GARVAN
INSTITUTE

Breakthrough Medical Research

PhD @ Garvan

2011 PROJECTS

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2011 PhD Projects

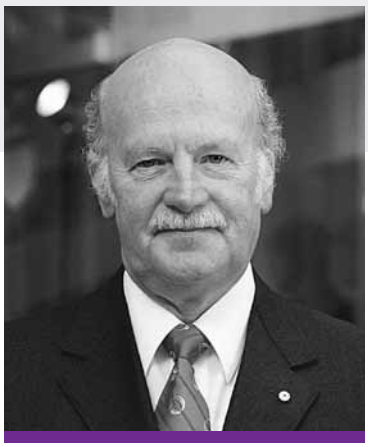
Introduction

The Garvan Institute of Medical Research is one of Australia's leading biomedical research centres, achieving major breakthroughs in the understanding and treatment of such diseases as cancer, diabetes and obesity, neurodegenerative disorders,osteoporosis, arthritis and asthma. Garvan is affiliated with St. Vincent's Hospital and The University of New South Wales. Annual research expenditure is in excess of $60 million. Students comprise nearly a quarter of our researchers. Postgraduate students contribute significantly to our scientific success and Garvan actively seeks high caliber people who demonstrate initiative and independent thought. Our research programs include neuroscience, cancer, bone, immunology, diabetes and obesity.

PhD Open Day 25th of August

Our postgraduate student open day will take place on the 25th of August from 10.30 am to 12.30pm. Interested applicants **must register** to attend at www.garvan.org.au. You will meet with prospective supervisors seeking students. Outside of this period, you may contact specific researchers directly or the graduate student coordinator. The following pages provide details of some of the available PhD projects.

Information about the postgraduate application process and application form can be found on the last page of this brochure or can be downloaded at http://www.garvan.org.au/education



From the Executive Director

Professor John Shine AO FAA
Executive Director

Many professional careers are rewarding, but none are more exciting and fulfilling today than that of biomedical research. With the completion of the human genome sequence and related technologies, the pace of acquisition of new knowledge about fundamental life processes is growing exponentially. Every day, amazing new insights into health and disease are emanating from research labs around the world.

Undertaking advanced postgraduate study in medical research in these exciting times is a very special opportunity. This includes the excitement of discovery, development of an international network of friends and colleagues and the satisfaction of success in your chosen field. The Garvan Institute is well placed nationally and internationally to ensure this success, with state-of-the-art technologies, leading research programs and importantly, a sole focus on research excellence. The institute's role as an integral partner on the St. Vincent's Campus also means that research discoveries have every opportunity to be closely linked to real improvements in the prevention and treatment of disease.

The Garvan Higher Degrees Committee

The Garvan Higher Degrees Committee provides pastoral care, monitors academic progress and well-being of students during their tenure here. The overall objective of the committee is to assist the executive director in the administration of matters relating to higher degrees. This committee is concerned with all full time and part time students who are undertaking postgraduate research at Garvan, and where the principal supervisor is a member of the Garvan scientific staff.

HIGHER DEGREES COMMITTEE MEMBERS:

Back (L-R): Dr Maija Kohonen-Corish, Dr Paul Baldock, A/Prof. Chris Ormandy; Front: Dr Ross Laybutt, Prof. Antony Basten, Dr Stuart Tangye, Dr Sharon Oleskevich ***(not pictured: A/Prof. Kathy Samaras, A/Prof. Trevor Biden)***

Diabetes & Obesity Research Program

Metabolic Systems Biology
Professor David James

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MAJOR RESEARCH INTERESTS AND TECHNOLOGIES USED

- Signal transduction (mass spectrometry, protein biochemistry)
- Systems Biology (mathematical modelling, bioinformatics)
- Vesicular transport (advanced imaging and microscopy)
- Diabetes and metabolic disease (animal models, clinical studies)

NEW PROJECTS

1. Digitising insulin action

We have used quantitative mass spectrometry approaches to map the insulin-regulated phosphoproteome. This is vast, comprising thousands of phosphorylation events. These events are regulated by a vast number of kinases and phosphatases, and occur over a dynamic range and with different temporal profiles. This represents a snapshot of the complexity of cell signalling and emphasises the limitations of more reductionist approaches involving the study of changes in protein phosphorylation of just one or two proteins. Here we aim to take a system-wide approach to understand how insulin works and how defects in this system might contribute to disease. This will involve a detailed study of the phosphoproteome as well as other omic data sets in a range of cell types. People with interests and/or expertise in protein biochemistry, metabolism, mass spectrometry, signal transduction or bioinformatics are encouraged to apply.

2. Targeted analysis of the proteome in type 2 diabetes

In collaboration with Dr Greenfield we have established a tissue bank of muscle biopsies from a range of individuals with variance in body weight and whole body insulin sensitivity thus providing a unique opportunity for mechanistic studies in this important disease. We aim to develop a cutting edge multiplex mass spectrometry based assay enabling us to quantitatively and simultaneously interrogate the levels of 100s of different proteins or changes in their posttranslational modifications in these tissues. This type of approach is unparalleled in the field and will provide a major advance in our understanding of this disease.

3. RabGTPases in insulin action

One of the major steps regulated by insulin is glucose entry into muscle and fat cells. Insulin stimulation triggers the movement of the facilitative glucose transporter GLUT4 from intracellular vesicles to the plasma membrane. A major interest of the lab is to define the regulatory machinery that controls this vesicular transport pathway. Rab GTPases play a major role in regulating all vesicle transport steps by recruiting interaction partners to define membrane identity in a temporal and spatial manner. One of our major interests is to identify the Rab GTPases that regulate GLUT4 trafficking and understand how these proteins co-ordinate the insulin-stimulated translocation of GLUT4 to the plasma membrane.

Insulin Signalling Group
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BACKGROUND

Type 2 diabetes is a major disease with debilitating complications, and its prevalence is reaching epidemic proportions. This group focuses on diabetes as a function of signal transduction pathways, and our work spans the key tissues involved in glucose homeostasis. Diabetes is strongly associated with obesity, and our strengths in signalling and lipid biochemistry, as well as in dietary models of glucose-intolerance, enable us to study the molecular mechanisms of lipid-induced insulin resistance in the target tissues of the hormone. These include the control of lipid metabolism in liver as well as the identification of key metabolites that accumulate in skeletal muscle, and how these processes disrupt insulin receptor signalling cascades. Emphasis is on the role of the lipid-activated protein kinase C (PKC) family of signalling enzymes and of the inhibitory lipid intermediate ceramide.

Project 1: PKC signal transduction and liver metabolism.

Using knockout mice, we have discovered new roles for PKC epsilon and delta, especially in the regulation of lipid metabolism in the liver. Our current aims are to understand the underlying intracellular mechanisms, using our expertise in gene chip technologies, gene over-expression or knock down, and protein phosphorylation and proteomics. We wish to identify the signalling molecules, protein substrates and binding partners which act in concert with PKC isoforms in order to define new pathways. Tissue-specific PKC knockout mice will be available to confirm the importance of these pathways ***in vivo***.

Project 2: Lipid-induced insulin resistance in skeletal muscle.

Our work using cultured skeletal muscle cells treated with fatty acids as a model of obesity and insulin resistance has identified novel lipid intermediates which inhibit insulin signal transduction, including ceramide. We are now employing mass spectrometry to determine the importance of individual ceramide species ***in vivo***, and will extend such lipidomic approaches to determine which ceramide-metabolising enzymes can be targeted to improve insulin action as a novel therapy for Type 2 diabetes.

