GARVAN INSTITUTE

Postgraduate Studies

2015 PhD Projects

Contents

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INSTITUTE

Postgraduate Studies at the Garvan

Prof John Mattick
Executive Director

In partnership with the University of New South Wales, Garvan Institute provides a learning and teaching environment of excellence for PhD students who are looking forward to being part of the next generation of great medical researchers.

As one of the world's leading medical research institutes with programs in cancer, diabetes and metabolism, immunology, neuroscience and bone biology, Garvan is playing a leadership role in translating the amazing developments in modern biomedical research into real improvements in health care and quality of life. The joint initiative with St Vincent's Hospital in establishing The Kinghorn Cancer Centre will enable Garvan's research discoveries to make a real difference in the prevention and treatment of this devastating disorder. This however is only the beginning - the future for Garvan will be to ensure that this paradigm is expanded to all of our research areas.

A focus on the promise of genomic medicine and new technologies such as next generation sequencing, and a complementary depth of expertise in cell biology, proteomics, systems biology, bioinformatics, epigenetics and translational research together make Garvan one of the most exciting places to be doing medical research right now and in the future.

As well as ensuring the development of scientific knowledge and skills for the future, postgraduate scholars undertaking their PhD at Garvan are valued as important contributors to the life of the Institute as a whole.

We look forward to you joining us.

John Mattick AO FAA FRCPA
Executive Director
Garvan Institute of Medical Research

Why Choose the Garvan

_ We offer a competitive salary top-up on eligible scholarships

_ The Garvan boasts state-of-the-art research facilities which incorporate a range of cutting-edge equipment and expertise

_ Students at Garvan (SAG), the student representative group within the Garvan Institute provides both academic support and social activities in our off-campus environment

If you would like to find out more about the fantastic opportunities that doing your PhD at Garvan Institute can provide, please email **study@garvan.org.au**
or visit
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Cancer

The Cancer Division at the Garvan Institute is the largest division at the Garvan and one of the most highly regarded cancer research teams in Australia and internationally. With complementary skills in cancer genomics, cancer epigenomics, cancer molecular and cellular biology, cancer biomarker and therapeutic target identification & validation and translational research, the division is focussed on understanding the causes of and developing new diagnostic, prognostic treatment and prevention strategies for the most commonly diagnosed and most lethal cancers including breast, prostate, pancreatic, colorectal, lung, and ovarian. Among the many succesful PhD graduates are Directors of major research institutes and academic departments, professorial heads of independent research groups and clinical units, and recipients of prestigious NHMRC and ARC Fellowships.

Prof David Thomas
Division Head

Human and Comparative Genomics Group

Biologically relevant biomarkers of prostate cancer risk and disease outcomes

Prostate cancer is not only the most common male cancer worldwide (affecting one in every six men) it is arguably the most heritable of the common cancers. Australia has one of the highest incidence rates, with marked global disparities in both incidence and mortality. The genetic etiology of prostate cancer and this global (ethnic) disparity is however poorly understood. Clinical management is hindered by lack of reliable biomarkers and the heterogenous nature of disease course (from asymptomatic to rapid metastasis and mortality). The goal of this PhD study is to apply high-throughput genome-wide genetic approaches (from genotyping to sequencing) to identify biologically relevant (statistically significant) biomarkers of PCa that may explain disparities in prostate cancer risk and disease outcomes globally, using large local and internationally relevant study cohorts. The genetic data will furthermore be correlated with environmental factors to identify confounding associations, while biological relevance will be further investigated using both computational and laboratory-based models.

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Co-supervisor: Dr Eva Chan
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Invasion and Metastasis Group

Cancer invasion and metastasis occur in a complex 3D-environment, with reciprocal feedback from the surrounding host tissue and stroma governing cancer cell behaviour. Understanding this behaviour in an intact host setting allows us to examine, in a physiological context, the aberrant regulation of critical events that lead to dissemination and spread of the primary tumour. Intravital *(in vivo)* imaging is providing new insights on how cells behave in their native microenvironment in real-time, thereby improving our understanding of disease progression (1).

Our group specialises in applying state-of-the-art imaging technology and 3D modelling to assess the spread of cancer in living tissue (2-4). Two PhD projects are available in our laboratory using novel fluorescent biosensors to monitor cancer cell response to anti-invasive drug targeting in live tumours. Both projects involve cutting-edge training in 3D culture of cancer cells with associated stromal tissue engineering and involve molecular intervention with regards to controlling extracellular matrix strength and stiffness (a key feature known to drive the aggressive nature of cancer and its response to current therapeutics (5)). Response of tumour cells to drug targeting in relation to their proximity to blood vasculature (imaged using quantum dots) will also feature heavily in each project.

Each project will involve the use of novel cre-inducible mouse models engineered to uncouple the metastatic process into key stages, to identify critical steps in the metastatic cascade that are aberrantly regulated by candidate genes previously identified in our screen for drivers of invasion.

Currently unavailable elsewhere, these models permit real-time, intravital imaging, ranging from whole body tumour progression to single-cell invasion events, and will help us to understand how:

_ tumour cell dissociation (E-cadherin-GFP; FRAP model),
_ invasion (Rac/RhoGTPase FRET reporter models) or
_ cell growth/survival (GFP model) are controlled and how this is linked to the development of metastasis in the native tumour tissue microenvironment.

New Approaches to a Complex Problem

Two sub-cellular applications currently in use in collaboration with pharmaceutical industry will form the basis of each project:

Project 1: *In vivo* FRAP

E-cadherin-based cell-cell contacts are prominent sites of remodeling during early stages of epithelial to mesenchymal transition (EMT). The deregulation of E-cadherin-based adhesions leads to the collapse of normal epithelial architecture that precedes the initial spread of tumours from their primary site and can therefore serve as an early molecular marker of invasion. We recently established the first application of Fluorescence Recovery After Photobleaching (FRAP) in live tumours to examine and predict E-cadherin cell-cell junction turnover during early stages of cancer dissemination. Importantly, we have now generated the world's first E-cadherin-GFP FRAP mouse and will use this pre-clinical model to assess the effects of therapeutic intervention on E-cadherin dynamics using clinically approved anti-invasive drug therapy, and investigate whether candidate molecules from our screen alter E-cadherin dynamics to drive early tumour invasion (3).

Project 2: *In vivo* FRET

Co-ordinated regulation of RhoGTPases is known to control actin-mediated cell movement that is central to tumour cell invasion. Recently, we used Fluorescence Resonance Energy Transfer (FRET), for the first time, at the sub-cellular level ***in vivo***, to examine RhoA activity during invasion in live tumours (4). Here, we identified at high resolution a small yet important pool of active RhoA at the poles of invading cells, not observed ***in vitro***, that correlates with invasion in live tumours. Expansion of this work led to the first use of FRET to monitor the activity of RhoA at a sub-cellular, rather than global level, upon therapeutic intervention. As above, we have now generated a fluorescent RhoA-FRET mouse and will use this pre-clinical model to examine whether candidate molecules from our screen/drug targeting alter actin-mediated cell movement and invasion in live tissue.

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4. Timpson et al Cancer Res 2011 Feb 1;71(3):747-57
5. Samuel et al Cancer Cell 2011 Jun 14;19(6):776-91

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Pancreatic Carcinogenesis Group
Pancreatic Cancer is the fourth leading cause of cancer death in our society. Almost 90% of the patients succumb within a year of diagnosis, unless detection is done at very early stage. Evidence also supports a long period in which preneoplastic lesions are present.

The Pancreatic Carcinogenesis team is focused on identifying key drivers and biomarkers of pancreatic cancer through studying the earliest changes in exocrine cell differentiation and proliferation using pancreas specific models (*in vitro* and *in vivo*).

The Pancreatic Carcinogenesis group sits within the Pancreas Cancer Group (Prof A Biankin) which co-leads the Australian Pancreatic Cancer Genome Initiative (APGI), a member of the International Cancer Genome Consortium (www.icgc.org). The APGI aims to fully characterize the genomic, epigenomic and transcriptomic aberrations in tumor samples of pancreatic cancer patients using the latest next generation sequencing technologies. As such, the APGI provides a unique resource to investigate molecular mechanisms involved in pancreatic carcinogenesis, to eventually reveal new targets for the development of novel detection methods, chemoprevention and chemotherapeutic strategies.

Specific projects available include:

Project 1
Investigating the expression and the role of candidate gene aberrations identified by APGI in models of early pancreatic cancer; genetically modified mouse models have been introduced and need to be further investigated. In addition, genetic manipulation is used *in vivo* and *in vitro* to define the functional consequences and molecular mechanisms of these novel gene aberrations in model systems of early pancreatic cancer.

Project 2
Investigating ENU-induced mutagenesis mouse models, including forward screens to identify new genes that can impact on exocrine pancreas cell differentiation and proliferation and reverse screens where the effects of a known mutation in a gene of our interest (as identified by APGI) are further investigated for a contribution to pancreatic carcinogenesis.

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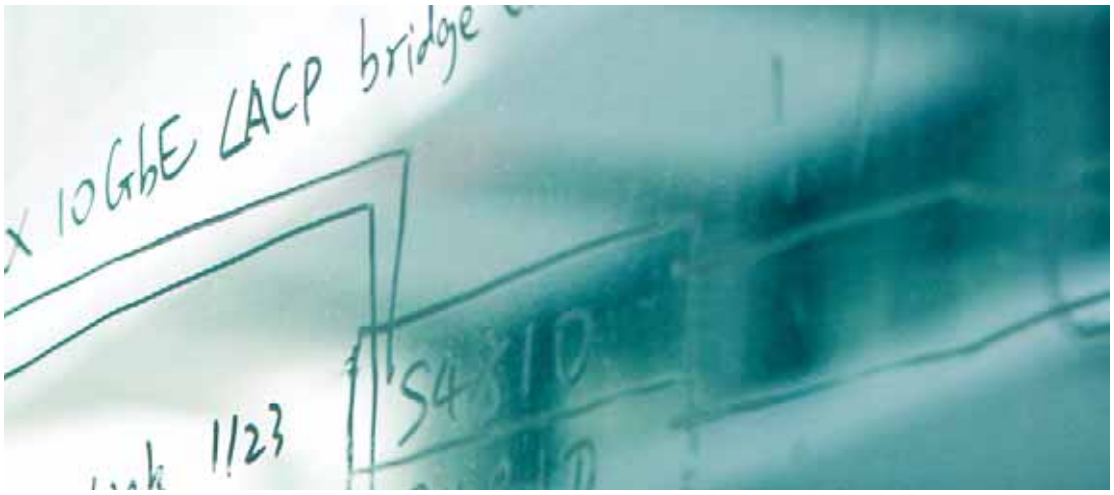

Ubiquitin Signalling Group

Project 1: Identification of novel ubiquitin targets

Ubiquitylation is one of the most abundant protein modifications in cellular signalling, controlling numerous cellular pathways such as cell cycle progression, DNA damage response, receptor endocytosis, and transcription. Ubiquitin (Ub) labels substrate proteins via a highly ordered multi-step enzymatic cascade. Defects in either the Ub conjugation pathway, or downstream effectors of Ub signaling, are linked with cancer pathogenesis. Accordingly, components of the Ub-proteasome system, and E3 Ub ligases in particular, have become an attractive target for development of novel therapeutic strategies to combat cancer, neurodegeneration and other diseases.

