symptoms (behaviours reduced compared to healthy subjects, e.g. flattened emotions and social withdrawal), and cognitive deficits. While current treatments are relatively effective for managing the positive symptoms of schizophrenia, cognitive deficits are not improved by these, and make the greatest contribution to functional impairment in patients.

Mir-137 is a microRNA that plays an important role in brain development and is also one of the leading genetic risk factors for schizophrenia. Adolescence is considered to be an important period for vulnerability for schizophrenia, however expression patterns of Mir-137 and other microRNA in brain regions relevant to schizophrenia across adolescence are poorly understood. Adolescence is an important period of protracted brain development in the prefrontal cortex, a region that is important for cognitive functioning, and it is possible that disruption of adolescent Mir-137 expression during adolescence in schizophrenia causes abnormalities in prefrontal cortex development that contribute to cognitive deficits in patients during adulthood. The goal of this project is to 1) characterize the expression of microRNAs across adolescent development in brain regions relevant to the pathophysiology of schizophrenia in that rat, and 2) determine the effects of adolescent disruption of Mir-137 on cognitive functions in adulthood. Successful applicants will receive training in molecular (gene expression)and behavioural testing to complete these studies, and will be exposed to stereotaxic surgery and calcium imaging methods that are likely to integrated in subsequent studies.

Laboratory Location

MSB (Callaghan campus)

PROJECT DETAILS

Project Title: How does the vagina keep itself fit? Defining the mechanisms of vaginal epithelial maintenance during homeostasis and regeneration.

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Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:
Intact vaginal epithelium is essential for women's reproductive health. Disruption of vaginal epithelial homeostasis due to aging, hormonal changes, and alterations in the microbiota predispose women to human papillomavirus (HPV), human immunodeficiency virus (HIV), and sexually transmitted infections. Despite the significance of the vagina in reproductive tract functions and diseases, we know very little how this epithelium maintains itself. The vagina is a highly regenerative organ and its epithelium undergoes cycles of proliferation and differentiation throughout the female reproductive life cycle. The experimental or agerelated decline in oestrogen levels in mice and humans leads to regression of the vaginal epithelium, which is reversed upon external supplementation of oestrogen. We hypothesised that this exceptional regenerative capacity of the vagina is due to the existence of a population of epithelial stem/progenitor cells. However, the identity and location of such cells in the vagina is currently unknown. We identified a rare population of highly proliferating stem cells in the basal compartment of vaginal epithelium that both self-renews and gives rise to the differentiated cells. This project aims to investigate how hormonal and local autocrine/paracrine signals affect the activity of these vaginal epithelial stem cells during homoeostasis and regeneration using both in vivo and in situ models.

Laboratory Location: LS229

PROJECT DETAILS

Project Title: Assessing the role of ovarian surface epithelium in developing high-grade serous ovarian cancer by using patient-derived organoid platform

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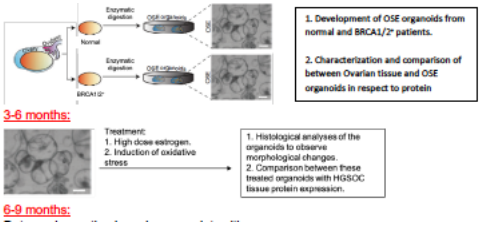
Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach: (MAX HALF-PAGE).

Background: High-grade serous ovarian cancer (HGSOC) is one of the most prevalent and deadliest gynaecological malignancy, which accounts 70-80% ovarian cancer mortalities (Gershenson, D. M. et al. 2006). This high mortality rate due to HGSOC is because of the late diagnosis, as when it gets diagnosed the tumour has already spread to the abdominal cavity. As a result, the early stages of HGSOC development are still unknown. For years, it is believed that HGSOC originates from the ovarian surface epithelium (OSE), which undergoes multiple rupture and repair processes during ovulatory cycles. It is assumed during this cyclical process OSE cells accumulates multiple irreversible DNA damage due to oxidative stress, which could consequently lead to malignancy (Fathalla, M. F. et al., 1971). Interestingly, women carrying germline mutations to BRCA1/2 genes are more susceptible to develop HGSOC. This shows, women carrying mutation in BRCA1/2 gene in their OSE possibly are more prone to accumulate DNA damage mutations during the ovulatory cycles, to transform into full-blown HGSOC. Thus, here in this project first time we want to investigate the role of OSE in developing HGSOC by using patient-derived organoids.
Hypothesis: Investigate the role OSE with germline mutation in BRCA1/2 gene in developing High-grade serous ovarian cancer.
Experimental approach:
0-3 months:



Data analyses, thesis and manuscript writing
References:
Gershenson, D. M. et al. Clinical behavior of stage II-IV low-grade serous carcinoma of the ovary. Obstet. Gynecol. 108, 361–368 (2006)
Fathalla, M. F. Incessant ovulation—a factor in ovarian neoplasia? Lancet 2, 163 (1971).
Lõhmussaar, K. et al. Assessing the origin of high-grade serous ovarian cancer using CRISPR-modification of mouse organoids. Nature Communications 11, 2660 (2020)

Laboratory Location
LS-229, Level-2, Life Science Building

PROJECT DETAILS

Project Title: Investigating immune pathways modulated by a high fruit and vegetable intervention in children with asthma

Supervisory Details:
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Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

The burden of asthma in children is unacceptably high, with more than 10% of Australian children affected. Current treatments for asthma are not effective in preventing asthma attacks in many children and alternative strategies are urgently needed.