SELECTED PUBLICATIONS

Diverse roles for protein kinase C delta and protein kinase C epsilon in the generation of high-fat-diet-induced glucose intolerance in mice: regulation of lipogenesis by protein kinase C delta.

Frangioudakis G, Burchfield JG, Narasimhan S, Cooney GJ, Leitges M, Biden TJ, Schmitz-Peiffer C ***Diabetologia*** 52:2616-2620, 2009

Inhibition of PKCε improves glucose-stimulated insulin secretion and reduces insulin clearance.

Schmitz-Peiffer C, Laybutt DR, Burchfield JG, Gurisik E, Narasimhan S, Mitchell CJ, Pedersen DJ, Braun U, Cooney GJ, Leitges M, Biden TJ ***Cell Metab*** 6:320-328, 2007

Akt mediates insulin-stimulated phosphorylation of Ndrg2- Evidence for cross-talk with protein kinase C theta.

Burchfield JG, Lennard AJ, Narasimhan S, Hughes WE, Wasinger VC, Corthals GL, Okuda T, Kondoh H, Biden TJ, Schmitz-Peiffer C. ***J Biol Chem*** 2004; 279:18623-32

Ceramide generation is sufficient to account for the inhibition of the insulin-stimulated PKB pathway in C2C12 skeletal muscle cells pretreated with palmitate.

Schmitz-Peiffer C, Craig DL, Biden TJ: ***Journal of Biological Chemistry*** 274:24202-24210, 1999

Assoc Professor Antony Cooper
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Cooper Group - Neurodegeneration / Cell & Molecular Biology / Genetics

The Cooper lab seeks to identify and understand the basis for Parkinsons Disease, a neuro-degenerative disease affecting >50,000 Australians who have already lost ~ 40% of dopamine producing neurons at time of diagnosis. Earlier diagnoses and new treatments are critically needed as current therapies are only partially effective. Discovering the cascade of events causing the loss of neurons will allow early diagnosis while the identification of the primary mechanistic defect will lead to targeted development of treatments/drugs. These projects involve a wide range of approaches including genome-wide screening, cell and molecular biology techniques, immunofluorscence microscopy, siRNA knockdown, cell culture and mice models.

Project 1: Identifying the underlying molecular mechanism(s) of Parkinson's Disease (PD)

αSynuclein is a natively non-toxic protein of unknown function that associates with synaptic vesicles. αSynuclein is also the central component in PD as its aggregated form is the main component of Lewy bodies, the primary pathological hallmark of PD. Although the underlying cause of PD is unknown in >90% of cases, the propensity of aSynuclein to become cytotoxic likely results from a complex interaction of unknown predisposing genes (risk factors). Identifying these risk factors will enhance our understanding of how Parkinson's disease develops and is an important step towards preventing the disease or to develop therapeutic agents that may inhibit the degeneration of neurons. Using a new model system expressing nontoxic levels of αSynuclein, we screened for αSynuclein dependent toxicity upon loss of function of 5000 genes (the potential risk factors). We have identified that defects in major signaling pathways play critical roles in αSynuclein toxicity. These genes/proteins will then be investigated in human neural cell lines using a broad array of genetic, cell and molecular approaches to both confirm their association with PD and identify the underlying molecular mechanism(s) responsible for contributing to PD prior to testing in human brain samples.

Project 2: The role of PARK9 in Parkinson's disease.

Some patients with early onset Parkinson's disease have mutations in PARK9. In a collaborative approach we have made an exciting discovery that PARK9 expression levels modify aSynuclein toxicity. Using our custom PARK9 antibodies we have recently discovered large differences in PARK9 protein levels between a PD patient and control, providing further evidence that PARK9 is playing a significant role in PD. This project will involve multiple PD model systems (cell culture, mice) and live human neurons derived from a PARK9 PD patient, to identify and dissect the mechanism by which PARK9 dysfunction contributes to PD.

Project 3: Mitochondrial dysfunction in Parkinson's disease.

Mitochondrial dysfunction is a common occurrence in Parkinson's disease- why? Although a number of hypotheses have been proposed (enhanced production of reactive oxygen species [ROS], diminished ATP production, enhanced apoptosis rates) our Parkinson's disease model system has found that these hypotheses are unlikely to be responsible. Instead this project focuses on using PD models to investigating our exciting novel hypotheses that would integrate several distinct aspects of Parkinson's disease and potentially identify a prime target(s) for therapeutic intervention.

SELECTED RECENT PUBLICATIONS:

Gitler et al. "Alpha-synuclein is part of a diverse and highly conserved interaction network that includes PARK9 and manganese toxicity." ***Nat Genet***. 41:308-15 (2009). Impact Factor = 25.5

Cooper et al "Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models." ***Science***. 313:324-8. 2006. Impact Factor= 30.927

Immunology & Inflammation Program

B CELL IMMUNOBIOLOGY GROUP

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The basis of allergy – how do IgE antibody responses occur?

Immunoglobulin E (IgE) is one of the four major classes of antibody produced by the immune system in response to the entry of foreign organisms or substances (foreign antigens) into the body. The unique activity of IgE is its ability to bind to and activate a subset of immune cells (mast cells and basophils) that release histamines and other inflammatory mediators. Whilst this response has evolved to combat infections by certain parasites such as helminths, activation of IgE production is a feature of most allergens and is a major cause of the bronchoconstriction associated with asthma.

Activation of B lymphocytes by foreign antigen results in their differentiation into antibody-secreting plasma cells. However, this most commonly results in the production of IgM, IgG or IgA rather than IgE antibodies. IgE is therefore the least abundant class of antibody in the body and the basis for its production is poorly understood. Although it is apparent that the processes underlying IgE production differ significantly from those that governing the production of conventional antibodies, the molecular and cellular basis of this unique response remain undefined.

We have developed unique strains of genetically modified mice and a panel of recombinant antigens based on the protein hen-egg lsozyme (HEL), that give us the ability to identify, characterise and isolate participating B and T cells from the very earliest stages of the immune response. We have recently modified this system so that the anti-HEL B cells, instead of producing IgG antibodies, produce a strong IgE response. We are now in a unique position where we can characterise in detail the cellular and molecular changes that take place over the course of an IgE antibody response.

The key objectives of this proposal are to:

- Identify the sequence of cellular events during the evolution of an IgE antibody response and how these differ from a conventional IgG response.
- Identify the molecules expressed differentially by key responding populations (T and B cells) in IgE versus IgG responses.
- Assess these molecules as potential targets for therapies aimed at controlling/eliminating IgE antibody responses.

This project promises to reveal new information on the molecular and cellular control of IgE production and therefore the genesis of asthma and other allergic diseases. Identification of molecules expressed during the early stages of these responses may indicate future targets for therapeutic intervention in these conditions.

B CELL IMMUNOBIOLOGY GROUP

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The role of subcapsular sinus (SCS) macrophages in LN melanoma metastases.

The primary function of the lymph node (LN) is to filter the lymph to trap and degrade any pathogens and cancer cells that may have infiltrated the host organism. Afferent lymph enters the SCS which forms an anatomical and functional barrier to the free diffusion of lymph borne particles. This barrier is formed by lymphatic endothelial cells and tissue-resident macrophages that express the sialic acid-binding C-type lectin CD169 (sialoadhesin). Lymph then reaches the medullary sinuses which is also lined by lymphatic endothelial cells and $CD169^+$ macrophages where the bulk of lymph-borne soluble and particulate antigen is trapped and catabolized. Cancer cells must therefore cross this lymph-tissue interface in order to invade the underlying parenchyma. While interest has focussed on the molecular steps involved in oncogenesis and tissue invasion, there has been surprisingly little research on the steps involved in the establishment of metastatic cancer cells once they reach the LN.