The overall objective of the proposed research is to identify novel cellular targets of the Ubiquitin-Proteasome System using an integrated approach employing proteomics, in situ detection of protein-protein interactions, and novel animal models. We are currently using this approach to understand disease pathology in a range of conditions including cancer, ALS, Alzheimer's, and Diabetes.

Project 2: Targeting metabolic reprogramming in pancreatic cancer

Pancreatic cancer has a devastating prognosis, with an overall five-year survival rate of less than 5%, and severely restricted treatment options reflecting a high degree of molecular heterogeneity. Advances in adjuvant and metastatic chemotherapeutic regimens have resulted in some improvement in outcome, but pancreatectomy remains the single most effective and the only potentially curative modality for PC for the ~20% of patients suitable for the procedure. The role of altered nutrient metabolism in cancer cells has attracted significant renewed interest, both in understanding tumourigenesis and as a potential therapeutic target. The mitochondria are key players in cellular metabolism. Although an accumulation of mutations in the mitochondrial genome has been observed in various tumour types, very little work has been done linking these observations to phenotypic changes through functional studies. We propose that a reciprocal relationship exists between somatic mitochondrial mutations and the shifting metabolic needs of tumour cells. We hypothesise that the heterogeneous genomic landscape of pancreatic cancer underlies a common metabolic phenotype, representing a novel therapeutic target. Hence, the objective of the proposed research is to characterise metabolic reprogramming in pancreatic cancer and determine the relative contribution of mitochondrial genetic mutations to these phenotypes. While others have identified mtDNA variants and observed metabolic phenotypes in various tumour types, this study will directly link these through functional studies. By integrating genomics, transcriptomics, metabolomics, and functional analysis on a panel of pancreatic cancer patient-derived tumour cell lines, we aim to directly link genotype and metabolic phenotype at a functional level.

Outcomes of this project include a detailed understanding of the genetic basis of metabolic reprogramming in pancreatic cancer and definition and pre-clinical validation of novel therapeutic strategies.

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Immunology

The work of the research team at the Garvan Immunology Division is divided between studying how a immune system functions in a balanced way during health and how this can goes wrong in diseases such as type I diabetes, asthma and immunodeficiency. Program Head Prof Robert Brink and the Group Leaders in the Immunology team regularly published in many high profile journals including *Nature, Cell, Nature Immunology, Immunity* and *J. Exp. Med*.

Many successful PhD students trained in the Immunology Division have published at least one highly cited first author paper in either *Immunity or J. Exp. Med.*; a number have also been awarded *New Investigator of the Year* honours at the annual conference of the Australasian Society of Immunology as well as the Garvan thesis prize. Since completing their PhDs, many Garvan Immunology Division alumni have successfully obtained NHMRC Fellowhips for further postdoc study both in Australia and overseas at such prestigious institutes as Harvard Medical School, Genentech, Max-Planck Institute in Berlin, Stanford University, Rockefeller University (New York) and Yale University.

Prof Robert Brink
Division Head

Intravital Microscopy Group

Project 1: Tracking the origin and fate of immune cells critical for vaccine-induced immunity

Immune responses to pathogens are characterised by a complex interplay of dynamic interactions between many different immune cells that are located in different anatomical compartments in the body. These interactions are tightly co-ordinated and depending on the nature of the interactions, it is possible for the same precursor cell population to give rise to multiple diverse cell types that serve different functions. For example, naive $CD4^+$ T cells may acquire the capacity to 'help' B cells make antibodies or activate $CD8^+$ T cells to kill infected cells, secrete different cytokines and migrate to different lymphoid organs and microanatomical locations. Similarly, germinal centre B cells may give rise to long-lived plasma cells or memory B cells.

To understand the origin of this heterogeneity, we have taken the novel approach of fluorescently 'tagging' antigen-specific immune cells and directly visualising the behaviour of these cells in real-time using intravital two-photon microscopy. Fluorescently tagged cells can be tracked for long periods of time over large distances within live animals. Two-photon microscopy will then be combined with multiparameter flow cytometry and phenotypic, functional and gene expression analyses at the single cell level to characterise the fates of these cells.

The project therefore involves the use of several cutting-edge technologies to answer questions that are fundamental to our understanding of the immune response and may reveal novel pathways that may be perturbed to promote vaccine-induced immunity.

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Project 2: Using real-time intravital two-photon microscopy of intestinal barrier function to determine the pathogenesis inflammatory bowel disease

Idiopathic inflammatory bowel diseases (IBD) comprise two types of chronic intestinal disorders: Crohn's disease and ulcerative colitis. This is a chronic relapsing disease with early onset in young adults with lifelong impact and considerable mortality and morbidity. Emerging evidence indicates that inflammatory bowel disease (IBD) results from an unrestrained immune response to intestinal microbial antigens in genetically susceptible individuals. Based on clinical data, we believe that baseline epithelial barrier defects (“leaky gut”) in predisposed individuals exposes the intestinal immune system to gut microbiota and initiates the inflammatory cascade that results in disease.

To test this hypothesis we have developed a novel pre-clinical mouse model of leaky gut through acute exogenous administration of recombinant murine TNF.

The aims of the project are to:
_ Establish the kinetics and cellular dynamics of epithelial cell shedding, immune cell activation and inflammatory cell recruitment in this model.
_ Determine how the intestinal immune system, particularly IntraEpithelial Lymphocytes (IELs), innate lymphoid cells (ILCs), dendritic cells and macrophages senses and responds to leak of luminal microbial antigens.
_ Determine the molecular interactions required for the initiation and amplification of intestinal inflammation as a pathway for drug discovery.

These studies will involve two-photon microscopy, multiparameter flow cytometry and gene function analyses as well as pharmacological studies using monoclonal antibodies and small molecular inhibitors to target steps in the pathogenesis of intestinal inflammation.

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Immunology and Immunodeficiency Group

Project: STAT3-mediated regulation of human antibody responses

The ability of B cells to differentiate into antibody (Ab)-secreting plasma cells (PC) is critical for host protection against infectious pathogens. This unique feature of B cells also underlies the success of most available vaccines. B-cell differentiation into PCs is regulated by the integration of signals provided by antigen, T-cell help and specific cytokines. Signal transduction pathways activated by these molecules converge to activate key transcription factors (TFs) that mediate the commitment of activated B cells to a PC fate.

The requirements for B cell differentiation have been gleaned from studies of humans and mice with mutations in key genes. Our studies of a series of monogenic primary human immunodeficiencies have identified the critical role of the IL-21-IL-21R/γc-STAT3 signalling axis in mediating the differentiation of naïve B cells into memory cells and PCs, and therefore the generation of robust humoral immune responses and establishing long-lived serological memory. The next step is to advance our understanding of how STAT3 controls human B-cell function, and translate this knowledge into improved vaccine development and drug discovery for various immunopathologies. To this end, it is imperative to determine the molecular mechanism by which IL-21/STAT3 signalling operates in the context of human B cells to induce their activation and differentiation.

Objectives

The objectives of this PhD project will be to:
_ Identify how targeting of genes by STAT3 is differentially regulated by cytokines in naïve and memory B cells
_ Determine how this contributes to enhanced responses by memory B cells; and
_ Elucidate the mechanisms by which disease causing mutations affect STAT3 function.

Outcomes and significance

Elucidating the mechanism by which STAT3 functions to regulate human B-cell differentiation is highly significant to human health and disease. First, it will shed substantial light on the molecular requirements for human B-cell function. Second, and more importantly, it will reveal molecules and pathways that could be targeted therapeutically to, on one hand improve humoral immunity in cases of immunodeficiency, immune suppression and vaccination, or, on the other, attenuate pathological Ab responses in the setting of B-cell mediated autoimmmunity. As this project focuses on human immunology, translation of findings to clinical settings will be immediately feasible, representing a substantial advance over studies of non-human species.

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Transplantation Immunology

The Transplantation Immunology Group studies the immunology of inflammatory diseases including autoimmune diabetes and rejection of transplants. Our research involves basic science research as well as clinical studies and trials in the field of human islet transplantation. A number of projects are available that would suit highly motivated and ambitious students. Our research includes analysis of gene expression and regulation, molecular signalling pathways that regulate inflammation, cellular immunology, and animal models of type I diabetes and organ graft rejection. Cutting edge technologies used in our research include use of animal knock and transgenic models; cell signalling and molecular biology, cellular immunology, as well as molecular genomic approaches including RNAseq, micro-array, methylation studies and histone modifications, and bioinformatics. This work covers the fields of immunology, diabetes, genetics and transplantation.

Project 1: Immunology of transplantation

Transplantation of organs is a life saving procedure that can only happen with the use of immunosuppression. However, even with immunosuppression most transplanted organs will not survive forever. Also immunosuppression can be toxic, cause cancer, and may prevent the ability of the body to accept (tolerance) the transplant. For these reasons we are looking at different ways to reduce the need for immunosuppression. We are interested in how signalling pathways in T cells, but also the transplant itself, cross talk to drive the destruction of the transplanted organ. By changing and re-wiring this 'rejection-circuitry' we can show that transplants survive longer with less immunosuppression. This idea may be beneficial in the case of pancreatic islet transplantation, one potential therapeutic option to restore normal blood sugar regulation in people with type 1 diabetes.