We have successfully completed a world first dietary intervention in children with asthma. This trial has shown that when children with a poor-quality diet increase their fruit and vegetable (F&V) intake for 6 months, then their lung function improves. In addition, the number of children who have multiple asthma attacks in a 6-month period declines.

HYPOTHESIS: Increased F&V intake improves childhood asthma, including improved lung function and reduced exacerbation frequency via increased intake of antioxidants and soluble fibre which enhance immune function.

AIMS: To identify changes in immune pathways that occur following a 6-month F&V intervention and to relate these changes to improvements in lung function and exacerbation frequency following a 6-month F&V intervention.

METHOD: This study will utilise stored peripheral blood mononuclear cell samples previously collected during an RCT.

Laboratory and statistical techniques: RNA will be extracted from PBMC's using the RNeasy Mini Kit (Qiagen, Hilden, Germany), quantitated using the Quant-iT RiboGreen RNA Assay Kit (Molecular Probes Inc, Invitrogen, Eugene, OR, USA). PBMC transcriptome analysis will be performed in baseline and 6-month samples using the Nanostring nCounter Analysis System Human Immunology v2 Panel (Nanostring Technologies, Seattle, WA, USA), where expression of 579 immunology-related genes and 15 internal reference controls will be measured. Nanostring data will be analysed using nSolver Analysis Software v2.5 (Nanostring Technologies). Gene profiles will be analysed for differential expression by paired T test for significance. Relationships of gene expression profiles will be examined using hierarchical clustering. Potential molecular mechanisms will be investigated by both gene ontology and pathways analysis. Associations between clinical improvements and changes in inflammatory pathways will be examined using multiple regression.

Laboratory Location
HMRI, Level 2 – West

PROJECT DETAILS

A) Project Title: Preclinical testing of novel combination therapies for acute myeloid leukaemia

B) Supervisory Details:

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Co-Supervisor Name: Dr Heather Murray
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Co-Supervisor Name: Dr Joshua Brzozowski
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C) Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

Acute myeloid leukaemia (AML) is a devastating blood cancer that kills 3 out of 4 patients within 2 years of diagnosis. Improved therapies are urgently required to improve the outcome for these patients. Our laboratory has recently carried out a world first discovery project combining genomics, proteomics and phosphoproteomics profiling of AML patients (Murray *et al*, 2020 *Leukemia* https://www.nature.com/articles/s41375-020-01050-y and unpublished data). This wealth of novel information has identified important biological pathways that are activated in specific subsets of AML patients.

We hypothesize that these pathways are essential for the survival of the leukaemia cells, and therefore that pharmacological targeting of the pathways will be therapeutically effective.

To test this hypothesis, we will test a range of novel drugs alone and in combination with standard AML chemotherapy, in AML cell lines, primary patient AML samples and a mouse model of AML. The efficacy of these inhibitors on cell proliferation, apoptosis and cell signaling will be examined using cytotoxicity assays, flow cytometry, western blotting and mass spectrometry. The student will be trained in these areas as well as cell culture techniques, animal handling and bioluminescent imaging, in a dynamic and passionate laboratory environment. The findings from this project will be published in a high impact international journal and translation of findings could lead to improved outcomes for AML patients.

Laboratory Location

Life Sciences Building LS3-26 and LS3-17

PROJECT DETAILS

Project Title: Deciphering a novel molecular mechanism of therapy resistance in breast cancer.

Supervisory Details:
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D) Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

Early detection and improved therapies have seen the survival rate for breast cancer significantly improve over the past few decades. Despite this, breast cancer still kills over 3,000 Australian women every year. This is primarily due to disease relapse and the development of resistance to therapy. Our laboratory has recently identified a novel mechanism by which breast cancer cells become resistant to breast cancer therapies – reduced expression or genetic loss of a gene called *PPP2R2A*. This gene encodes a regulatory subunit of the protein phosphatase 2A (PP2A), PP2A-B55α, that is a master regulator of cellular growth, survival and differentiation pathways. Through analysis of human breast tumours and molecular inhibition studies, we have discovered that reduced PP2A-B55α appears to confer resistance to endocrine therapy (e.g. Tamoxifen) and targeted anti-HER2 and anti-EGFR therapies. However, to confirm this we must now test this in a mouse model of breast cancer. To do this we have developed the world's first PP2A-B55α knockout mouse and have bred these mice with a genetically modified mouse model of breast cancer.

These unique mice are the ideal tool to test our hypothesis that reduced PP2A-B55α confers anti-breast cancer therapy resistance by activation of specific PP2A substrates leading to constitutive activation of estrogen signalling growth and survival pathways.