The project will therefore use genetic and pharmacological approaches to determine the role of $CD169^+$ SCS macrophages in LN metastases in an ***in vivo*** mouse model. It will involve:

- Transducing B16-F10 melanoma cells with a retroviral vector expressing green fluorescent protein to generate B16-eGFP cells
- Growing B16-eGFP cells in tissue culture and then implanting them by intradermal (i.d.) injection in the flanks of anesthetised mice.
- Tracking early metastases to the draining LN in wildtype mice by detection of green fluorescent cells by confocal laser scanning microscopy and fluorescence activated cell sorting
- Tracking early metastases to the draining LN in B cell-deficient mice and wildtype mice treated with lymphotoxin blocking agent. These mice selectively lack $CD169^+$ SCS but not medullary macrophages.
- Directly visualising melanoma cell-macrophage interactions ***in vivo*** in real-time by intravital two-photon microscopy in wildtype mice and mice lacking candidate adhesion molecules.
- Correlating the findings in the ***in vivo*** mouse model to clinical studies of sentinel LN biopsies in patients with metastatic melanoma.

These studies will provide a molecular basis for understanding the earliest steps in LN metastases and drive the development of novel therapeutic strategies to prevent LN metastases not only in melanoma but other cancers.

B CELL IMMUNOBIOLOGY GROUP

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Dynamic visualization of immune responses to tumours

Our group is interested in understanding the role of the immune system in cancer. One of the most promising approaches for the treatment of established cancers is harnessing the immune system, either through boosting the function of immune cells that combat cancer, or inhibiting cells that subdue immune responses. Multiple complex mechanisms govern the interactions between tumours and the immune system. We have developed an innovative system to visualize immune cells in vivo in intact tumours and to 'tag' tumour-infiltrating cells. With the aid of 2-photon microscopy, a cutting edge technique that allows to 'see' cells hundreds of microns below the surface of intact organs, we will monitor the interactions between the tumour and immune cells taking place below the tumour surface. This technology in combination with a novel transgenic mouse allows us to follow the fate of immune cells as they leave the tumour. We can then investigate whether these cells participate in anti-tumour immune responses or whether they aid tumour metastasis, one of the most fearsome aspects of cancer. This project should provide important insights into the interplay between the tumour and the host's immune system.

CELLULAR IMMUNITY GROUP

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Project: Regulatory T cells in cancer, autoimmunity and transplantation

Regulatory T cells (Tregs) are a specialised population of CD4+ T cells whose role it is to suppress the action of other immune cells. By regulating the response to self- and foreign-antigen, they prevent autoimmunity and also excessive inflammation. Insufficient numbers or poorly functional Tregs have been linked with the development of autoimmune disease. Conversely, a high number of Tregs can prevent the immune system from eliminating cancerous cells.

Recent work in our lab has identified an exciting novel approach to expanding Treg numbers in mice. The combination of the cytokine interleukin-2 (IL-2) with an anti-IL-2 antibody was able to dramatically boost these cells; an effect not seen with any other ***in vivo*** approach. This spike in Treg numbers perturbed the development of autoimmunity and also led to long-term acceptance of transplants. This project will further analyse the role of these expanded Tregs in disease models, particularly transplantation and autoimmunity in contrast to a tumour model. In each disease state, we will investigate the interactions between Tregs and other immune cells (both regulatory and effector), examine the consequences of transient Treg depletion on immune cell kinetics, and dissect the mechanisms of IL-2/ IL-2 mAb function.

Selected publications:

1. Webster, K.E., S. Walters, R.E. Kohler, T. Mrkvan, O. Boyman, C.D. Surh, S.T. Grey, and J. Sprent. 2009. In vivo expansion of T reg cells with IL-2-mAb complexes: induction of resistance to EAE and long-term acceptance of islet allografts without immunosuppression. ***J Exp Med*** 206:751-760.
2. Boyman, O., M. Kovar, M.P. Rubinstein, C.D. Surh, and J. Sprent. 2006. Selective stimulation of T cell subsets with antibody-cytokine immune complexes. ***Science*** 311:1924-1927.

MUCOSAL AUTOIMMUNITY RESEARCH GROUP

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Project: 1: Research project: Regional specification of IL-21-producing T helper cells in autoimmunity.

Background to the research: Diabetes (medically known as diabetes mellitus) is the name given to disorders in which the body has trouble regulating its blood glucose, or blood sugar, levels. Type 1 diabetes, also called juvenile diabetes or insulin-dependent diabetes (T1D), is a disorder of the body's immune system—that is, its system for protecting itself from viruses, bacteria or any "foreign" substances. When our immune system damages our own tissues, this is termed autoimmune disease. The cytokine IL-21 is an important soluble messenger in the immune system. IL-21 is necessary for the development of T1D in mice. Our lab has shown that the increased production of IL-21 in T1D prone mice was found to result from two single nucleotide polymorphisms within the distal promoter region that conferred increased binding affinity for the transcription factor Sp1. We have also shown that the cells that produce IL-21 within autoimmune lesions of the pancreas and salivary glands are defined by their expression of the chemokine receptor CCR9. Tccr9 cells are increased in the blood of humans with Sjogrens syndome and we think that this is a major autoimmune disease inducing T cell subset that exhibits regional specification for organs of the digestive system.

Research Plan: Specific Aims: (1) utilizing a transcription and translation system available in our Lab to confirm functional significance of polymorphisms in regulatory regions of NOD versus B6 IL-21 gene (2) Use of a number of knock-out mice and reagents that are exclusive to the Garvan, including NOD.IL-21-/-, NOD.IL-21R-/-, NOD. 8.3 IL-21R-/-, IL-21R.Fc and IL-21R.Fc to

confirm relevance of IL-21 production to T1D in vivo. (3) Phenotypic analyses of Tccr9 cells in human autoimmune disease.

Significance and relevance to human disease: These findings will define the role for IL-21 in autoimmunity.

Skills developed: Multiparameter flow cytometric analyses of immunostained cell populations, tissue procurement and immune cell purification. Immunohistochemistry of pancreata. Islet isolation, purification and culture. Luciferase expression assays. Mouse models and treatment with Garvan produced pharmacologic agents.

Project: 2: Lineage specification of self-reactive CD4+ T helper cells in autoimmune diabetes.

Background to the research: Both transcription factors and epigenetic mechanisms are critical in regulating cellular differentiation. Posttranslational modifications of histones are implicated in regulating gene expression by controlling chromatin structure and DNA accessibility. Epigenetic modifications influence the binding of transcription factors to promoter regions of genes, contributing to the heritability of TH lineage decisions and evidence is emerging that these decisions remain open to revision. Through the analysis of the chromatin state in resting and effector T cells (including TH1, TH2, TH17 cells cultured in vitro and Treg subsets) a recent study has revealed the retention of both permissive and repressive transcription factor binding (bivalent) marks in TH cell specific genes, including those of transcription factors. Transcription factor genes in a bivalent state have the potential for subsequent activation or silencing, suggesting that TH cells retain the potential for functional revision. This project Aims to define the subset of CD4+ T cells that is the sole source of IL-21 in the autoimmune lesions of the NOD mouse. We will use the exciting new ChIP-Seq technique to determine how this novel TH subset is related to other well-defined lineages. This information will provide suitable therapeutic targets for the control of diabetogenic TH cells.