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Project 2: The role of NF-kappaB in pancreatic islet biology

NF-kappaB is a transcription factor that controls cellular pro-inflammatory response genes but also genes like TNFAIP3 (otherwise known as A20). We have found that the TNFAIP3 gene is regulated by NFkappaB signalling axis in pancreatic islet cells. TNFAIP3 functions as a negative feedback to dampen NF-kappaB activation and prevent cell death. These data suggest that NF-kappaB controls anti-inflammatory and protective responses, whilst simultaneously elaborating pro-inflammatory responses. Thus, it can be seen that a tissue involved in inflammation undergoes a complex series of signalling responses dependent upon these multiple actions of NFkappaB, which could contribute to the final outcomes of tissue destruction or tissue survival and repair. Understanding this signalling axis may be important in the case of autoimmune disease and transplant rejection - situations where tissue inflammation drives a destructive immune response. Indeed, TNFAIP3 has been identified in a number of human GWAS studies for autoimmune disease. Using cell specific deletions of NF-kappaB genes and state of the art genomics to map the islet transcriptome and methylome, we are unravelling these complex signalling pathways in the context of transplantation and diabetes. Understanding the molecular networks that control tissue inflammatory responses may lead to novel ways to prevent transplant rejection and autoimmunity.

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Antibody Therapeutics Group

Our laboratory is working on the development of novel antibody-based therapeutics.

The following PhD projects are available:

Project 1: Molecular engineering of antibody therapeutics

Therapeutic monoclonal antibodies are among the fastest growing class of drugs in the pharmaceutical sector with more than $30 billion sales in 2012. Examples include the breast cancer drug Herceptin and the anti-inflammatory drug Humira. Unfortunately, many human antibodies display poor stability and a tendency to aggregate. This greatly hinders the development of therapeutics and results in high failure rates in pre-clinical drug development. Our group has pioneered approaches to increase the stability of human antibody therapeutics using high-throughput phage display and X-ray crystallography methods.

Project 2: Targeted cancer therapy

Monoclonal antibodies hold great promise for the treatment of cancer. Unfortunately, the majority of current antibody drugs are directed against a limited number of well characterized cell surface receptors (such as HER2, EGFR). New targets and therapeutics are urgently required. Our group is developing new antibody therapeutics against targets emerging from the Garvan research programs.

Supervisor: A/Prof Daniel Christ
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Recent Publications

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Obesity and Metabolism

Obesity is a major risk factor for many other diseases including diabetes, cardiovascular disease, Parkinson's disease and cancer. This indicates that these diseases are mechanistically linked. Our division takes a very broad approach involving basic and clinical research to tackle the complexity of metabolic disease. This by definition requires interdisciplinary research so that we can integrate various layers of information that depict the behaviour of mammals as they respond to changes in their environment. We have expertise in islet, fat cell, liver and muscle biology. We use a combination of molecular, cellular, biochemical and physiological approaches to dissect the metabolic wiring in these different organs with the ultimate goal of pinpointing major regulatory features that both cause disease and/or may be manipulated therapeutically.

Most of our students publish first author papers in top level journals and end up doing postdoctoral fellowships in some of the best labs throughout the world. Many have gone on to successfully establish their own labs around the world.

Prof Greg Cooney
Acting Division Head

Molecular Metabolism Group

Increased body fat (obesity) is one of the most important current health problems because obesity is associated with the development of a number of serious and common diseases such as heart disease, stroke, type 2 diabetes, liver disease, arthritis and cancer. The broad aim of our projects is to understand how different tissues and different genes contribute to the way the body balances food intake and energy expenditure to maintain healthy body weight and what goes wrong when this balance breaks down and obesity develops.

Project: Circadian rhythms and energy metabolism

Many important genes of metabolism are expressed in a circadian rhythm synchronized with the light/dark cycle and feeding/sleeping patterns. The modern lifestyle is associated with disrupted eating and sleeping patterns and an increase in obesity but whether this is accompanied or caused by a disruption in the circadian rhythms of gene expression is not known. This project investigates whether circadian gene expression is altered in situations of obesity and insulin resistance and what metabolic processes these genes regulate in different tissues. Our recent studies have established that altering feeding patterns can have significant and different effects on energy metabolism in liver and muscle (1). Future studies will involve altering the expression of specific genes that control circadian rhythms in liver and muscle and examining how this alters the way liver and muscle deal with glucose and lipid metabolism.

Recent publications

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Islet Biology Group

The current epidemic of type 2 diabetes represents a major global health problem, with over 7% of the Australians suffering the disease. While there is a well-established relationship between obesity and insulin resistance, the majority of overweight individuals do not develop type 2 diabetes because their pancreatic ß-cells compensate with enhanced insulin secretion. It is the failure of ß-cell compensation that is fundamental to the development of diabetes. The ß-cell is a highly specialised cell with a unique metabolic profile and differentiation specifically geared towards making these cells able to sense fluctuations in circulating glucose levels and secrete insulin accordingly. We propose that in susceptible individuals, a gradual rise in blood glucose (hyperglycaemia) and lipid levels resulting from increasing obesity and insulin resistance leads to a loss of the unique expression pattern of genes necessary for appropriate insulin secretion. This exacerbates hyperglycaemia, which causes further ß-cell dedifferentiation and eventually the death of ß-cells by apoptosis. Our group has recently found evidence in several models of diabetes that supports this hypothesis. We have identified and are investigating novel candidate genes that link hyperglycaemia to the development of impaired ß-cell function. Furthermore, we are investigating endoplasmic reticulum (ER) stress as a potential mechanism for ß-cell destruction in type 1 and type 2 diabetes.

We are using *in vivo* and *in vitro* systems to investigate the following hypotheses important for our understanding of ß-cell failure and progression to diabetes:

_ The loss of ß-cell phenotype (dedifferentiation) underlies the loss of insulin secretory function in type 2 diabetes.
_ Hyperglycaemia plays a critical role regulating the progression to ß-cell dedifferentiation.
_ The overexpression of key candidate gene products play an integral role linking hyperglycaemia to the loss of ß-cell differentiation and secretion.
_ ER stress is necessary and contributes to ß-cell death in type 1 and type 2 diabetes.

Identifying the mechanisms of ß-cell failure in diabetes is of critical importance considering that the incidence of newly diagnosed diabetes is growing to epidemic proportions. Our studies will make a major contribution to our understanding of why ß-cells fail in diabetes and aim to provide novel therapeutic targets in the treatment of diabetes.

Recent Publications

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Chan JY, Biden TJ, Laybutt DR. Cross-talk between the unfolded protein response and nuclear factor-∫B signalling pathways regulates cytokine-mediated beta cell death in MIN6 cells and isolated mouse islets. *Diabetologia* 2012; 55:2999-3009

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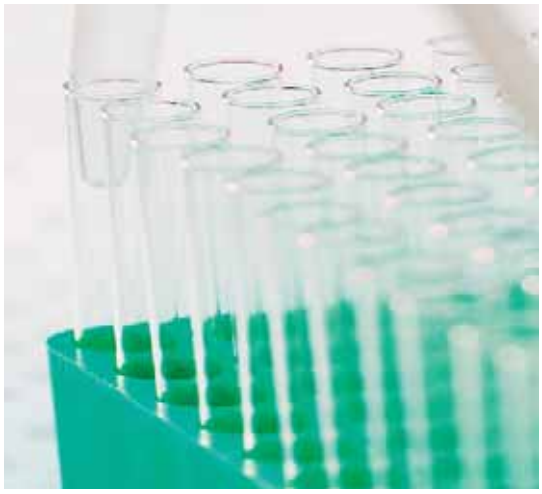

Beta Cell Regeneration Group

The common forms of diabetes are characterized by the destruction (type 1) or an insufficiency (type 2) of insulin secreting pancreatic beta cells. We are taking an interdisciplinary approach to devise novel strategies for beta cell replacement therapy. Our primary experimental system is the zebrafish embryo, a model that is at the intersection of genetic and pharmacological research.

Project 1: Directed reprogramming of acinar cells

We are applying insights from developmental biology to use the abundant pancreatic acinar cell type as a source of progenitors for beta cell regeneration. We have established an in vivo model to induce acinar cell reprogramming and track the fate of the cells as they transition to insulin producing beta cells. This project will focus on increasing the efficiency and specificity of cellular reprogramming. We are particularly interested in developing a protocol that is responsive to the metabolic dysfunction associated with diabetes.

Project 2: *in vivo* drug screening

Traditional drug screens have targeted single molecules or cell types. While the targets are often well justified, it is difficult to predict how the hits will behave *in vivo*, which has contributed to the poor success rate for new drugs in recent years. We have developed a number of transgenic models that allow us to monitor metabolic parameters in intact embryos (glycemia, beta cell mass, etc.) to help identify the next generation of antidiabetic drugs. Projects in this area would include assay development and screening as well as mechanistic analysis of previously discovered lead compounds.

Selected Publications

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Appetite and Adiposity in Type 2 Diabetes and Prader-Willi Syndrome Group

Project: Advancing therapeutical management of obesity in Prader-Willi syndrome

Prader-Willi syndrome is one of the most common known genetic causes of obesity, occurring in 1:15,000 live births. Besides some behavioural and endocrine abnormalities, this syndrome is known for the insatiable appetite and hyperphagia which leads to severe obesity if access to food is not restricted. There are no tested pharmacological treatments available so far, and strong behavioural restraints with locking the fridge are usually the only options to prevent these patients from gaining weight.

Our PWS Research Group at the Garvan has a considerable track record of doing research in PWS, usually recruiting patients from the PWS Clinic at RPAH and through the PWS Society. PWS is an excellent human model to understand and learn more about appetite dysregulation and obesity in general. Our published research includes assessment of systemic inflammation, body composition, hunger and satiety ratings during a meal, and assessment of autonomic nervous system. Furthermore, we have conducted the first clinical trial testing a GLP-1 analogue for its use to control appetite and body weight.

Based on the successful pilot study where we tested a short acting GLP-1 analogue, a project is now being built up aiming to test different clinically available GLP-1 analogues for their efficacy and safety in PWS. A theoretical risk with these agents remains the still open question whether gastric emptying is delayed in PWS, which is still controversial, and whether it could be further delayed by GLP-1, increasing the risk of the potentially lethal complicating of gastric necrosis.

This project aims at recruiting 8-10 PWS subject for a randomised cross-over trial testing once daily and once weekly GLP-1 agonists. Gold standard methods of gastric emptying will be used to rule out significant alteration in gastric emptying. If this study can prove the safety and efficacy of these pharmacological agents in PWS, then these would be the first effective treatments available for hyperphagia in PWS, and represent a huge milestone for the patients, families and carers.

The project would involve conducting clinical research using state-of-the-art techniques for metabolic phenotyping, with much opportunity for learning standard laboratory techniques.