To test this hypothesis the project aims are to:

1. Test the anti-breast cancer efficacy of anti-HER2/EGFR therapy in wildtype and PP2A-B55α knockout mice bearing HER2+ breast tumours
2. Perform proteomic and phosphoproteomic analyses of breast tumours from the wildtype and PP2A-B55α knockout mice
3. Determine the functional importance of proteins/pathways identified in aim 2 using pharmacological and/or molecular inhibition in human breast cancer cells

The student will be part of a passionate and dynamic research team and will learn a range of techniques including state of the art mass-spectrometry based proteomics and phosphoproteomics, bioinformatics analyses, cell culture, cytotoxicity assays, western blotting, animal handling and molecular techniques. The findings from this project will be published in a high impact international journal and could identify novel therapeutic approaches for treating therapy-resistant breast cancer.

Laboratory Location Life Sciences Building LS3-26 and LS3-17

PROJECT DETAILS

Project Title: Central role of a master regulator of cellular signalling in development, function and disease of the epidermis

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Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

Protein phosphatase 2A (PP2A) is a master regulator of cellular signalling, controlling over 50% of serine/threonine dephosphorylation in cells. We recently generated the first constitutive (full body) knockout mouse model of the *Ppp2r2a* gene encoding the B55α regulatory subunit of PP2A which allowed us to reveal some of its essential functions. Homozygous knockout of *Ppp2r2a* is embryonic lethal and associated with strongly impaired skin formation, as well as limb and neural defects. The skin defect is characterized by incomplete epidermal barrier acquisition, associated with poorly differentiated stratified epithelium with weak attachment to the underlying basement membrane (Panicker *et al* 2020 https://www.frontiersin.org/articles/10.3389/fcell.2020.00358/full) .

These findings led us to hypothesize that B55α is involved in the regulation of keratinocyte cell adhesion to the basement membrane, a crucial component of skin integrity and the wound healing process. However, the expression of B55α in several cell types of the skin and the severe phenotype of the mice prevented us from further studying its role on epidermal function and disease in the constitutive knockout.

This project will contribute to testing our hypothesis through the following aims:
Aim1- Generate and characterize keratinocyte specific *Ppp2r2a* knockout mouse models.
Aim2- Characterize the keratinocyte adhesion defect to the underlying basement membrane using a combination of skin tissue from the mice and human keratinocyte cell lines to allow preliminary wound healing *in vitro* studies.
Aim3- Perform proteomic and phosphoproteomic studies to decipher the network of signalling pathways controlled by B55α in keratinocytes.

Overall, the study will delineate the role of B55α in epidermal development, adhesion and wound healing, and thus could lead to identification of new targets for a range of debilitating skin disorders. The student will be trained in cutting edge biochemistry, cell biology and animal techniques in a dynamic laboratory environment, and the findings published in a high impact international journal.

Laboratory Location Life Sciences Building LS3-26 and LS3-17

PROJECT DETAILS

Project Title: The impact of stroke on cerebrospinal fluid efflux from the cranial space.

Supervisory Details:
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Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

Background: The main goal of stroke therapies is to restore blood flow to prevent further loss of brain tissue. It has been demonstrated that increased pressure in the skull after stroke disrupts blood flow to the stroke site. This means that more tissue is damaged, and outcomes are worse for the stroke patient. We have identified that this increase in pressure appears to be due to changes in the movement of cerebrospinal fluid after stroke with less cerebrospinal fluid managing to exit the skull after stroke. As the amount of cerebrospinal fluid in the skull increases, so too does the pressure.

Hypothesis: We hypothesise that intracranial pressure rise after stroke occurs due to decreased efflux of cerebrospinal fluid from the cranial space.

Experimental approach: To investigate this, adult mice will be exposed to a photothrombotic stroke or sham surgery (6 per group). At 24 hours or two weeks after stroke a fluorescent tracer will be injected into the cerebrospinal fluid via the cisterna magna. After allowing the tracer to circulate for 30 minutes, animals will be euthanised and their skulls and brains will be collected. Collected brain tissue and skulls will be cut, mounted on slides, and analysed using confocal microscopy to determine the routes cerebrospinal fluid normally takes and how this is altered after stroke. Particular attention will be given to the meningeal lymphatic vessels and the cribiform plate.

Laboratory Location

This project will take place at the Hunter Medical Research Institute and the University of Newcastle Callaghan campus as required.

PROJECT DETAILS

Project Title: Changes in cerebral perfusion during ischemic stroke and reperfusion

Supervisory Details:

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Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

Ischemic stroke involves blockage of a blood vessel in the brain that disrupts cerebral blood flow. Following an ischaemic insult, a penumbral region exists around a core of irreversibly damaged tissue. This penumbral region is supplied by collateral/'bypass' blood vessels allowing the cells to survive for a limited amount of time. If blood flow is restored to this region, the penumbra may be salvaged, improving recovery outcomes for the patient.

Patients who appear to be recovering from their stroke sometimes have expansion of their stroke and clinical deterioration 24-48-hours after their stroke. Published data from our lab has identified that, even for small stroke, an increase in pressure within the skull (intracranial pressure, ICP) can hinder the delivery of blood to the penumbra via collateral vessels, because normal cerebral autoregulation is impaired. Increasingly, patients with large strokes are treated with endovascular clot retrieval to reperfuse and hopefully save the penumbra. However, reperfused penumbra may also have impaired autoregulation. We have shown that reperfused patients have significant ICP elevation, and imaging data suggests that the amount of dead brain tissue (infarct) may expand after reperfusion. It is not yet known if ICP elevation following reperfusion is the cause of infarct expansion.