Research Plan: Specific Aims: (1) Isolation of IL-21-producing CD4+ TH cells through their unique surface marker expression and isolation of T follicular heper cells, Tregs as well as in viitro differentiation of Th1, Th2, Th17 cells (2) ChIP of TH subsets and delivery of DNA to core facility for genomic sequencing (3) Data analyses.

Significance and relevance to human disease: These findings will define a link between diabetes-causing T cells and other T cell subets.

Skills developed: Multiparameter flow cytometric analyses of immunostained cell populations, tissue procurement and immune cell purification. Chromatin Immunoprecipitation assay (ChIP). Islet isolation, purification and culture.

Project: 3: The relationship between IL-2 and IL-21 in type-1 diabetes.

Background to the research: Diabetes (medically known as diabetes mellitus) is the name given to disorders in which the body has trouble regulating its blood glucose, or blood sugar, levels. There are two major types of diabetes: type 1 and type 2. Type 1, also called juvenile diabetes or insulin-dependent diabetes (T1D), is a disorder of the body's immune system—that is, its system for protecting itself from viruses, bacteria or any "foreign" substances. When our immune system damages our own tissues, this is termed autoimmune disease. IL-2 and IL-21 are two cytokines with great potential to affect autoimmune destruction of pancreatic tissue and are contained within the strongest non-MHC-linked locus for T1D susceptibility on the NOD mouse (Idd3). IL-21 is necessary for the development of diabetes in the type-1 diabetes prone mouse strain, but a number of important studies argue that decreased expression of IL-2 explains Idd3. We have demonstrated that the amount of IL-21, but not IL-2, correlated with T1D incidence. The increased production of IL-21 in type-1 diabetes prone mice was found to result from two single nucleotide polymorphisms within the distal promoter region that conferred increased binding affinity for the transcription factor Sp1.

Research Plan: Specific Aims: (1) utilizing a transcription and translation system available in our Lab to confirm functional significance of polymorphisms in regulatory regions of NOD versus B6 IL-21 gene (2) Use of a number of knock-out mice and reagents that are exclusive to the Garvan, including NOD.IL-21-/-, NOD.IL-21R-/-, NOD. 8.3 IL-21R-/-, IL-21R.Fc and IL-21R.Fc to confirm relevance of IL-2 and IL-21 production to T1D in vivo.

Significance and relevance to human disease: These findings will define a link between regulation of IL-2 and IL-21 and type-1 diabetes.

Skills developed: Multiparameter flow cytometric analyses of immunostained cell populations, tissue procurement and immune cell purification. Immunohistochemistry of pancreata. Islet isolation, purification and culture. Luciferase expression assays. Mouse models and treatment with Garvan produced pharmacologic agents.

Cancer Program

CELL CYCLE GROUP

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The Cell Cycle Group's research is aimed at better understanding how female hormones like estrogen, their receptors and signalling pathways are involved in the normal control of cell proliferation and differentiation, how these control mechanisms are deregulated in cancer and how these pathways can be manipulated to treat and prevent breast cancer.

Estrogen regulates cell proliferation and survival in the normal breast and breast cancer and consequently the antiestrogen tamoxifen has been the most widely used adjuvant endocrine therapy for estrogen receptor-positive breast cancer patients for over 20 years. Unfortunately, resistance to tamoxifen limits its clinical utility. Cyclin D1 and Myc are estrogen target genes that can mimic estrogen's ability to promote cell cycle progression and cause antiestrogen resistance when overexpressed in breast cancer cell lines in culture. To dissect the relative contributions of pathways downstream of cyclin D1 and Myc to estrogen action, and potentially to antiestrogen resistance, we have used transcript profiling to identify suites of genes regulated by cyclin D1 and Myc. We are also undertaking large-scale functional genetic screens to identify genes whose increased or decreased expression modifies sensitivity to antiestrogen-mediated growth arrest in cultured breast cancer cells.

We now have a PhD project available to characterise the roles of individual candidate genes in estrogen action using our well-characterised breast cancer cell line models and to investigate their role in the response to antiestrogen therapy in breast cancer. This will involve determining how the candidate gene is regulated by estrogen, determining its normal cellular role, and defining how its overexpression or knockdown affects the ability of estrogens and antiestrogens to regulate the cell cycle machinery. We will also test whether expression of the candidate gene in breast cancers is correlated with patient outcome or response to antiestrogen therapy.

INTEGRIN & CELL BIOLOGY GROUP

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Project 1: Regulation of stem cells in cancer & development

Stem cells play a critical role in the normal development and functioning of organs. In addition, 'cancer stem cells' may be responsible for tumour growth and resistance to therapeutics. Integrins are cell-matrix receptors that through both adhesive and signalling roles play a critical role in normal development and cancer. Integrin expression has also been used to purify breast and prostate stem cell populations, along with cancer stem cells. This project will determine the role of integrin signalling in the regulation of stem cell function during development and cancer progression using conditional knockout mice, breast and prostate cancer mouse models and alteration of signalling in normal and human cancer cells.

Project 2: The role of Runx2 in prostate development & cancer

In addition to its expression in breast and bone, Runx2 is also expressed in the prostate. As human prostate cancer progress to metastatic disease the expression of Runx2 is lost, indicating that Runx2 may act as a tumour-suppressor in the prostate. This project will determine the role of Runx2 in prostate development and cancer using new transgenic mouse models to delete or overexpress Runx2 specifically in the prostate, experimental prostate cancer models and by manipulating Runx2 in xenografts and cancer cells in vitro.

Project 3: Targeting bone cells in inhibit breast cancer induced bone metastasis

During bone metastasis a 'vicious cycle' occurs where tumour cells secrete growth factors that activate and perturb normal bone cell function, in turn 'activated' bone cells secrete and release growth factors that further support tumour growth. The role of bone cells, especially as therapeutic targets, has largely been over-looked in this process. This project will undertake a completely novel approach. Rather than targeting breast cancer cells, we will target bone cells (by inhibit Runx2 specifically in bone cells in metastatic models) to prevent them from responding to the oncogenic signals from tumors cells. By preventing subsequent bone degradation and further growth factor release, we hope to inhibit the 'vicious cycle' of bone metastasis.

SAUNDERS GROUP

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Current cancer therapies use drugs that target both tumour cells and rapidly growing normal cells – causing side effects and limiting effectiveness. Newer treatments aim to target molecules that are unique to tumour cells, leaving normal cells unharmed. Hence, the identification and validation of novel molecular targets is crucial for development of new therapeutic strategies in cancer. However, defining the functions of proteins encoded by disease-related genes and potential therapeutic targets presents a significant challenge. This problem will become increasingly important as large cancer genome sequencing projects now underway identify potential molecular targets for cancer diagnosis/therapy. A major bottleneck in these studies is the functional validation of putative novel tumour genes and mutations, which is critical for understanding the huge wealth of information being generated, and translating the results into improved patient outcomes. Our research is focused in two broad areas:

1. Identification and characterization of targets and components of the Ubiquitin-Proteasome system, a cellular recycling and garbage disposal system; and
2. Novel functions of the Serine Protease Inhibitor family (SERPINS).