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Personalised Medicine in Diabetes Care Group

The increasing prevalence of obesity and type 2 diabetes (T2D) has reached epidemic proportions, and new ways of prevention and effective treatment are urgently needed. T2D is a chronic disease accounting for 95% of diabetes worldwide, and is characterized by an insufficient compensatory insulin secretion to insulin resistance. In contrast to other diseases, it consists of a number of subgroups differing in phenotype, manifestation and disease mechanisms. Personalised medicine represents a novel approach for defining both disease subtypes and biomarkers that could identify those patients who are most likely to benefit from a specific treatment. It can be used in prevention, detection, individualised treatment and monitoring of diseases. In today's treatment algorithms, little attention is given to the huge variation in phenotype in these patients, who might have significant differences in insulin resistance, beta-cell function, gut hormone levels, systemic inflammation and central control of metabolism including the autonomic nervous system. No treatment decision is currently made on this basis, but rather on the available evidence as to which treatment the biggest numbers of patients respond. However, phenotypic assessments complemented with genomic data may well enable identification of a subtype with specific treatment options which could avoid ineffective and time wasting treatments, and ultimately lead to a more targeted and rational treatment strategy in T2D.

In addition to the above, many patients are thought to be misdiagnosed for type of diabetes, which results in suboptimal treatment and worse clinical outcome. This includes many cases of monogenetic forms of diabetes, most commonly Maturity Onset Diabetes of the Young (MODY) who never had genetic testing and are presumed either T1D or T2D. Many of these patients present clinically in-between T1D and T2D, and treatment allocation is most commonly based on clinical judgment and also on their response to the chosen treatment regimen. Monogenetic diabetes may make up 1-5% of patients in a large diabetes clinic. There are clear advantages of diagnosing these monogenetic forms of diabetes: In many patients, insulin may not be required and could be substituted with oral agents. In addition, families should be screened and additional affected patients could be identified and appropriately treated at an early stage. However, genetic testing is still not widely available, is expensive, and routine tests may not detect all the known genetic mutations if they are not specifically looked for.

The aims of this project include novel approaches to carefully phenotype individual patients with T1D or T2D to:

_ Identify the most commonly affected mechanisms of disease in T2D, leading to the definition of specific disease-subtypes
_ Screen for gene variants associated with these phenotypic features. Identification of specific risk genes could eventually assist or even replace future clinical assessment for stratifying these patients into their specific subgroups.
_ Test the effect of allocating these T2D patients to suitably targeted treatment options considering the specific pathophysiology.
_ Prospectively collect data to track treatment responders and non-responders in order to identify gene polymorphisms which could serve as predictors and be used to build new treatment algorithms, using a pharmacogenomic approach.
_ Set up a genetic testing facility in our clinical genomics centre to be able to screen patients for monogenetic diabetes
_ Analyse genome data and try to find new candidate genes for T1D

In summary, these new strategies could test a new methodology of personalized medicine which could prove to be more health protective and cost effective in the long term, and provide a new treatment algorithm for T2D for the future. Screening for the most common forms of monogenetic diabetes would allow identifying so far undiagnosed patients and allow tailoring their treatment specifically for their gene defect. The project would involve conducting clinical research with a strong link to the genome sequencing and bioinformatics facilities.

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Neuroscience

The Garvan Neuroscience Division is an active, collaborative research community that investigates how the brain functions. Research undertaken by the Division looks at the brain at many different levels, from genes and molecules to synapses, neurons, brain regions and behaviour. A wide range of models from flies, mouse to humans and state-of-the-art molecular and biochemical techniques are employed to address both basic and medically relevant problems in neuroscience. The Division's goal is to understand how the brain works and to improve understanding, diagnosis, and ultimately develop novel therapies for neurological disorders. We are particularly interested in conditions like Parkinson's Disease, Alzheimer's Disease and general conditions of dementia in which the natural ability of the brain to regenerate itself (via neuro-stem cells) is compromised. Furthermore, we investigate the role of the nervous system in pain perception as well as how the brain communicates with other organs and tissues in the body, for example to control bone formation; and in the regulation of energy balance (intake and expenditure), which affects fertility, mood, weight gain, physical fitness and how this can lead to obesity.

Prof Herbert Herzog
Division Head

The majority of the PhD students trained in the Neuroscience Division are supported by Australian Postgraduate Awards or NHMRC scholarships, and have received numerous presentation awards and travel fellowships to national and international meetings. Research produced by our students is published in high-ranking journals such as *PNAS, J.Biol.Chem, J.Clin.Invest., JBMR, Nat. Med, PlosONE, Cell Metabolism, J. Neurosci, Cell* and *Nature*.

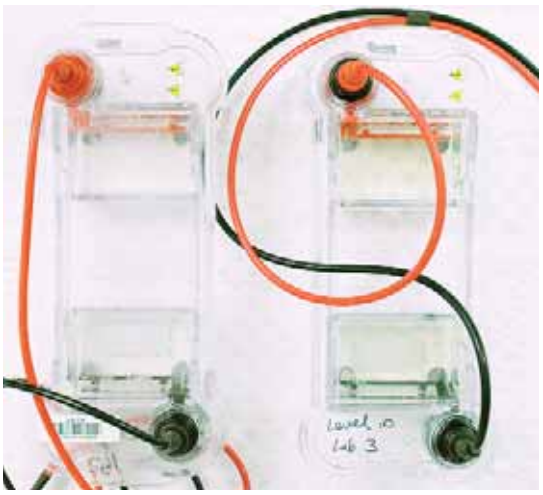
Eating Disorders Group

Project 1: Novel neuropeptide regulators of energy homeostasis

The worldwide prevalence of obesity is increasing at alarming rate, and is a major risk factor for type 2 diabetes and other diseases. Although the benefits of losing excess weight are undisputed, there currently exists no effective non-surgical treatment for obesity. Body weight and body composition such as fat tissue mass are regulated by an interactive complex of energy homeostatic system. Thus to meet the urgent and desperate need for the development of novel pharmacological tools for treating obesity, researchers need not only to know the identity and functions of individual molecules and pathways involved in the regulation of energy homeostasis, but also to understand how these molecules and pathways interact. Among these, neuropeptide Y (NPY), - one of the most widely expressed molecule in the brain, is a known player critically involved in the regulation of body weight ad adiposity via its control on every aspects of energy homeostasis, such as appetite, energy expenditure, physical activity and fuel partitioning[1]. Recently, our unpublished studies show that neuropeptide FF and NPFF receptor 2 (NPFF2R) are the novel players in the energy homeostatic complex. Interestingly, our preliminary results suggest that NPFF system may exert its control on energy homeostasis via interacting with NPY pathway.

Therefore, this project is to:
_ further investigate the mechanism by which NPFF system regulates energy homeostasis;
_ to investigate how the NPFF and NPY systems interact in these regulations.

To achieve this, we will examine aspects of energy homeostasis and factors in controlling them in multiple mouse models where either or both NPFF and NPY system have been genetically altered. Such mouse models include mice with NPFF overexpression by delivering the NPFF-containing adeno-associated viral vector to the adult mouse brain, germline NPFF2R knockout mice, and mice with adult-onset specific deletion of NPFF2R from NPY neurons. By utilizing cutting edge internationally competitive technology and unique germline and conditional knockout and transgenic mouse models, this project will make highly original and high-impact contributions to the understanding of the role of NPFF system in energy homeostasis and its interactions with the NPY pathway, and will demonstrate whether targeting NPFF2R could provide the basis of novel anti-obesity treatment.



Major techniques involved in this project

Indirect calorimetry, infrared imaging, stereotactic brain injection, oral glucose tolerance test, intraperitoneal insulin test, dual-energy X-ray absorptiometry, tissue dissection, *in situ* hybridyzation, Western blotting, immunohistochemistry, various serum assays.

Selected recent publication

Zhang L et al. The neuropeptide Y system: Pathological and implications in obesity and cancer. *Pharmacol Ther.* 2011 Jul:131(1):91-113

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Project 2: Insulin action in the brain

The prevalence of obesity has reached epidemic levels and is further increasing at an alarming rate. Currently there are no effective therapeutic treatments for obesity, however it is generally recognized that any treatment must be associated with a reduction in energy intake, an increase in energy expenditure or ideally both. Therefore, defining how the central nervous system coordinates information to regulate energy balance is important for understanding the pathology of obesity as well as for designing treatments to combat this disease. Insulin is a potent anabolic hormone, secreted by the pancreas in response to the increase in blood glucose levels. Recently, insulin has been reported to have effects not only in the peripheral tissues, but also in the brain to regulate satiety and glucose and energy balance. Previous studies from our lab and others have established the importance of the central neuropeptides Y (NPY) system in the regulation of food intake and energy expenditure, with hypothalamic NPY mRNA levels elevated in several rodent models of obesity. Increased NPY levels contribute to the development of obesity in a two-fold way by increasing food intake and also reducing energy expenditure. Although insulin is known to influence energy balance, the precise neuronal action and population(s) of neurons that mediate insulin action remains unknown. Thus, the major aim of this project is to understand and define the role of insulin in NPY neurons in the regulation of energy homeostasis. This research will not only help to get more mechanistic insights in the etiology of obesity, but also contribute to the precise understanding of central insulin action in the NPY-ergic pathway in the regulation of energy homeostasis.

Specific Aims

_ To generate and characterize NPY-neuron specific IR-deficient mice.
_ To investigate the molecular mechanisms by which central insulin action regulates energy homeostasis.

Summary of techniques to be used

Conditional knockout mouse models, indirect calorimetry, metabolic measurements, real-time PCR, in situ hybridization, western blotting, patch clamp electrophysiology, immuno-histochemistry.

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Project 3: SNORD RNA's and their role in obesity

Obesity is a major global public health concern, with Australia being one of the most affected countries. Although great effort has been placed on identifying treatments for obesity and critical players and pathways that control appetite have been characterised, hardly any effective drugs are on the market. Therefore there is a desperate need to identify new alternative targets to treat obesity. One way to learn more about the critical pathways that control food intake and energy homeostasis is by investigating naturally occurring mutations that lead to obesity. The identification of the gene mutation in the leptin gene that causes the massive obesity in the ob/ob mouse was a landmark discovery, which has and still provides us with important information about the control of this complex system. While mutations in the leptin gene in humans are actually very rare there are other genetic variations that also lead to massive increase in appetite and the development of obesity that have much higher frequencies like the one causing Prader-Willi-Syndrome (PWS), which is the most common known genetic cause of obesity, with a prevalence of 1 in 25,000 to 1 in 10,000 live births.