Aim: Determine whether ICP elevation causes perfusion reduction and infarct expansion in reperfused ischaemic penumbra.

Hypothesis: Reperfused penumbra exhibits impaired autoregulation after reperfusion and is susceptible to critical hypoperfusion and infarct expansion from ICP elevation.

Experimental approach design:

Male, adult Wistar outbred rats will undergo temporary occlusion of the middle cerebral artery using the intraluminal thread model. Laser Speckle Contrast Imaging will be used to calculate the blood perfusion of the two hemispheres prior to stroke (baseline), immediately after stroke to determine the location of the ischemic penumbra and at 18 to 24 hours after stroke (the period of time in which increased pressure within the skull has been demonstrated in our stroke model). ICP will be monitored at the same time, and in one cohort of animals ICP will be artificially lowered by either acetazolamide infusion (shown to reduce cerebrospinal fluid production and thus ICP) or a subdural CSF drain. The brain will be collected 24 hours post stroke for histological analysis of infarct size. A total of 24 animals (12/group) will be analysed.

The surgical procedures in this project are quite complex and training of a student to competency in the short honours time frame is likely not to be feasible. Surgeries will be performed by Dr Daniel Beard with assistance from the student. The student will be in charge of blood flow imaging recording, physiological monitoring, ICP and cerebral blood flow analysis and histological assessment of infarct size (staining and tracing).

Laboratory Location: Medical Science Building (MSB) 504

PROJECT DETAILS

Project Title: Epigenetic programming increases the of risk of preterm birth

Background and Summary of Proposed Research:

Proinflammatory changes in uterine tissues form key steps in preparation for birth. The proper timing of this transformation ensures the birth of a healthy mature baby. In contrast early onset of these changes result in premature birth with potentially devastating consequences that include long term disabilities or death. The epigenetic control of the proinflammatory trigger processes remain poorly understood.

This Honours Project will explore how activation of uterine tissues is regulated at the time of birth. The underlying hypothesis is that epigenetic changes during pregnancy programme proinflammatory process to begin prematurely and this involves changes of chromatin structure in key inflammatory genes.

The laboratory work will include isolating and culturing epithelial and mesenchymal cells from uterine tissue and treating the cells with bacterial endotoxin and cellular damage products to model infectious and sterile inflammation, respectively. The expression of a panel of inflammatory genes will be measured in the two cell types novel assay systems. Further, epithelial cells will be induced to adopt a activated phenotype and changes of responsiveness will be measured. Epigenetic histone modifications at inflammatory gene promoters will be determined by chromatin immunoprecipitation, and chromatin structure at the responsive genes will be monitored by FAIRE assays. Clarifying the role of epigenetic processes in the proinflammatory transformation of uterine tissue will reveal new for therapeutic targets aimed at reducing the risk of preterm birth.

Laboratory Location: Hunter Medical Research Institute and John Hunter Hospital Department of Maternity and Gynaecology

Supervisor and Co-supervisor contact information (phone number and email address):

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Laboratory Location HMRI 3502

PROJECT DETAILS

Project Title: Discovering and developing novel cardioprotective therapies to mitigate cardiovascular complications of cancer therapies.

Supervisory Details:
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Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

Cancer survival rate has greatly improved in the last two decades due to the emergence of next-generation anti-cancer agents. However, several of these therapies may also lead to several unexpected complications that can be detrimental to patients' health, quality of life and survival. In particular, some anti-cancer treatments cause cardiotoxicity and lead to cardiovascular complications (e.g. heart failure). This has led to early disruption or discontinuation of potentially life-saving anti-cancer therapy and consequently harms cancer survival rate. Therefore, there is an urgent need to identify novel cardioprotective therapies to mitigate cardiotoxicity whilst preserving the effectiveness of current anti-cancer therapies.

Sodium-glucose co-transporter 2 (SGLT2) inhibitors, such as dapagliflozin and empagliflozin, demonstrated a surprising cardioprotective effect (reduced mortality and heart failure hospitalisation risk) in patients with type 2 diabetes mellitus. The mechanisms responsible for the cardioprotective effects of SGLT2 inhibitors remain incompletely understood and yet to be explored in the context of cancer therapies. Therefore, the proposed project will examine the potential of SGLT2 inhibitors in mitigating cardiotoxicity induced by cancer therapies such as carfilzomib. We **hypothesised** that these novel SGLT2 inhibitors will rescue primary human cardiomyocytes from cancer therapies-induced cardiotoxicity. The **aims** of the study are to 1) assess the cardioprotective potential of SGLT2 inhibitors in primary human cardiomyocytes (HCMs) exposed to anti-cancer agents known to induced cardiotoxicity and 2) elucidate the underlying mechanism(s) and targets for future drug discovery. **Experimental approach**:

Aim 1: A dose response study will be performed to examine the effect of SGLT2 inhibitors (dapagliflozin and empagliflozin) on HCMs with or without exposure to anti-cancer agents (carfilzomib and bortezomib). Cardiotoxicity will be assessed in terms of cell viability at four timepoints (0, 24, 48 and 72 hrs) using the Cell-Titre Glo™ and/or LDH cytotoxicity assays.