Our research uses the emerging technology of functional genomics, a powerful, cutting-edge approach to understand gene function and define biological pathways involved in cancer and other diseases. For example, using a technique called bimolecular fluorescence complementation (BiFC), we are able to look for differences in protein modifications and interactions between tumour and normal cells. BiFC makes use of the reconstitution of fluorescence from two non-fluorescent components of a protein such as GFP. A major advantage of this approach over biochemical techniques is the detection of interactions within live mammalian cells, providing information about the cellular context of the interaction (e.g. sub-cellular location). These approaches will help us to understand how normal cells become cancerous through changes in their protein content, and also identify new molecules that can be targeted by anti-cancer drugs.

APOPTOSIS RESEARCH GROUP

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Human cancers are characterised by a disruption of normal cellular growth due to defects in the control of both cell proliferation and cell death (apoptosis). Apoptosis is a physiological form of cell death with distinct morphological and biochemical characteristics. Current therapies for the treatment of human cancers, including ionising radiation and chemotherapeutic drugs, kill tumour cells by inducing apoptosis, so understanding how the process of cell death is regulated in normal and cancerous cells is an important goal for effective treatment.

Project 1: The role of survival/apoptosis regulation in the development of endocrine resistance.

In collaboration with the Cell Cycle Group, we have recently identified a group of genes regulating cellular survival/apoptosis that are associated with the response to tamoxifen in breast cancer patients[1]. More recently, we have been using mass spectrometry-based phospho-proteomics to further define the signalling pathways controlling apoptosis that are dysregulated in ***in vitro models*** of tamoxifen-resistance. This project aims to determine how individual genes in this 'signature' such as the pro-survival protein, BAG-1 can influence both the development of breast cancer and the response to therapy[2], and delineate the underlying mechanisms involved[3].

Project 2: Regulation of apoptosis in normal and malignant breast epithelial cells by the oncoprotein, c-Myc.

The oncoprotein, c-Myc is frequently overexpressed in breast cancers and can confer resistance to antioestrogens ***in vitro***. However, sustained c-Myc expression not only stimulates proliferation, but can also induce apoptosis directly and sensitise to apoptotic stimuli. Consequently, much interest has centred upon determining how these intrinsic aspects of Myc function are mediated in both the normal and tumorigenic setting, and how they may modulate the response to therapy. Recent studies have identified regions of Myc that can negatively (Myc Box III) or positively (MBIV) regulate its apoptotic function. This projects aims to determine how these domains influence Myc's apoptotic function and subsequently the malignant process in the breast.

[1] Musgrove EA, CM Sergio, S Loi, CK Inman, LR Anderson, MC Alles, M Pinese, M Gardiner-Garden, CJ Ormandy, G McArthur, AJ Butt & RL Sutherland (2008) Identification of functional networks of estrogen- and c-Myc-responsive genes and their relationship to response to tamoxifen therapy in breast cancer. ***PLoS One*** 3 (8): e2987

[2] Millar EKA, LR Anderson, CM McNeil, SA O'Toole, M Pinese, P Crea, A Morey, AV Biankin, SM Henshall, EA Musgrove, RL Sutherland & AJ Butt (2009) BAG-1 predicts patient outcome and tamoxifen responsiveness in ER-positive invasive ductal carcinoma of the breast. ***Brit J Cancer*** 100: 123-33

[3] Anderson LR, RL Sutherland & AJ Butt (2010) BAG-1 overexpression attenuates luminal apoptosis in MCF-10A mammary epithelial cells through enhanced RAF-1 activation. ***Oncogene,*** 29 (4):527-38

Neuroscience Program

HEARING RESEARCH GROUP

Dr Sharon Oleskevich
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Phone: 02 9295 8290

Project 1 Using Adult Stem cells to Repair Hearing Loss

Ever wondered if your music player is too loud? Studies have shown that listening to loud music for extended periods can cause long-term hearing loss. The hearing loss results from damage to the sensory receptors for hearing, the hair cells in the inner ear. This project will explore whether adult stem cells can replace the damaged hearing cells. Mouse sensory stem cells from the nose, tongue and balance organ will be transplanted into the inner ear of deaf mice. Hearing levels will be tested before and after cell transplantation. All techniques are well established and currently functioning in our laboratory. Future studies with human stem cells offer a clinically relevant progression from animal studies.

Project 2: Brain Pathways for Locating Sound in Space

Can't hear in a noisy pub or restaurant? Hearing loss affects one in five Australians, many of whom experience difficulty in understanding speech in noisy spaces. The pathways in the brain that help us locate sounds and recognise speech are not fully understood. This research project will study the brain pathways and nerve cells involved in hearing using electrical recordings in brain slices. The goal is to improve treatment strategies for individuals with hearing loss, users of hearing aids, and deaf persons with cochlear implants.

A wide range of skills is available to students, including stem cell biology, immunohistochemistry, molecular biology, cell culture, electrophysiology, fluorescent confocal microscopy, and hearing testing. Laptop and conference travel are guaranteed.

NEURODEGENERATIVE DISORDERS GROUP

Dr Bryce Vissel
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Phone: 02 9295 8293

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,physiology, animal behavior, 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

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: (1)Stereotaxic survival surgery and gene therapy approaches, (2) Immunohistochemistry combined with advanced confocal microscopy and stereology for analysis of regeneration. (3) Use of in vitro cell systems, including neural stem cells, for studying neurogenesis. (4) Behavioural testing to determine the capacity for functional recovery in animal models (5) 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: Neurodegeneration research and studies of synapses

When neurons in the brain that secrete dopamine die, this causes Parkinson's disease, which leads to a profound loss of motor function and eventually death. The biology of dopamine neurons, which is absolutely critical to a range of diseases such as Parkinson's disease, is poorly understood. We are researching dopamine neuron synapses to develop a greater understanding of the role that dopamine synaptic function in normal behavior and in disease and we have several cutting edge projects available for students. The student will learn advanced techniques in studying dopamine synaptic function, including: (1) Culture of

primary cells from mouse neural tissue, including glial cells, dopamine neurons and hippocampal neurons (2) Immunocytochemistry combined with advanced confocal live imaging microscopy (3) Advanced methods in microscopy for analysing active synapses formed by live neurons. (4) New concepts and techniques that few labs worldwide are able to achieve. The available research projects have significant potential to lead to important discoveries.

NEUROSIGNALLING GROUP

Dr Adam Cole
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Phone: 9295 8289

Project 1: Function of new Cdk5/GSK3 substrates in Alzheimer's disease

Background: Dementia is a debilitating disease that affects almost 250,000 Australians and costs the health service over $6 billion per year. There are over 100 different types of dementia, with Alzheimer's disease (AD) being the most common. At present, it is impossible to distinguish AD from other forms of dementia with absolute certainty until after the patient has died (autopsy). Also, it is usually diagnosed ***after*** symptoms become more severe. Biomarkers that could detect AD in its early stages are urgently required, since this is the period that is most responsive to current drug treatments.

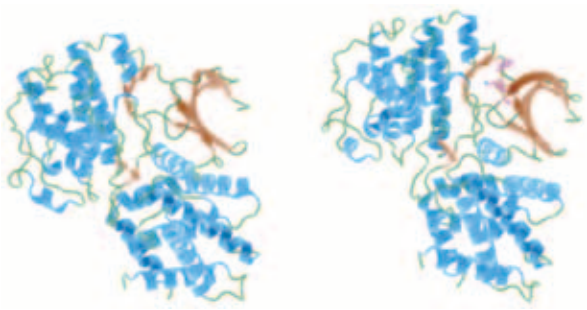
Cdk5 and GSK3 are closely-related enzymes (Ser/Thr protein kinases) that are critical for normal brain function, and deregulation of their activity is known to be involved in AD. Mice that have been genetically modified to increase the activity of Cdk5 or GSK3 develop symptoms similar to human AD patients, while another mouse model of AD responds favourably to treatment with drugs that reduce GSK3 activity. These observations clearly implicate Cdk5 and GSK3 as potential therapeutic targets for the treatment of AD patients. However, knowledge of the direct ***substrates*** of Cdk5 and GSK3 is essential to: 1) identify the pathologic targets of these enzymes, 2) to predict potential side effects of treatments using Cdk5/GSK3 inhibitors, and 3) to identify biomarkers of AD for diagnostic purposes.