PWS is characterized by severe infantile hypotonia with poor suck and failure to thrive in the first 1 to 2 years of life. This initial lack of feeding drive changes then dramatically and subjects with PSW develop an obsession with food leading to an un-saturable appetite, which if not controlled, will lead to early-childhood onset obesity. PWS is due to the absence of paternally expressed imprinted genes at 15q11.2-q13. Interestingly, single deletion of known genes in this region in mice although showing some effects related to the PWS phenotype, do not result in a phenotype that would resemble the classical features of overeating and development of obesity seen in human PWS subjects. Importantly, several recent studies have identified subjects with PWS that have only micro-deletion in this locus on chromosome 15, but still show many of the major features such as increased appetite and early onset of obesity characteristic for this syndrome. The different deletions vary in size but all contain the entire 27 copies of the SNORD116 locus.

Astonishingly hardly anything is known on how the genetic variations in Snord genes cause the incredible high-level of appetite and massive obesity in affected individuals. Therefore the major aim of this study is to identify the underlying mechanism that leads to increased appetite and body weight of a particular mutation in this PWS locus, called Snord116 using various genetically modified mouse models.

Specific Aims

_ Determine the effect of adult onset SNORD116 deficiency on food intake and energy homeostasis
_ Investigate whether re-introduction of SNORD116 can rescue the hyperphagia of SNORD116 KO mice
_ Identification of SNORD116 downstream affecter pathways

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Project 4: Anorexia Nervosa–the starving brain

Anorexia nervosa is a debilitating disorder affecting as many as 1 in 100 young women. Approximately 10% of people suffering with anorexia are male.

Without treatment, up to 25% of people with anorexia nervosa die. With treatment, about 20% of patients make only partial recoveries, remaining too focused on food and weight to be able to participate fully in life. An additional 20% of sufferers do not improve, even with treatment. They are seen repeatedly in emergency rooms, eating disorders programs and mental health clinics. Clearly, new treatments for anorexia nervosa are desperately required.

The precise causes of anorexia nervosa are unknown, but environmental and psychological factors often cited as playing a role. However, emerging evidence strongly suggests genetic causes for anorexia nervosa. For instance, most people simply cannot diet down to an unhealthily low body weight. That is because weight loss activates strong physiological mechanisms that protect against further weight loss. This 'famine reaction' is triggered by natural brain chemicals in a part of the brain called the hypothalamus, with effects include irrepressible hunger, lethargy and sharp reductions in metabolic rate.

Paradoxically, people with anorexia nervosa do not demonstrate these expected responses to weight loss, suggesting perturbations in the natural brain chemicals responsible for the famine reaction. If we understood exactly which chemicals in the brain were responsible for mediating the famine reaction, how they worked, as well as how these molecules are perturbed in anorexia nervosa, then we could develop novel treatment strategies to target the physical causes of this debilitating disorder, and possibly therefore help people who do not respond to conventional treatments.

Using sophisticated genetic engineering techniques, we have developed mice with perturbations in genes encoding substances that act on the brain to mediate the famine reaction, such as neuropeptide Y, peptide YY, and dynorphins. Intriguingly, these transgenic mice demonstrate metabolic features characteristic of people with anorexia nervosa, notably an enhanced ability to lose weight and burn body fat. However, in order to fully investigate the role of these substances in the development and treatment of anorexia nervosa, we need to investigate their effects on food intake and body composition other eating related behaviours.

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Project 5: Altering thermogenesis as weight-loss strategy

Obesity-associated cardiovascular diseases and diabetes are leading causes of death and are expected to increase as the obesity epidemic worsens. Current weight-loss therapies mainly target reduction of energy intake, providing only a transient or partial solution with limited effectiveness. Alternatives are needed to combat this problem and one potential promising approach is to target the other side of the energy balance equation, energy expenditure.

The therapeutic potential of brown adipose tissue (BAT) in weight reduction via the regulation of energy expenditure has emerged as a conceivably promising yet underexplored area. Whilst previously believed to be small animal-specific and exclusively neonatal in mammals including humans, the abundance of functional BAT in adult humans has been recently confirmed to be widespread by positron emission tomography (PET) marking it a promising target for anti-obesity therapy. However, little is known about the control of BAT activity and function. BAT is the main tissue that harbours uncoupling protein 1 (UCP1), the major component that is responsible for mediating metabolic thermogenesis. Our preliminary data demonstrates that elevated neuropeptide Y (NPY) levels specifically in the arcuate nucleus (ARC) of the hypothalamus, which is known to be a major driver for marked reductions in energy expenditure, also influences UCP1 expression in the BAT. We thus aim to investigate the specific role of the NPY system in integrating hypothalamic functions with energy expenditure specifically focusing on BAT activity. To achieve this, we will utilize a set of novel and unique mouse models that allow for the neuron-type specific conditional deletion or over-expression of NPY in an inducible adult-onset fashion. A wide range of laboratory techniques will be employed, including but not limiting to in-situ hybridization, immunohistochemistry, high-sensitivity infrared thermal imaging, histological examination, cell cultures, quantitative real time-PCR and Western blotting, to determine the key regulators of thermogenesis and mitochondrial function and mechanistic central pathways possibly involved. All of the mouse models, methods and experimental paradigms are well established in our laboratory as demonstrated by our extensive publication record on these topics in highly ranked journals like *Nature Medicine* and *Cell Metabolism* (1,2,3,4,5).

Results from this study will provide critical new insights on NPY's role in the control of BAT-mediated energy expenditure. These results will also provide valuable contributions to the development of potential therapeutics to increase energy expenditure, likely being a more effective way for the treatment of obesity.

Selected Recent Publications

1. Johnen H, Lin S, et al. Tumor-induced anorexia and weight loss are mediated by the TGF-beta superfamily cytokine MIC-1. *Nat Med*. 2007 Nov;13(11):1333-40

2. Lin S, Shi YC, et al. Critical role of arcuate Y4 receptors and the melanocortin system in pancreatic polypeptide-induced reduction in food intake in mice. *PLoS ONE*. 2009;4(12):e8488

3. Cox HM, Tough IR, et al. Peptide YY Is Critical for Acylethanolamine Receptor Gpr119-Induced Activation of Gastrointestinal Mucosal Responses. *Cell Metab*. 2010 Jun 9;11(6):532-42

4. Shi YC, Lin S, et al. NPY-neuron-specific Y2 receptors regulate adipose tissue and tranbecular bone but not cortical bone homeostasis in mice. *PloS ONE*. 2010;5(6):e11361

5. Shi YC, Lin S, et al. Peripheral-specific Y2 receptor knockdown protects mice from high-fat-induced obesity. *Obesity*. 2011 Nov; 19(11): 2137-48

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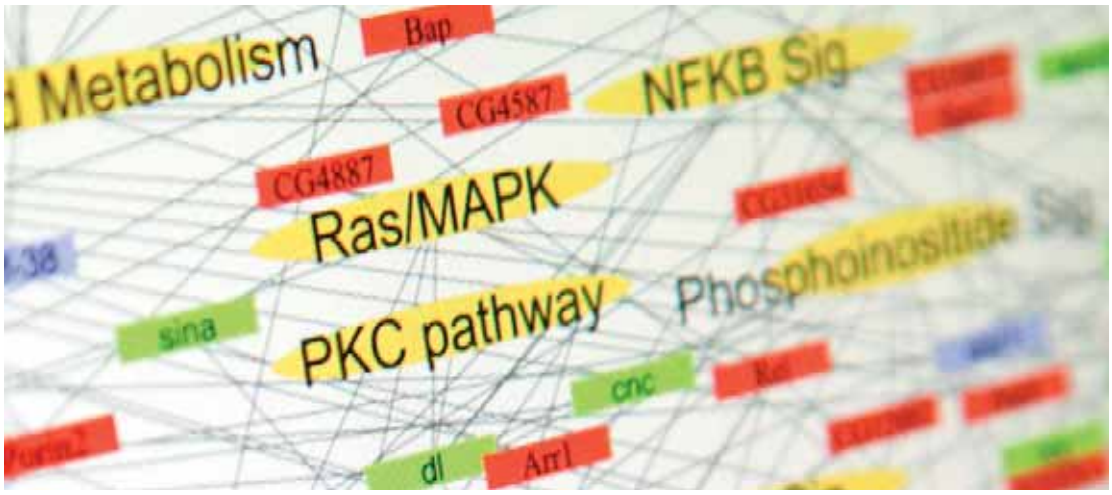

RNA Biology and Plasticity Group

Over 98% of the human genome does not encode proteins, yet over 80% is transcribed into RNA in a developmentally-coordinated and tissue-specific manner. With the recent advances in genome sequencing technologies, understanding how human genetic variation contributes to the onset and development of complex diseases is more feasible than ever. The RNA Biology and Plasticity lab studies how non-protein coding regions of the genome function at the level of RNA through a combination of molecular biology, pioneering next generation high-throughput sequencing technologies, and bioinformatics. We, and others, have shown that non-coding RNAs (ncRNAs) play a major role in human development by directing the epigenetic complexes that control chromatin structure and gene expression, and are associated with many complex diseases including cancer and neurological disorders. We have also shown that non-coding regions of the genome are conserved throughout evolution in both primary sequence and secondary structure, suggesting that there is still much to discover about the regulatory programming of complex organisms.

Project 1: RNA structures regulating development and disease

We currently have a PhD project available using next generation sequencing techniques to resolve the structure-function relationship of long ncRNAs. The project will investigate the molecular mechanisms of how genetic variation contributes to disease onset, focusing on the role of higher-order RNA structures in the regulation of epigenetic states. This will involve techniques in tissue culture, molecular biology, biochemical assays, and cutting-edge next generation sequencing technologies. Furthermore, the project will potentially involve bioinformatics analyses, although experience in computational biology is not a pre-requisite.

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Project 2: Deciphering the epitranscriptome

A major challenge now lies in deciphering the language used by ncRNAs that determines their function. Our hypothesis is that RNA modifications are part of a vital and yet undeciphered epigenetic code used by the RNA world to transmit primary sequence information into dynamic biological function. Over 100 epigenetic modifications of RNA have been documented, of which >90% affect non-coding RNA species. Several RNA modifications are highly enriched in brain, and mutations in three RNA methyltransferases, cause intellectual disability in humans and affect cognitive ability in other species. This project will use a combination of well-established cutting-edge experimental techniques, transgenic mouse models and bioinformatics to analyse the distribution of certain modifications in human and mouse, to essentially identify and characterise the RNA modification events that when deregulated, lead to intellectual disability.