Aim 2: The study will be repeated based on the best dose and timepoint determined at Aim 1. Cell lysates will be collected for RNA and protein isolation. The expression levels of mRNA and protein targets associated with cardiotoxicity (e.g. DNA damage, oxidative stress and mitochondrial dysfunction) assessed by qPCR and/or Western blot analysis.

Laboratory Location: Hunter Medical Research Institute, Level 3 East Cardiometabolic/Cardio-oncology Research Group

PROJECT DETAILS

Project Title: Targeting oxidative stress in fat to fight against cancer and cardiovascular disease

Supervisory Details
Primary Supervisor Name: A/Prof Doan Ngo
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Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

Obesity is associated with two of our biggest killers: cancer and cardiovascular disease (CVD). Adipose tissue (AT) in obese individuals can affect distant organs, contributing to increased oxidative stress levels in both the gut and the heart. Excess reactive oxygen species (ROS) can promote carcinogenesis in the colon, while in the heart, ROS is associated with heart failure due to apoptosis, necrosis and hypertrophic signalling. With oxidative stress being a common link between obesity, CVD and cancer, we have sought to counteract ROS in AT by overexpressing the ROS scavenger mitochondrial-targeted catalase (mCAT) in AT of obese mice that are induced to develop colorectal cancer (CRC). Preliminary results show that mCAT expression in AT can delay CRC development in these mice. The aims of the study are:

(1) To determine whether AT-targeted overexpression of mCAT in a CRC mouse model can cause molecular changes detrimental to CRC development;
(2) To determine whether these changes can be reproduced in a human CRC cell culture model by manipulating mitochondrial ROS *in vitro* and
(3) To determine whether AT-targeted overexpression of mCAT can affect signalling pathways to protect the heart.

Hypothesis: Manipulation of reactive oxygen species levels in mouse and human models of colorectal cancer will cause molecular changes associated with delayed colorectal cancer development and changes that protect the heart.

Experimental Approach:
Aim 1: RNA and proteins from stored CRC tissue samples from wild-type or mCAT mice fed either a standard diet or a western style diet will be analysed by qPCR and Western blot. Colon tissue sections will be analysed by H&E and immunostaining.
Aim 2: Human CRC cell line HCT116 will be transfected with an mCAT overexpression plasmid or treated with pharmacological ROS scavenger Mito-TEMPO. The effect on cell proliferation and cell migration will be assessed by Cell-Titer Glo assays and wound healing assays respectively. Expression levels of protein and mRNA targets identified in Aim 1 will be analysed by Western blot and qPCR.
Aim 3: RNA and proteins from stored heart tissue samples from the mouse groups in Aim 1 will be analysed by qPCR and Western blot. Heart tissue sections will be analysed by Masson's Trichrome and immunostaining.

Laboratory Location: Hunter Medical Research Institute, Level 3 East Cardiometabolic/Cardio-oncology Research Group

PROJECT DETAILS

Project Title: The Mitochondrial origin of Fetal Growth Restriction

Supervisory Details:

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Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

Fetal growth restriction (FGR) is defined by the inability of the fetus to reach its predetermined growth potential. FGR impacts immediate fetal and neonatal health and also has lasting implications on cardiovascular, renal and metabolic health later in life. FGR affects approximately 10% of births each year in Australia and accounts for 7% of stillbirths, a figure equating to 1 in every 150 stillbirths. In spite of these alarming statistics the cause of this disorder remains unknown and a robust method of early detection elusive. While the mechanistic cause remains uncertain, FGR is often attributed to placental insufficiency and dysfunction. A key indicator of which, is mitochondrial function, with mitochondrial dysfunction associated with many pregnancy complications. While often referred to as the “powerhouse” of the cell, the mitochondria have a complex role in regulating homeostasis which includes regulating metabolic function, nutrient utilisation and oxygen sensing.

This project will examine the potential for mitochondria within the placenta to sense oxygen via electron transport chain (ETC) proteins and alter metabolism accordingly. Ultimately, determining if this mechanism is dysregulated in pregnancies which progress to fetal growth restriction.

Primary placental tissue from uncomplicated pregnancies will be compared to FGR utilising a multitude of techniques. Mitochondria will be isolated by centrifugation and assessed in real time via respirometry to measure mitochondrial function. This will enable evaluation of the bioenergetic capacity within pathology and provide clear assessment of metabolic pathway usage. Key ETC proteins known to sense and initiate responses to oxygen will be examined to determine the gene and protein expression levels via PCR and Western blotting. To comprehensively examine the mitochondrial differences between the two groups visualisation of the protein localisation will be conducted by Immunohistochemistry.

This project will provide the opportunity to develop a fundamental knowledge of metabolism and mitochondrial physiology, develop skills in numerous lab techniques while maintaining a clinical and translatable focus.