Project: We have previously identified a novel target of Cdk5/GSK3 called CRMP2 that it is excessively modified (hyper-phosphorylated) in the brains of AD patients. This occurs early in the disease process and might be specific for AD, since it does not occur in other forms of dementias tested so far. Therefore, CRMP2 is a potential biomarker for early and specific diagnosis of AD. Unfortunately, CRMP2 is only detectable in the brain, which is not a suitable tissue for diagnostic purposes. Therefore, we initiated a search for other targets of Cdk5/GSK3 that are likely to be abnormally modified in AD brains, but also detectable in peripheral tissues that are better suited to routine testing, such as blood. In order to achieve this, we developed a new screening method called the BIPPS technique (BioInformatic Prediction of Phosphorylated Substrates). This method was successfully used to identify 6 new target proteins of Cdk5/GSK3 that are highly expressed in the brain, but also detectable in peripheral tissues. One protein in particular (b-adducin) is expressed ***only*** in the brain and erythrocytes (red blood cells). It displays several similarities to CRMP2, raising the exciting possibility that it might be an effective blood-based biomarker for early and specific diagnosis of AD. Opportunities exist to investigate the role of any of the 6 new Cdk5/GSK3 substrates in healthy and AD brains. A variety of techniques will be employed, including protein biochemistry, ***in vitro*** phosphorylation assays, recombinant DNA technology, immunoflourescence microscopy and neural cell culture.

Benefits: Very little is known about the function of these new Cdk5/GSK3 substrates, providing great opportunities for discovery. Importantly, if these substrates are excessively modified in AD, it will be the first time that AD-associated proteins will have been predicted, providing a major step forward in our understanding of this disease. Many essential reagents have already been generated and are ready for use, greatly expediting this research. The Garvan is an exceptionally well-equipped medical research institute with state of the art facilities and modern laboratories. Students in the Neurosignalling Group will be provided with attentive supervision, superior research facilities, exposure to a wide range of experimental techniques and will participate in national/international conferences and collaborations.

Project 2: Function of PCTK2, the forgotten brain kinase

Background: Almost all cellular functions involve communication via protein phosphorylation. This process is mediated by enzymes called kinases, which covalently modify their direct target proteins (substrates) on Serine, Threonine or Tyrosine residues with the g-phosphate from a molecule of ATP. Phosphorylation of a substrate alters its activity by inducing a change in its structure, localization, binding properties or enzymes kinetics. This in turn influences downstream cellular functions, such as cell shape, survival, gene transcription, etc. Therefore, protein phosphorylation signalling pathways are an important mechanism used by the cell to transmit signals that regulate vital cellular functions.



3-dimensional structure of PCTK2

PCTK2 is a Serine/Threonine kinase that is exclusively expressed in neurons of adult brains, suggesting that it might perform important brain-specific functions. Surprisingly, it has been largely ignored, so its brain-specific functions remain to be discovered. A small number of preliminary studies have implicated PCTK2 in protein exocytosis/cell surface expression and neurite outgrowth, indicating a potential role in regulating neurotransmission (transfer of information between neurons). However, these studies were performed in non-neuronal cells. To date, no studies have been performed in neurons, which is the only place that PCTK2 is expressed in.

Project: This project will focus on 3 key issues: 1) How is PCTK2 activity regulated? That is, are there other protein kinases that directly phosphorylate PCTK2 or co-factors that bind to PCTK2 to regulate its activity? 2) What are the physiological substrates of PCTK2? 3) What is the functional effect of PCTK2 kinase activity in primary neurons? These questions will be investigated using a variety of experimental techniques, including protein biochemistry, ***in vitro*** phosphorylation assays, recombinant DNA technology, liquid chromatography, mass spectrometry, immunoflourescence microscopy, primary neuronal cell culture and phospho-specific antibody production. In addition, new substrates of PCTK2 will be identified using 2 new specialist techniques; the proteomics-based KESTREL screen and the bioinformatics-based BIPPS technique.

Benefits: Very little is known about the function of PCTK2 in the brain, providing great opportunities for discovery. Many essential reagents have already been generated and are ready for use, greatly expediting this research. The Garvan is an exceptionally well-equipped medical research institute with state of the art facilities and modern laboratories. Students in the Neurosignalling Group will be provided with attentive supervision, superior research facilities, exposure to a wide range of experimental techniques and will participate in national/international conferences and collaborations.

NEURO-BONE GROUP

Dr Paul Baldock
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Project 1: Neuropeptide function in bone and fat cells?

The brain controls the actions of peripheral tissues through a number of processes, including hormonal outputs from the pituitary and direct neural outputs via the peripheral nervous system. Our group, in collaboration with the Eating Disorders Group has been instrumental in examining the action of a family of neuropeptides in the control of peripheral tissues such fat and bone, revealing novel aspects of conditions such as obesity and osteoporosis. The identification of peptide signalling molecules in the brain is a rapidly expanding field, with new candidates and new functions constantly increasing our knowledge of the scope of centrally-mediated processes.

One aspect of this research that has very recently become apparent is that these neuropeptides, formerly thought to signal predominantly with the central nervous system, are also expressed with target tissues. The role of this peripheral neuropeptide expression is unclear; however, the potential to modulate this expression in a tissue-specific manner has great therapeutic possibilities.

Our Program has made extensive contributions to the study of one such neuropeptide family, the Neuropeptide Y (NPY) system. NPY has rapid and powerful effects to regulate peripheral homeostasis of adipose and skeletal tissue. To date, the examination of this pathway has focussed upon action in the hypothalamus of the brain. However, NPY is also expressed in the cells that produce fat and bone, the adipocyte and osteoblast. Until now no models have existed to study the action of NPY in these tissues. We have developed a mouse model where NPY can be deleted in a tissue or temporal specific manner. Moreover, this will be combined with comparison of an opposing neuropeptide, cocaine and amphetamine related transcript (Cart), controllable in a similar manner.

This project will map, for the first time, the function of NPY and Cart in the adipocyte and osteoblast and compare results to central and global deletion models. Moreover, it will compare actions of NPY and Cart, as well as their combination.

These two neuropeptides are powerful modulators of fat and bone, and understanding of their peripheral action will be a major advance in our understanding of the regulation of these two tissues. And give insight into the relationship between central efferent neural pathways and their peripheral target tissues.

Project 2: Is Anorexia all in your brain?

Anorexia nervosa (AN) is the most lethal psychiatric condition, with up to 20% mortality stretching over a 20 year period. Sadly, An is an increasing health issue, with patients presenting at younger ages and with more frequency than in the past. To date, treatment options are severely limited, however, recovery and survival rates are markedly improved (70% recovery at 12 months) if nutritional therapy is included with normal psychiatric care. This is clear evidence for the importance of nutritional elements to this condition, formerly treated as a purely psychiatric disease. It is very difficult to model AN in animals; however, we have constructed a number of unique genetic mouse models in the Neuroscience Program that will enable us to examine a critical aspect of the nutritional aspect of AN.