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Project 3: What makes us human?

Our brains have evolved higher cognitive abilities through fine-tuned and dynamic regulation of gene expression. Characterising the genomic and molecular mechanisms of human-specific neural function is an essential step to further our understanding of intellectual disability and neurodegenerative diseases. A PhD project is available to investigate human-specific RNA transcripts involved in neural activity. This will involve generating neurons from induced pluripotent stem cells (iPSC) taken from patient skin samples, a technology that has recently revolutionised neuroscience. This approach allows for effective exploration of human neural mechanisms from both unaffected control and disease-affected patients (e.g. schizophrenia). Furthermore, we will use Next Generation Sequencing (NGS), already established in our laboratory, to take a comprehensive view of the dynamic neural transcriptome. The project will require collaboration with computational biologists to analyse the data, including new approaches for circumventing repeat-mapping problems.

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http://garvan.org.au/research/neuroscience/rna-biology-and-plasticity/@@publications

Parkinson's Disease and Neurodegeneration Group

Parkinson's disease (PD) is a debilitating neurodegenerative disorder that affects ~80,000 Australians and >6 million people worldwide. The prevalence of PD is expected to increase significantly in our aging population and currently there is no test for early diagnosis, no cure and no long-term effective therapy. The lack of knowledge of the underlying mechanisms responsible for causing PD and its progression is the major impediment to therapeutic advances. To achieve earlier diagnoses and development of treatments and drugs, our research, currently funded by both the Australian NHMRC and Michael J Fox Foundation, centers on discovering the cascade of molecular and cellular events that cause the loss of neurons in PD.

Attaining these goals will:

_ provide critically needed biomarkers for PD that will allow significantly earlier diagnosis, screen for people at risk for PD, and to monitor both disease progression and therapeutic effectiveness.

_ provide mechanistic insights that will result in the development of treatments and drugs, and potentially a cure.

Project 1: The role of long non-coding RNAs in Parkinson's Disease

Long non-coding RNAs (lncRNAs) are RNA transcripts of >200 bases (distinct from microRNAs) that typically do not encode proteins and instead function by binding RNA, DNA or proteins to perform a wide range of activities that include modulating transcription, alternative splicing, mRNA stability, mRNA translation, and epigenetic events, such as chromatin remodeling. Currently >15,000 human lncRNAs have been preliminarily identified and the number is predicted to exceed the number of protein coding genes. The rapid increase in the number of lncRNAs within the primate lineage suggests that lncRNAs have played a part in the evolution of human brain form and function. lncRNAs represent a burgeoning field of regulatory elements that play diverse regulatory roles in gene expression while their dysregulation is associated with human diseases including Alzheimer's and Huntington's disease. lncRNAs represent a new frontier in molecular genetics and molecular biology that have tremendous potential for a transformative advance in our understanding of PD by integration of the genetics, cellular pathways and potential environmental impacts of this disease. Integrating human PD patient genetic data with bioinformatics analysis has led us to identify a number of human lncRNAs that are clearly contributing to the disease. This project offers a wide range of interesting aspects to investigate including modulating their expression as therapy, identifying their targets and functional mechanisms, and their use as informative biomarkers.

Project 2: The inter-relationship of αSynuclein and dysfunctional mitochondria in Parkinson's Disease

The molecular mechanisms responsible for PD development remain unclear but diverse approaches have shown that abnormalities in the synaptic protein αSynuclein play a central role in PD. Our whole genome functional screening approaches have identified αSynuclein to cause defects in several major cellular pathways. This project will dissect the inter-relationship(s) between αSynuclein, dysfunctional mitochondria (a common feature in PD), endoplasmic reticulum and reactive oxygen species to identify the initiating events in PD as they provide excellent points for therapeutic intervention.

Project 3: Exosomes- mediators of cell-cell communication and their role in Parkinson's Disease progression

Current treatments do not prevent the inevitable and insidious progression of PD as new regions of the brain degenerate and expand the symptoms to include depression, anxiety and severely reduced cognitive function. Recently, toxic forms of αSynuclein has been found to be capable of transferring from within a degenerating neuron into neighbouring healthy neurons and trigger their degeneration. Such inter-neuronal transmission of αSynuclein is proposed to be responsible for the progression or "spread" of PD. Exosomes are small vesicles released extracellularly by cells including neurons, that can be taken up by recipient cells and evoke biological responses and as such provide a form of cell-cell communication. Exosomes have also been found to contain αSynuclein and are proposed to mediate this inter-neuronal transmission of αSynuclein. Therefore preventing either the release and/or uptake of αSynuclein containing exosomes offers a real opportunity to stop disease progression. This project involves a whole genome screening approach to identify the molecular mechanism responsible for the biogenesis of αSynuclein containing exosomes and testing the findings in rodent PD models for their effectiveness as a disease treatment.

Our research projects utilise a wide range of approaches including genome-wide screening, Next Generation sequencing, bioinformatics, cell and molecular biology techniques, fluorescence microscopy, qRT-PCR, lipodomics, proteomics, metabolomics, siRNA knockdown, gene knockouts, FACS analysis, cell culture, virus mediated gene expression, primary neurons, transgenic mice models and human PD patient brain samples. Further information regarding these projects can be obtained by contacting Antony Cooper.

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Functional Genomics Group

The focus of our group is to use a "systems biology" approach, combining fruit fly, mouse, and human genetics to identify novel conserved regulators of human disease. We are currently involved in using single nucleotide polymorphism genotyping and next generation sequencing technologies to identify genes and rare mutations that contribute to human disease, which we then validate in model organism and/or human cells.

Project 1: Bird and Swine Flu research

Highly pathogenic influenza, such as bird or swine flu, represents a major health concern and social risk to our society. The dangers of new highly pathogenic and transmissible influenza is clearly exemplified by the pandemic of 1918, killing 50-100 million people or ~10% of those infected, resulting in the death of ~3% of the world population, and this devastating outbreak occurred before global air travel was commonplace. Despite major advances in medicine over the 20th century, we are still extremely limited in our ability to treat highly pathogenic influenza infection, using most of the same strategies that were employed in 1918. We have a long-standing interest in host defence to pathogens, and have developed a rapid, safe, *in vitro* method for assessing the innate immune response to bird and swine flu. In this project we will assess and model the innate immune response to bird/swine flu infection at the genomics level, and use bioinformatics to predict key regulators of innate immunity. We will then identify candidate small molecule compounds predicted to block these key innate immune regulators and test these compounds for efficacy in suppressing the human innate immune reaction to influenza *in vitro* and eventually in mice as well. The successful applicant will receive training in analysis of large genomics data sets and deep sequencing efforts, basic training in handling human peripheral blood cells and instruction in assessing innate immune responses to influenza *in vitro* (human) and *in vivo* (mice). Some previous experience with bioinformatics analysis or basic tissue culture will be preferred. At the end of this project we hope to have identified novel, FDA approved therapeutics ready for trial in human patients with highly pathogenic influenza infections.

Project 2: Sensory perception - chronic pain

There is currently a lack of effective therapies to treat chronic pain diseases such as neuropathic pain. In this project, we will perform a genome-wide association study (GWAS) for chronic pain in fruit flies. These data will then be subject to bioinformatics analysis and compared to parallel unpublished human pain genomics approaches. We will then validate candidate GWAS loci using *in vivo* transgenic RNAi. Efforts will also be made to identify key neural circuitry required for higher order pain processing in the brain. The successful applicant will receive training in delicate surgical manipulation of neural populations in live fruit flies as well as *in vivo* electrophysiology.

Project 3: Longevity and lifespan extension

In this project we have combined data from a large human genetics study on aging (The Framingham study) with functional validation of longevity in the fruit fly. We have identified multiple new "life extension" genes and this project will involved characterising these genes in more detail and mechanistically. The overall goal of this project is to target these genes therapeutically to extend lifespan with a specific focus on preventing dementia and improving cardiac performance.

Project 4: regulation of synaptic transmission and motor neurone disease

In this project we have screened the entire fruit fly genome for factors required for proper neurological development. We found about 1000 new neurological genes, and with subsequent studies focused on the synapse we have identified about new synaptic genes. This project will involved characterising some of these genes using molecular biology, electrophysiology, real-time calcium imaging, assessment of neurodegeneration and various behavioural tests, confocal microscopy, and electron microscopy. The overall goal of this project is to characterise new proteins acting on synaptic vesicles to regulate neurotransmission, with the long-term goal of developing treatments for motor neurone disease.

Project 5: Functional genomics of cancer drug resistance and metastasis

We have developed novel techniques to identify new drug targets to kill tumour cells or block metastasis. This project involves working with a team of molecular biologists and a bioinformatician to screen multiple tumour cells for genes that activate and suppress tumour growth and metastasis. This work will also involve analysis of next generation sequencing data sets, as well as validation of drugs and targets in mouse cancer models.

Project 6: Pathways regulating cell fate and transdifferentiation

We have developed novel techniques to identify gene combinations that drive dedifferentiation or transdifferentiation of cell types. This project will involve identifying gene combinations and pathways that are sufficient to alter cell fate. From a technical perspective this project will involve advanced molecular biology, cell culture, FACS, and next generation sequencing data collection and analysis. The overall goal of this project is to identify novel genes, pathways, and small molecules we can use to generate human cells and tissue for therapeutic purposes.

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7. Pospisilik et al, Targeted deletion of AIF decreases mitochondrial oxidative phosphorylation and protects from obesity and diabetes. *Cell.* 2007 Nov 2;131(3):476-91. (Cover)

Neurodegenerative Disorders Research Group

PhD Studies in Dr Bryce Vissel's group allow you the opportunity to learn and develop cutting edge technologies and approaches that will contribute to a deeper understanding and treatment of Parkinson's disease, Alzheimer's disease or spinal cord disorders. The group uses sophisticated approaches to understand how synaptic dysfunction leads to neurodegeneration and to identify potential approaches to reverse the disease process. In addition to studying mechanisms of neurodegeneration, the group studies stem cells and the mechanisms underlying regeneration in the nervous system. The goal of this work is to identify approaches that could drive recovery in the brain in diseases such as Parkinson's and Alzheimer's disease. All our projects will train you in a wide range of cutting edge approaches, including anatomy, molecular biology, gene therapy, physiology, animal behaviour, cell culture, high end microscopy, surgery and so on. Our group is helpful, friendly and highly motivated. These are kinds of studies you could undertake:

Project 1: Neural regeneration research and studies of stem cells in Parkinson's and Alzheimer's disease

Students will have the opportunity to study neural regeneration in our group. Adult neurogenesis is the process by which the brain generates new nerve cells in the adult central nervous system (CNS) from stem cells that naturally exist in the brain. Stimulating neurogenesis may potentially offer a therapeutic approach for neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and spinal disorders. In our group, we are working to identify mechanisms that regulate adult neurogenesis (neural repair mechanisms) in the normal and diseased brain, to determine if manipulating these mechanisms may offer therapeutic potential. The students who are interested in research projects in this area will learn advanced techniques in the study of neurogenesis and neural stem cells.