Laboratory Location: HMRI Level 3 East

PROJECT DETAILS

Project Title: Evaluating potential therapeutic side effects following pregnancy compromise

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Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

Pregnancy compromises, such as maternal stress exposure and preterm birth, can have serious effects on the neurodevelopment of the offspring. Behavioural disorders such as attention deficit hyperactivity disorder (ADHD) and anxiety are relatively common in children who were born preterm or exposed to stress *in utero*. We have found that key steroids from the placenta are reduced following stress and preterm birth. These processes involve steroid-$GABA_A$ receptor interaction that directly promote proper neurodevelopment. We propose that targeting the $GABA_A$ receptor may be an effective approach to improving outcomes following these pregnancy problems. We have used preclinical models of preterm birth and prenatal stress to show that $GABA_A$ receptor agonists given one week following birth lead to improved outcomes. This project will focus on evaluating the possible effects these therapies have on the steroid producing capacity and drug metabolising ability of the neonate. We hypothesise that there will be clear differences in the response of neonates of differing birth ages (term vs preterm), differing pregnancy compromise (stress vs preterm birth) and potentially of differing sexes. Thus, tailored approaches to administering this therapy may be required. To determine the effect of postnatal administration of our novel treatments on endogenously produced steroids (such as cortisol), adrenal tissue from treated and untreated neonates will be used for gene expression analyses using the high throughput Fluidigm system. Steroid concentrations will also be quantified by enzyme immunoassay and correlated with gene analyses. Overall, this project will identify a clinically feasible therapy with minimal off-target effects to improve outcomes for vulnerable newborn infants.

Laboratory Location: Level 3 East HMRI

PROJECT DETAILS

Project Title: Investigating the relationship between hypoxia and reduced sperm quality in mice

Supervisory Details

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Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

Background, Aims and Hypothesis: Hypoxia is a condition in which the body or a region of the body is deprived of adequate oxygen supply at the tissue level. Variations in arterial oxygen concentration can be part of normal physiology e.g strenuous physical exercise, however it is often considered a pathological condition. Hypoxia is associated with many conditions including sleep apnoea, stroke and pneumonia, and can be induced by cigarette smoking or breathing in polluted air. Male infertility is a medical condition affecting one in 20 men in the western world. Of concern, over the past 40 years, sperm counts have halved and the proportion of men at risk of requiring fertility treatment has risen from 12.4% in 2004 to 21.3% in 2017. The proposed project will be part of a larger body of work aiming to understand why sperm counts continue to fall. The hypothesis of this project is that hypoxic conditions are detrimental to sperm development. In pilot data we have already shown, very unexpectedly, that hypoxia exposure results in structural changes to the seminal vesicle in the form of "crystalline" structures. Given this, the specific aim of this project is to test the effect of different hypoxic conditions on sperm production.

Experimental Design: Students will subject mice to hypoxic conditions, ranging from 12%-16% for a period of 2-4 weeks. A comparable number of mice with normoxic conditions (21% O_2) will be used as a control. Following exposure, the mice will be sacrificed, the male reproductive tract taken and sperm removed from the cauda epididymis. We will measure:

Testis:Body weight changes; Epididymis:Body weight changes; Testis Body weight changes; Sperm motility using computer assisted semen analysis; Sperm concentration though a haemocytometer; Sperm morphology through phase contrast; Seminal vesicle weight

Laboratory Location: All experiments will be conducted at HMRI (Level 3 East), the lab located in the Life Sciences Building (Level 4, L54.21) Both labs are equipped officers.

Any changes to the male reproductive tract will be further investigated by performing a quantitative proteomic analysis and comparing the protein abundance between the control and hypoxic samples. For example, it is anticipated already that large scale changes occur with the seminal vesicle of hypoxic mouse. As such, the protein composition will be compared by quantitative mass spectrometry. Changes will then be ratified through orthologous methods including immunoblotting and immunohistochemistry.

Significance: The results of the project will enable us to understand the impact of hypoxia on male-fertility. This will enable us to better advice infertile couples (where male-factor is diagnosed) on lifestyle choices that may help better sperm production.

PROJECT DETAILS

Project Title: Investigating the relationship between hypoxia and reduced sperm quality in mice

Supervisory Details:
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Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

Background, Aims and Hypothesis:

Stroke is a leading cause of death and disability worldwide. One of the most common neurological processes in the chronic phases of stroke is the development of secondary neurodegeneration (SND). SND develops in brain regions remote from, but synaptically connected to, the primary site of stroke damage. Whilst scientists are still investigating the underlying mechanisms of SND our lab has made several important findings that link SND with cognitive decline and motor impairment both of which significantly affect stroke survivors. Furthermore, we have shown an association between post-stroke cognitive decline and the accumulation of neurotoxic proteins incl. amyloid beta (Aβ). Excitingly, our group has shown that exposure to a low oxygen environment after stroke (low oxygen post-conditioning; LOPC; 11% O_2 8 h/day) can enhance recovery when administered in the first 2 weeks post-stroke. Not only have we shown that LOPC improves cognition and motor function but we have shown the first evidence that LOPC is capable of inducing robust decreases in the total level of Aβ post-stroke. This extremely effective non-pharmacological approach has numerous advantages over current treatment strategies including its well characterised and acceptable safety profile, relative low cost and ease of delivery. However, before we can translate this to humans however, we need to determine the optimal treatment paradigm for exposure i.e 8h vs 4h vs 1h/day. Therefore, the aim of this project will be *to determine the minimum effective dose of LOPC that will still improve outcomes post-stroke.*

Experimental Design: Students will subject mice to hypoxic conditions (11% O_2), ranging from 1-8 h/day for a period of 2-4 weeks post-stroke in our established mouse model of stroke. A comparable number of mice with normoxic conditions (21% O_2) will be used as a control. Following exposure, the mice will be sacrificed, and brains collected for immunohistochemistry and immunoblotting.