One circulating protein, Peptide YY (PYY), has emerged as being closely associated with a number of characteristic changes in AN. Elevated serum PYY is known to correlate with loss of fat mass

and bone mass in AN patients, two critical aspects of poor short term and long term recovery, respectively. Moreover, PYY has been used successfully to modulate body weight in human studies, indicating it's exciting therapeutic potential. We have developed several mouse models in which PYY expression can be increased in a time, or cell-specific manner. Initial investigations suggest that this PYY over expression is capable of inducing changes characteristic of AN.

It is our hypothesis that elevated PYY alters the signalling of critical pathways in the brain that control feeding, behaviour, hormone production and bone mass, thereby producing a cascade critical to the aetiology of AN.

It is our aim to dissect the central and peripheral actions of PYY in an effort to isolate a target for therapeutic intervention. The receptors, both central and peripheral, by which PYY act are known and our Program has extensive experience and tools for modulating their activity. Initial studies will aim at understanding the role of the PYY Y2 receptor in the hypothalamus and transmission of the serum PYY signals to higher brain regions and the neuropeptide changes that are involved..

This basic research will complement a clinical study being organised with collaborators in the Adolescent Health Unit at the Children's Hospital at Westmead. Together we aim to provide critical insight into the treatment of this important health issue.

PAIN RESEARCH GROUP

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NEURONAL SYSTEMS BIOLOGY

The human genome project was a major advance allowing for molecular foot hold towards an understanding of human diseases. The real question now is "what do these genes do, and how do they participate in human disease?" 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.

Project 1: Conserved regulators of appetite

Eating disorders are a significant medical concern in the western world. To gain insight into novel neuronal mechanisms regulating appetite, we have recently performed a genome-wide, neuronal specific RNAi screen for appetite in the fruit fly. In this screen, all genes were knocked-down one by one in the nervous system, and food intake was indirectly measured. This approach has revealed ~250 candidate regulators of appetite, many of which have unknown functions. A continuation of this project will involve further phenotyping of these candidate appetite genes, comparison of candidate fly appetite genes with unpublished human genome-wide association studies, followed by a more detailed biochemical workup on selected conserved appetite genes. In some cases this may involve gene targeting in mice for candidate conserved regulators of appetite.

Project 2: Conserved regulators of neurodegeneration

Therapies for neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, represent an unmet clinical need. We have recently completed a genome-wide, neuronal specific RNAi screen for neuronal cell death in the fruit fly. In this screen, all genes were knocked-down one by one in the nervous system, and degree of neuronal cell death was measured. This approach has revealed ~250 candidate regulators of neurodegeneration and neuronal cell death, many of which have unknown functions. In addition, we have access to an unpublished list of about 500 candidate human "Parkinson's disease" genes uncovered in a recent human genome-wide association study. A continuation of this project will involve further phenotyping of these new conserved neurodegeneration genes using neuronal-specific RNAi ***in vivo***, comparison of fly vs. human genes implicated in Parkinson's disease, use of existing models of Alzheimer's and Parkinson's disease in the fruit fly, and a more detailed biochemical workup on selected genes. In some cases this may involve gene targeting in mice for candidate conserved regulators of neurodegeneration in mice.

Project 3: Conserved regulators of the sleep deprivation response

The purposes and mechanisms of sleep are only partially understood and are the subject of intense research. The fruit fly has recently become a useful model for studying sleep, and sleep deprivation is lethal in fruit flies, mice, and humans. We are currently developing novel high throughput strategies to study sleep in the fruit fly. In conjunction, we have access to an unpublished list of candidate human "sleep" genes, some of which have already been validated in the fly. A continuation of this project will involve setting up and optimizing our sleep and sleep deprivation systems, screening candidate "sleep" genes in the fruit fly using neuronal-specific RNAi ***in vivo***, and a more detailed biochemical workup on selected genes. In some cases this may involve gene targeting in mice for candidate conserved regulators of sleep in mice.

Selected recent publications:

1. **Neely** et al, A global in vivo Drosophila RNAi screen identifies NOT3 as a conserved regulator of heart function. **Cell**. 2010 Apr 2;141(1):142-53.(Cover).

2. Pospisilik et al, Drosophila genome-wide obesity screen reveals hedgehog as a determinant of brown versus white adipose cell fate. **Cell**. 2010 Jan 8;140(1):148-60.

3. Cronin et al, Genome-Wide RNAi Screen Identifies Genes Involved in Intestinal Pathogenic Bacterial Infection. **Science**. 2009 Jul 17;325(5938):340-3.

4. Imai et al, Identification of oxidative stress and Toll-like receptor 4 signaling as a key pathway of acute lung injury. **Cell**. 2008 Apr 18;133(2):235-49.

5. 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).

Bone Research Program

EPIDEMIOLOGY AND GENETICS GROUP
OSTEOPOROSIS AND BONE BIOLOGY

Professor Tuan V. Nguyen
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Phone: 02 9295 8277

Clinical studies

Osteoporosis is a systemic skeletal disease characterized by low bone mass and degenerative microarchitectural deterioration of bone tissue which consequently increase bone fragility and susceptibility to fracture risk. A pre-existing fracture increases the risk of subsequent fracture and mortality.

We are interested in identifying risk factors that contribute to fracture and mortality in men and women. Specific risk factors considered are: hormones, bone turnover markers, genetic factors, bone strength, bone quality, and lifestyle factors. Some specific projects include:

- Development and validation of a composite outcome that captures all clinical aspects of osteoporosis;
- Role of biochemical markers of bone turnover in the prognosis of fracture risk;
- Relationship between obesity, diabetes, and osteoporosis.

Genetics of osteoporosis

We are interested in searching for novel genes that are involved in the regulation of bone phenotypes and fracture risk. We use both genome-wide linkage and population-based genetic association analyses. This line of research requires expertise in medical science, genetics, biostatistics, and bioinformatics. Specific projects included, but not limited to:

- Candidate gene association studies;
- Influence of gene-gene and gene-environmental interactions on bone phenotypes;
- Development of clinico-genetic prognostic models for individualising fracture risk.

Assoc Professor Jackie Center
& Assoc Professor Grahame Elder
Email: j.center@garvan.org.au
Phone: 02 9295 8271

Dr Grahame Elder has collected data on patients on dialysis about to undergo kidney and kidney-pancreas transplantation. It is a tremendous resource and there are some very exciting questions that can be explored through this database with issues related to bone and renal bone disease, vascular calcification, diabetes and so on. Projects are available for PhD, Masters and Honours students.

Project

Since 2003, patients entering the kidney and the national kidney-pancreas transplant program at Westmead Hospital have been comprehensively assessed for abnormalities of bone and mineral metabolism, both immediately before and following transplantation. Over 430 patients have now been entered into this data base, which provides cross sectional data on a relatively healthy (fit for transplantation) dialysis population as well as longitudinal data from just pre-transplant onwards.

Bone densitometry and spinal X-rays (including lateral lumbar spine / abdomen; which is a validated means of assessing vascular calcification) are undertaken yearly and multiple laboratory investigations including bone turnover markers and hormonal levels are collected at baseline, 1, 3 and 12 months and at 2 and 5 years. Quality of life assessments at baseline, 3 and 12 months after transplant, using a KDQOL-SF that is validated for dialysis and transplant patients, have recently been added to the data collected.