Techniques learned will include:

_ Stereotaxic survival surgery and gene therapy approaches,
_ Immunohistochemistry combined with advanced confocal microscopy and stereology for analysis of regeneration.
_ Use of in vitro cell systems, including neural stem cells, for studying neurogenesis.
_ Behavioural testing to determine the capacity for functional recovery in animal models
_ molecular biology. Research into mechanisms and role of neural regeneration is a cutting edge area of research worldwide and the research has significant potential to lead to important discoveries.

Project 2: The role of immune processes in learning and memory, and in Parkinson's and Alzheimer's disease

Our lab's studies are identifying a critically important role for inflammatory processes in brain plasticity and disease. In our group, we are working to identify mechanisms that regulate the interaction between inflammatory cells and neurones in specific brain regions, with a view to understand how these mechanisms ultimately lead to normal bran function, or abnormal brain function in diseases such as Parkinson's and Alzheimer's disease. The students who are interested in research projects in this area will learn advanced techniques in the study of neurodegeneration and neuroinflamation.

Techniques learned will similarly include:

_ Stereotaxic survival surgery and gene therapy approaches,
_ Immunohistochemistry combined with advanced confocal microscopy and stereology for analysis of regeneration.
_ Use of in vitro cell systems.
_ Sophisticated learning and memory and movement studies in mice
_ Molecular biology. Research into mechanisms and role of neurodegeneration and neuro-inflamation is another cutting edge area of research worldwide and the research has significant potential to lead to important discoveries.

Project 3: Post-transcriptional events that regulate neural plasticity, memory and diseases

We have recently identified RNA editing of specific neuronal RNAs as a novel mechanism that can affect Parkinson's disease, Alzheimer's disease and behaviour in mice. This is an exciting and novel project that offers interesting possibilities for significant new insights into brain function. The experiments will use similar methods to those described for the projects above.

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Bone Biology

Research at the Bone Biology Division is focused on understanding the causes and development of new treatments for major diseases of the skeleton, particularly osteoporosis and cancers, such multiple myeloma, and breast and prostate cancers that metastasise to bone. The latest cutting edge technology in genomics, proteomics and contemporary imaging approaches are being applied to address critical clinical questions in skeletal medicine. Students in the Division have made fundamental discoveries that are having a real impact in skeletal medicine. Garvan researchers were the first to show the importance of genes in regulating the skeleton; have identified critical molecular pathways that regulate bone; and recently discovered the importance of neurological control of bone. Work undertaken at the Garvan has also led to the development of new approaches to predicting who will fracture their bones, an example of laboratory discoveries being translated directly into the clinic for patient benefit. Research from the Bone Biology Division has been published in high ranking journals such as *Nature, Nature Genetics, Blood, JAMA,* and *N.Engl J. Med*. PhD students participate and present their work at major international scientific meetings and attracted numerous awards. Our postgraduate students are highly regarded, gaining their own fellowships and have established their own independent scientific careers often at prestigious Institutes and Universities in the US, UK and Europe.

Prof Peter Croucher
Division Head

Bone Biology Group

The Croucher lab's research interests are in the major diseases of the skeletal system, particularly in diseases such as osteoporosis and tumours that grow in bone, including multiple myeloma, or those that metastasise to bone, such as breast and prostate cancer. Our research interests are in understanding the cellular and molecular mechanisms that lead to these conditions with the aim of developing new approaches for clinical intervention. In recent years we have developed new screening tools that have allowed us to identify new genes that control bone strength and developed new approaches to increasing bone mass. We have also developed novel high-resolution imaging technologies that allow us to visualize individual metastasis-initiating cells as they colonise the skeleton. Building upon these discoveries we are now utilizing the latest next generation genomic technology, bioinformatic and systems biology approaches, and the latest high-resolution imaging to take these projects forward.

We have a number of projects available in the following areas:

Project 1: New gene targets for anabolic therapy in osteoporosis

Treatments for osteoporosis prevents further bone loss but have a limited ability to restore bone mass so patients continue to fracture. In collaboration with Professor Graham William and Dr Duncan Bassett at Imperial College London, we have screened knockout mice from the Wellcome Trust Sanger Institute Mouse Genetics Programme and identified strains with increased bone strength resulting from deletion of genes not previously known to have a role in the skeleton. This project will establish the role of these pathways in controlling bone strength and identify new therapeutic targets for treating osteoporosis.

Project 2: Targeting 'metastasis initiating cells' in breast and prostate cancer

Bone metastases are a devastating clinical consequence for patients with breast and prostate cancer. The mechanisms leading to their development are poorly defined, and approaches to prevention and treatment limited. We have developed new high-resolution imaging technology that allows us to visualize the tumour initiating cells, at a single cell resolution, in the skeleton. Projects in this area will use the latest imaging technology and next generation genetic and bioinformatic tools to establish a genetic and molecular fingerprint of these tumour-initiating cells and utilise this knowledge to develop new therapeutic approaches to preventing the development of bone metastasis.

Project 3: Defining the tumour initiating cells in multiple myeloma

Multiple myeloma is a B-cell neoplasm characterised by the growth of tumour cells in the skeleton and the development of a devastating bone disease. We have developed novel *in vivo* imaging technology to study single myeloma cells and their interactions with bone *in vivo* and discovered new molecules implicated in myeloma bone disease. We will use this new technology and next generation genetic and bioinformatic approaches to define a genetic and molecular fingerprint of these cells, establish the role of osteoblasts in regulating their behaviour and utilise this knowledge to develop new therapeutic approaches.

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Bone Therapeutics Group

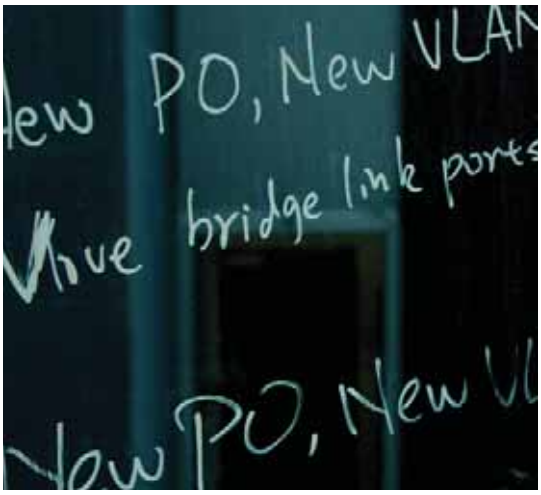
Bisphosphonates are a "blockbuster" class of drugs used worldwide for the treatment of common bone diseases such as post-menopausal osteoporosis, cancer-induced bone loss and Paget's disease. My lab is a world-leader in characterising how these drugs work at the cellular and molecular level. We made the breakthrough discovery a few years ago that most of these drugs act by inhibiting FPP synthase, a critical enzyme involved in the biosynthesis of cholesterol and a variety of lipid intermediates necessary for the lipid modification (prenylation) and hence normal function of essential signalling proteins. Several PhD projects will be available in my group and in collaboration with other research groups at Garvan. All of the projects will address clinically important questions about the pharmacology and biological actions of these drugs, particularly their anti-tumour actions and effects on myeloid immune cells.

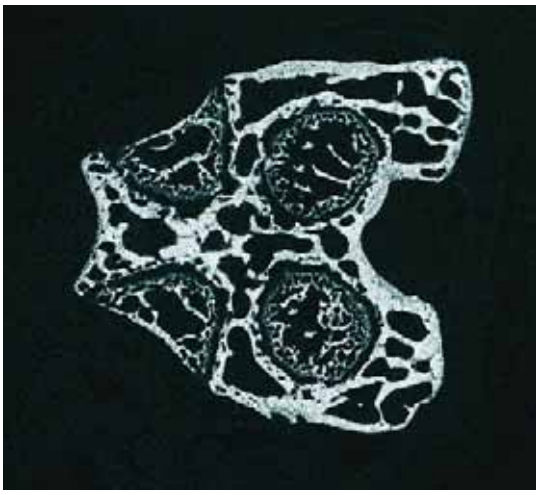
Project 1: Using biosensors to visualise the effects of bisphosphonate drugs on small GTPase signalling

In collaboration with Dr Paul Timpson (Cancer Division) we will use state-of-the-art imaging techniques to visualise novel biosensors that reflect the activity of small GTPase signalling proteins. This approach will provide new insights how bisphosphonate drugs, which inhibit the prenylation of small GTPases, affect the subcellular localisation and activity of small GTPases and how this influences the function of a variety of cell types, including bone-resorbing osteoclasts, macrophages and other immune cells. The project will provide training in various techniques including cell culture, transfection, microscopy and transgenic animal models.

Project 2: New insights into the regulation of the mevalonate pathway and its inhibition by bisphosphonate drugs

In this project we will use a variety of next-generation sequencing, bioinformatic and proteomic approaches to examine the importance of isoforms and newly-discovered post-translational modifications of FPP synthase, the enzyme target of bisphosphonate drugs. Differences in the expression or activity of this enzyme may account for differences in drug responsiveness between patients and resistance to bisphosphonate therapy. We will also seek to test, in well-established cell and organ culture models, a mathematical model of the mevalonate pathway that we have developed. This could be used to predict the effect of bisphosphonates and other drugs on lipid metabolites and enzyme expression using a range of molecular biology techniques including mass spectrometry and quantitative PCR.





Project 3: Anti-tumour effects of bisphosphonates

Bisphosphonate therapy has been shown to prolong the survival of patients with multiple myeloma and has anti-tumour activity in various animal models of cancer, including myeloma, but the exact mechanism remains unknown. In collaboration with Prof Peter Croucher, this project will seek to identify the mechanism underlying the anti-cancer activity of these drugs in the context of myeloma, breast cancer and other types of cancer, focusing on effects of bisphosphonates on immune myeloid cells. *In vivo* and *ex vivo* 2-photon microscopy and flow cytometry will be used to determine the distribution and cellular uptake of fluorescently-tagged bisphosphonates in mouse models of cancer. We will also use a wide variety of cell culture and molecular biology techniques to examine whether bisphosphonate treatment affects the immune-suppressive function of myeloid cells in mice and in cancer patients, and whether such effects are mediated via inhibition of protein prenylation.