Significance: The results of the project will enable us to determine the optimal treatment paradigm for LOPC after stroke. This will be an important step in the clinical translation of LOPC.

Laboratory Location: This honours project will be conducted at HMRI (Level 3 East).

PROJECT DETAILS

Project Title: A rapid approach to overcoming chemoresistance in high-grade serous ovarian cancer

Supervisory Details
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Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

Most patients with high-grade serous ovarian cancer (HGSOC) succumb to disease recurrence, largely due to resistance to standard combination of platinum and taxol chemotherapies. Platinum chemotherapies induce DNA damage, if extensive, causes double-strand breaks (DSBs) in the cancer cells, which then undergo apoptosis. If cells respond by repairing the damage or accumulating mutations, then the effectiveness of therapy declines. When cells are treated with platinum chemotherapy, single-strand breaks occur, which can be repaired via processes that rely on poly (ADP) ribose polymerase (PARP) enzymes. If the action of PARP is blocked, single-strand breaks convert to double-strand breaks and cell death occurs.

We therefore hypothesize that PARP inhibitors (PARPi) will enhance the effects of standard combination of platinum and taxol chemotherapies and thus improve treatment of HGSOC. Whilst several PARPi have been trialled in HGSOC patients, only limited patients within selected patient groups, mainly those with DSB repair deficiencies (BRCA mutations) have seen clinical benefits. Therefore, we are treating many patients with drugs for which it is unknown who will actually benefit exposing all patients to toxicity, an approach that is also costly to the health care system.

We have identified other drugs with PARPi activity that have been in clinical use for many years and as a result are also inexpensive, and may enhance the action of existing chemotherapy. The drugs to be tested have been selected by in silico modelling, from a database of existing FDA/TGA-approved drugs. The selected drugs have potential PARPi activity in addition to their mechanism for their primary indication or therapeutic use. The aim of this project is to test these drugs on HGSOC cells to determine if they can sensitize the tumour cells to respond better to the standard platinum and taxol chemotherapies using live-cell fluorescent assays. This proposed project is innovative in meeting the challenges in this setting as the drugs have already been previously and extensively studied and used in humans. This provides valuable information around toxicity and likely magnitude of efficacy, plus means they could be rapidly trialled in patients with HGSOC.

Laboratory Location: Hunter Medical Research Institute Level 3 West DNA Repair Group

PROJECT DETAILS

Project Title: Harnessing upper respiratory tract innate immunity to protect against respiratory virus infections

Supervisory Details:
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Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

Respiratory viruses cause a multitude of diseases. The most common – rhinovirus (RV), is the most frequent cause of the common cold as well as triggering more severe disease in susceptible populations including asthma and COPD. COVID-19 is a disease caused by a coronavirus (CoV) SARS-CoV-2 and is the third major CoV outbreak within the last two decades, preceded by SARS-CoV and MERS-CoV. Combined RV and CoV continue to represent a serious threat to human health, highlighting the need to develop pan-virus-effective treatments. This has led us to focus on treatments that licence innate immunity at the site of respiratory virus infection (upper respiratory tract) and develop a novel synthetic TLR2 agonist (INNA-X) [1]. In the current climate, such broadly protective antiviral therapeutic approaches are of critical importance and would be well-positioned to compliment vaccines and bridge the gap between outbreak and vaccination with control of virus infection by the innate immune system. Prior to COVID-19, INNA-X development was based on primary human bronchial epithelial cell- and mouse-RV infection models[1]. We have now developed a novel human CoV mouse respiratory infection model that we anticipate will be a major boost to understanding the pathogenesis of CoV-induced disease and anti-viral drug/vaccine development. This project will utilise our novel mouse models of upper respiratory tract (URT) RV and CoV infection in conjunction with prophylactic intranasal treatment with novel therapeutics (such as INNA-X) and assess the efficacy and mechanism of action of treatments such as INNA-X that reduce viral load in the upper respiratory tract, preventing the spread of infection to the lung, and reducing virus-induced lung inflammation.

Hypothesis: Prophylactic stimulation of upper respiratory innate immunity primes resistance to respiratory virus infection (RV and CoV), accelerating viral clearance in the URT, preventing lung infection and inflammation.

Aims:

1. Quantify viral load and antiviral responses in the upper and lower respiratory tract over time by qPCR (viral RNA in nasal and lung tissue) and infectivity assay (infectious virus in nasal- and bronchial washes)
2. Assess upper and lower respiratory tract immune responses (interferon production, cytokine response profile and cellular recruitment) in response to treatment and infection.

Laboratory Location: HMRI, level 2 east and west wing laboratories

1. Girkin, J., et al., *TLR2-mediated innate immune priming boosts lung anti-viral immunity.* Eur Respir J, 2020. **In Press**.

PROJECT DETAILS

Project Title: Defining the role of TLR2-agonist (INNA-X) induced NF-κB activation in priming anti-viral immunity of human airway epithelial cells

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Background and Summary of Proposed Research, including a clear hypothesis, aims and experimental approach:

The broad diversity of rhinovirus (RV) subtypes represents a challenge for the development of anti-viral treatments and vaccines. An alternative approach to therapy for RV infection is the modulation of the host innate immune response. Toll-like receptors (TLRs) regulate and initiate the immune response to microbes. We hypothesize that therapeutically targeting TLR2 would boost the immune responses to RV thereby reducing the capacity of this virus to cause disease. In collaboration with Ena Respiratory (Melbourne), we are working with a TLR2 agonist INNA-X in clinical development, which has demonstrated significant reduction in RV viral load in differentiated airway epithelial cells (Girkin, Loo, Bartlett European Respiratory Journal, 2020 in press) (1). However, the mechanism of action underpinning efficacy requires further investigation. Immune transcriptome analysis has revealed that INNA-X treatment increased early, rapid expression of NF-κB and NF-κB -regulated genes encoding cytokines (e.g. IL-1β), chemokines (e.g. CXCL1, CXCL2) and anti-microbial molecules (e.g. IDO1, calprotectin/S100A8-S100A9, interferon-λ) during RV infection[1].

While early, rapid up-regulation of NF-κB-mediated gene expression in airway epithelial cells is associated with improved control of RV infection and reduced virus-induced inflammation we are yet to demonstrate this empirically. We hypothesise that INNA-X treatment boosts RV-induced NF-κB activation and expression of innate immune proteins that suppress viral replication in airway epithelial cells. We aim to utilise conditionally reprogrammed-expanded airway epithelial cells differentiated at air liquid interface for 28-35 days. These cultures will be pre-treated with INNA-X, infected with RV-A1 or RV-A16 and NF-κB activation (p65 nuclear translocation, IκB degradation) and expression of NF-κB-regulated proteins will be analysed using western blot (intracellular proteins) and ELISA (secreted proteins). This will provide evidence at the level of protein expression to support transcriptomic data and confirm that INNA-X-innate immune priming involves rapid NF-κB-activation and NF-κB-regulated protein expression. Pharmacological inhibition will be used to confirm the role NF-κB activation for expression of chemokines, antimicrobial proteins and type III IFNs (2, 3). The specific role of type III IFN-λs will be determined using antibodies that block the type III IFN receptor or neutralise IFN-λ.

Laboratory Location: Level 2 West, HMRI

1. Girkin J, Loo S-L, Esneau C, Maltby S, Mercuri F, Chua BY, Reid A, Veerati P, Grainge CL, Wark PAB, Knight D, Jackson DC, Demaison C, Bartlett NW. TLR2-mediated innate immune priming boosts lung anti-viral immunity. *Eur Respir J* 2020; In Press.
2. Bartlett NW, Slater L, Glanville N, Haas JJ, Caramori G, Casolari P, Clarke DL, Message SD, Aniscenko J, Kebadze T, Zhu J, Mallia P, Mizgerd JP, Belvisi M, Papi A, Kotenko SV, Johnston SL, Edwards MR. Defining critical roles for NF-kappaB p65 and type I interferon in innate immunity to rhinovirus. *EMBO Mol Med* 2012; 4: 1244-1260.
3. WIlliams TC, Jackson DJ, Maltby S, Walton RP, Ching YM, Glanville N, Singanayagam A, Brewins JJ, Clarke D, Hirsman AG, Loo S-L, Wei L, Beale JE, Casolari P, Caramori G, Papi A, Belvisi M, Wark PAB, Johnston SL, Edwards MR, Bartlett NW. Rhinovirus induced CCL17 and CCL22 in asthma exacerbations and differential regulation by STAT6. *Am J Respir Cell Mol Biol* 2020; In Press.

List of Approved Projects (*have a student involved as at 1/12/20*):

Project Title: Characterising a new regulator of breast cancer cell invasion
Supervisors: A/Prof Kathryn Skelding, A/Prof Lisa Lincz

Project Title: The cancer neuroimmunology of breast cancer
Supervisors: A/Prof Phil Jobling, A/Prof Jay Horvat, Prof Hubert Hondermarck

Project Title: Targeting BCL6 in high-risk paediatric cancers
Supervisors: Dr Matt Dun, Dr Geoff De Iulius

Project Title: Investigating the potential of inhibiting bromodomain proteins for the treatment of diffuse intrinsic pontine glioma
Supervisors: Dr Matt Dun, Dr Ryan Duchatel

Project Title: Understand the basis of menses: how uterus performs scarless repair.
Supervisors: Dr Shafiq Syed, A/Prof Pradeep Tanwar

Project Title: Retrospective assessment of impact of statins on infarct size using clinical MRI data.
Supervisors: Dr Kirsten Coupland, Prof Neil Spratt, Dr Carlos Garcia-Esperon