Project 4: Physiological regulation of myeloid-derived suppressor cells during cancer development

Myeloid-derived suppressor cells (MDSCs) are a type of immature cell of the monocyte/granulocyte lineage that increase dramatically in number during the development of tumours in mice and humans and could be targets of bisphosphonate drugs. These cells promote tumour growth and metastasis, by producing factors that promote angiogenesis and by suppressing T cells that normally remove tumour cells by immune surveillance. Little is known about the mechanisms involved in the expansion of MDSCs in the bone marrow, but disabling MDSCs has been shown to decrease tumour growth by enabling normal T cell function. In collaboration with Dr Paul Baldock, we will use a wide variety of animal models to examine the influence of hormones and other physiological factors on MDSCs and their expansion and activation in the bone microenvironment during tumour development and metastasis.

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Bone Metabolism Group

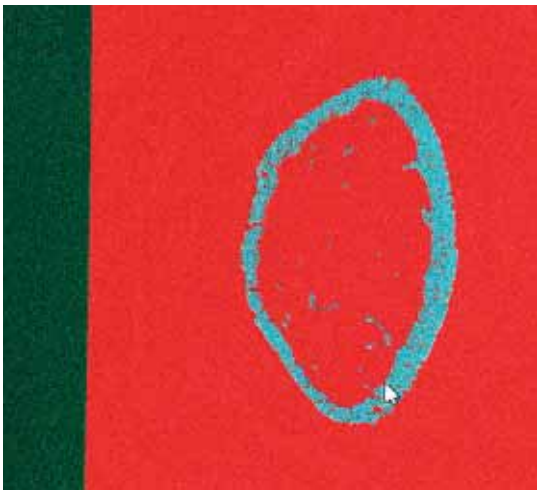
Project 1: Inter-organ signalling: A new level of regulatory control

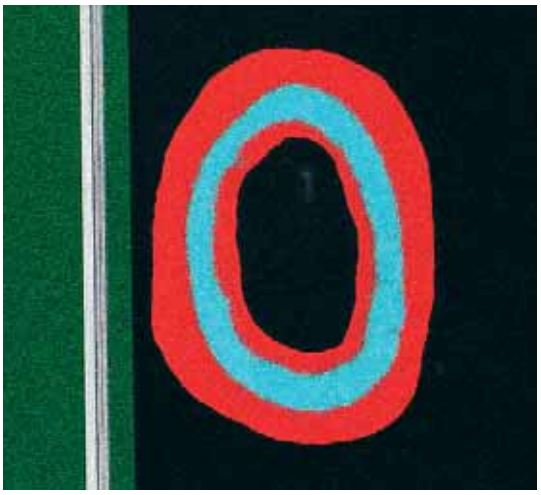
Our laboratory has a long-standing interest in defining the brain's role in controlling and co-ordinating peripheral tissue homeostasis. Using sophisticated genetic studies in mice, we have demonstrated that the hypothalamus regulates the behaviour of numerous organ systems, through modulation of specific neuropeptide pathways.

Our primary focus has been upon the powerful, multi-system responses that surround starvation and obesity, with a particular emphasis upon the neuropeptide Y (NPY) system. NPY is one of the most powerful regulators of energy homoeostasis throughout the body, and our group, in collaboration with the Eating Disorders Group, is amongst only a few in the world able to dissect the activity of this crucial pathway, through unique animal models made at the Garvan.

Using specific tissue responses, such as adipose, skeletal and pancreatic tissue, we have defined the mechanism whereby specific NPY pathways from the brain act within the periphery. These pathways are extremely potent, altering fat mass by over 4-fold and the production of bone by 7-fold, as well as altering endocrine function through altered production or end-organ responses. Moreover, these signals are co-ordinated across many organ systems, demonstrating a level of integration between organ systems, not fully appreciated previously.

Excitingly, during the course of these studies, we have uncovered unique signalling pathways, that indicate an additional layer of communication not previously appreciated, acting between the organs themselves. Endocrine regulation has typically been viewed as a top-down process, from the hypothalamus via the pituitary to the circulation. Our research will focus upon emerging inter-tissue communications, defining entirely new signalling molecules and axes of communication.





Our initial studies have identified actions entirely novel to science. For example, using tissue-specific neuropeptide models, we have demonstrated bone's signalling to the brain to control its own production, as well as bone's regulation of both adipose and glucose homeostasis.

Post graduate projects within the lab will involve investigations of inter-organ communication, concerning, but not restricted to:
_ Coordination of energy, skeletal and glucose homeostasis
_ Feedback signals from bone to brain
_ Regulation of tumour cell growth by marrow and skeletal tissue
_ Consequences of chronic obesity/leptin resistance
_ Neuropeptide regulation of central endocrine function

These studies represent the forefront of our understanding of how tissues communicate and co-ordinate their activities, and offer the potential for entirely new modalities for disease control. Every student within our lab has won at least one international young investigator award for oral presentation of their studies, and gone on to international positions. Our lab is integrated with many others within the Institute, including ongoing clinical studies, and offers a rewarding, productive and enjoyable experience for those eager to explore this emerging field.

Project 2: Sclerostin and Dickkopf-1 in regulation of bone mass

The WNT pathway is a powerful regulator of bone cell differentiation and formation. Two WNT modulators, sclerostin ad Dickkopf 1, are under development as the next generation of therapeutic agents for metabolic bone disease and represent the most exciting development in bone active drugs in decades. Decreases in the activity of Dickkopf 1 (Dkk1) and Sclerostin (Scl) are associated with marked increases in bone formation. Inactivating mutations of the sclerostin gene in humans results in extreme bone mass gains (+9 SD). In light of this enormous bone anabolic potential, antibody therapies are in Phase II trials for osteoporosis and are producing powerful and unprecedented increases in bone mass. Such agents based on Dkk1 and Scl are also potential therapies in osteogenesis imperfect, multiple myeloma and orthopaedic applications.

However, critical questions remain unanswered:
_ Are these agents safe during growth?
_ Is long term therapy effective?
_ Do circulating levels predict and /or regulate bone mass?
_ Are Scl and DKK1 responses equivalent?

The answers to these questions represent important outcomes in terms of:
_ Their use in children, particularly as therapy for osteogenesis imperfects, a crippling bone disease.
_ The treatment strategy, cyclical dosing vs long term therapy.
_ The monitoring/interpretation of patient levels and the basic mode of action of these agents.
_ The tuning of particular agents to specific patients.

To answer these questions specific animal models are required, enabling conditional deletion of Dkk1 and Scl expression. These have been developed at the Garvan and will be ready for experimentation in 2014. This study will employ these unique mouse models to clarify these outstanding questions regarding the mode of action and application of these pivotal emerging therapies, with a view to maximising their application, while ensuring patient safety.

The project will involve cutting edge *in vivo* imaging technology for tracking the skeletal response to temporal, spatial and lineage-specific Scl and DKK1 deletion. This will be combined with next generation transcriptomics and detailed histological assessment to detail the structural, cellular and molecular changes evoked by the loss of Scl/DKK1 expression. This project represents the most detailed and systematic analysis of these WNT modulators conducted in bone to date.

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Bioinformatics

In the last decade new technologies like massively parallel sequencing have transformed biology and medical research from the study of individual genes or proteins to system-wide approaches. These technologies provide us with unprecedented insights into the biology of disease. However, they also generate enormous amounts of data and the role of Garvan's Informatics group is to make sense of these data. We do this on our locally housed data using high-performance computing methods, but as datasets have grown so large that we cannot bring them in-house, we also mine them on remote locations. Much of what we do is the writing of software to analyse the data, but because of its complexity we also use and develop innovative visualisation methods to gain deeper insights into disease. We work very closely with bench scientists at Garvan who use our analyses to test hypotheses that we develop. Our students are an integral part of achieving this vision. As bioinformatics is a new field with very few practitioners having been trained as bioinformaticians we look for talented individuals from the worlds of mathematics, physics, chemistry, computer-science and biology to achieve these aims.

PhD Opportunity: Visual Analytics applied to Biological Data

General Research Project Area

The Garvan has recently started a new group focused on developing methods and tools for visualizing biological data. Jointly associated with CSIRO, the group focuses primarily on using principles of usability, data visualization, human-computer interfaces, and graphic design to develop state-of-the-art methods and tools that address cutting edge challenges in biological and biomedical research. A second focus of the team is on using these methods to analyse experimental datasets in collaboration with groups at the Garvan. Projects will include the VIZBI initiative (http://vizbi.org), the Reflect system for enhancing scientific literature (http://reflect.ws), and developing methods for integrating macromolecular 3D structures with genomics, proteomics, and other systems biology data. For further details see http://odonoghuelab.org/.

Techniques Used

Bioinformatics software development, graphics design, 3D graphics, 3D animation, Human-computer interface development. Applications to data from genomics, proteomics, systems biology and scientific literature databases.

Required Background

These positions could be filled by students from a range of backgrounds, including bioinformatics, biochemistry, chemistry, physics, or computer scientists and an interest in using data visualization, human computer interaction, usability, or design to address key challenges in basic biomedical research. Of particular interest would be students with strong JavaScript or Java3D skills, as well as a strong interest in using HTML 5 to build the next-generation of visualization tools for analysing omics datasets for systems biology. There is also scope for students wishing to focus not on tool development, but on applying visualization techniques to data from various disease areas, possibly in collaboration with other groups at Garvan.

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How to Apply

Applications are submitted online at:
http://www.garvan.org.au/students/postgraduate-studies

All applications are considered by the Garvan Higher Degrees Committee (HDC).

Closing dates for applications are:
_ 31 October for admission in Semester 1 (March commencement)
_ 30 April for admission in Semester 2 (July commencement)

Applications outside these times will only be considered in exceptional circumstances.

As Garvan is a not-for-profit organisation, it is unlikely that a research program would have the funds to take on a postgraduate student without scholarship funding of some kind. However, there are many different sources of funding available for postgraduate research students, such as UNSW APA, UIPA, IPRS and NHMRC.

Prospective students must also lodge an application for admission to UNSW online at:
http://research.unsw.edu.au/doctor-philosophy-phd

As part of this process, you will need to have agreed a potential research project with your supervisor.

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