This is one of the most comprehensive bone resources available for patients following kidney and kidney pancreas transplantation and is a useful cross sectional database for patients on dialysis; in particular, those with type 1 diabetes. It provides opportunities to study changes to BMD, biochemistry, sex hormones, bone regulating hormones, vascular calcification, prevalent and incident fracture and QOL from dialysis through transplantation. A post-transplant bone biopsy study, together with high resolution pQCT, is planned to commence in 2009-2010.

The patients on dialysis study (which has been approved and funded) aims to assess the value of supplemental vitamin D vs placebo in patients who are vitamin D deficient and on dialysis. Endpoints include patient-level outcomes such as muscle strength and mobility, in addition to biochemical endpoints and the evaluation of novel bone turnover markers.

Joint Project

DIABETES AND OBESITY RESEARCH PROGRAM

Assoc Professor Greg Cooney
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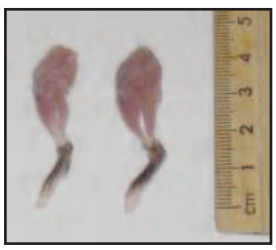
Phone: 02 9295 8209

Project: Regulation of body composition and glucose homeostasis by the adaptor protein Grb10

An important risk factor for Type 2 diabetes is the development of insulin resistance. Many factors contribute to insulin resistance including the decrease in muscle mass associated with reduced physical activity and ageing. Consequently, understanding how the signalling pathways involved in insulin action and maintenance of muscle mass are regulated is of major significance. We are focusing on two adapter-type signalling proteins, Grb10 and Grb14, which bind directly to the insulin receptor.

Our work on how the Grb10 and Grb14 signalling proteins regulate insulin action made the front cover of 'Molecular Endocrinology'.

We have recently demonstrated that Grb10 gene knock-out mice exhibit increased insulin signalling in skeletal muscle and adipose tissue. Furthermore, $Grb10^{-/-}$ mice also display increased skeletal muscle mass and reduced adipose tissue content.



Hindlimb musculature in wildtype mice (left) and Grb10-/- mice (right).

CANCER RESEARCH PROGRAM

Professor Roger Daly
Email: r.daly@garvan.org.au
Phone: 02 9295 8333

Since these mice have 'global' Grb10 ablation (ie Grb10 is absent from all tissues) it is unclear whether Grb10 has roles in both muscle and adipose tissue, or whether the effect in one tissue is an indirect consequence of its role in the other. In addition, if Grb10 is to be targeted therapeutically, it is important to determine whether the beneficial effects of ablating Grb10 require the absence of Grb10 during development, or whether they can be achieved via more 'acute' ablation of this adaptor in adult mice.

To address these issues we will utilize a conditional Grb10 allele ($Grb10^{fl/fl}$) to determine how Grb10 ablation in a tissue-specific and developmental stage-specific manner affects phenotype. The corresponding mouse strain is under development and should be available in late 2010. $Grb10^{fl/fl}$ mice will be crossed with mice expressing Cre recombinase, or tamoxifen-regulated Cre, in muscle or adipose. This will enable us to 'knock-out' Grb10 expression in muscle and adipose throughout development and adulthood, or alternatively from a particular developmental stage (by timed addition of tamoxifen, which induces the gene deletion). The resulting strains will be characterized for their muscle, fat and metabolic phenotypes, as well as for effects on signalling by insulin and other hormones/growth factors. This will determine whether the effects on body composition in $Grb10^{-/-}$ mice reflect autonomous roles for Grb10 in muscle and/or adipose, and whether an increase in relative lean mass and improvement in glucose homeostasis can be achieved by Grb10 ablation during adulthood.

You have a postgraduate application form and want to study at Garvan: What now?

1. **Complete the application and send it in after discussions with your proposed supervisor. Remember to include your CV and a copy of your most recent academic transcript.**

   All applications are considered by the Garvan Higher Degrees Committee (HDC) twice a year at times that coincide with the lodgement of Australian Postgraduate Award (APA) applications and National Health and Medical Research Council (NHMRC) scholarships. *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.

   Prospective students must also lodge an application for admission to UNSW online at: http://www.grs.unsw.edu.au/futurestudents/apply.html

2. **Apply for postgraduate funding.**

   Scholarships such as APA, UPA and IPRS should be applied for through the UNSW Graduate Research Office which will also provide additional information about other research scholarships offered by the university.

   NHMRC scholarships should be applied for through the Garvan grants administration office once you have been accepted by the Garvan. For more information email: grants@garvan.org.au.

KEY DATES	
20 April	Garvan HDC Application deadline
Late April (TBA)	HDC PhD application interviews & notification to students of acceptance (subject to APA success)
30 April	APA lodgement
June	APA notification
August	NHMRC (Dora Lush) scholarship due first Friday in August
20 October	Garvan HDC Application deadline
Late October (TBA)	Garvan HDC PhD application interviews & notification to students of acceptance (subject to APA success)
31 October	APA lodgement
December	APA notification

Garvan Institute of Medical Research

Application to undertake postgraduate study

If you are interested in commencing postgraduate study at Garvan, please fill in the details on this form, attach the following information, and send back to Garvan.

- A copy of your most recent academic transcript
- A CV and synopsis
- 2 academic referee reports (sealed)

Personal Details

Title: .....

Name: .....

Address: .....

.....

Phone: .....

Email: .....

DOB: .....

Education

Please list all university qualifications achieved:

Qualification	University	Date Completed

List any University qualifications currently being undertaken or completed:

Qualification	University	Subject Majors

If applicable, please provide a title and short description of your honours project (or you can attach your thesis abstract to this form):

Other Information:

List any awards you have received:

List any relevant memberships:

Provide details of any scientific work experience (paid or unpaid) you have gained outside your university studies (include details of any publications):

Please indicate fields of research interest (you may tick more than one):

- ☐ Cell Biology
- ☐ Biochemistry
- ☐ Genetics
- ☐ Immunology
- ☐ Clinical Medicine
- ☐ Physiology
- ☐ Pharmacology
- ☐ Bioinformatics
- ☐ Proteomics
- ☐ Neuroscience
- ☐ Others (Please specify) .....

Are there any Garvan researchers you has discussed your proposed research with or research groups you would like to work with in particular?

1.....

2.....

3.....

What funding have you, or do you intend on applying for?

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How did you hear about the Garvan Institute postgraduate program?

- ☐ Garvan PhD Student Day
- ☐ Garvan website
- ☐ Other (please specify) .....

PLEASE RETURN WITH REQUIRED INFORMATION FOR CONSIDERATION TO:

Higher Degrees Committee
C/- Dr Ebi Cocodia
Garvan Institute of Medical Research
384 Victoria Street
Darlinghurst NSW 2010, Australia

REFEREE REPORT

Postgraduate Application

SECTION A – FOR THE APPLICANT TO COMPLETE

Please complete Section A and send the form to your nominated academic referee.

Please note that it is your responsibility to ensure that 2 referee reports are returned to the Garvan Institute with the application form, in a sealed envelope, by the due date.

Title: .....

Family Name: .....

Given Name(s): .....

Please tick what type of degree you intend to undertake: ☐ PhD ☐ Masters by Thesis

SECTION B – FOR THE REFEREE TO COMPLETE

Please complete Section B, including the questions that follow, and return the completed report to the applicant, **in a sealed envelope.**

Title: .....

Family Name: .....

Given Name(s): .....

Institutional Name & Adress: .....

.....

Telephone: .....

Facsimile: .....

Email: .....

1. How long have you known the applicant?

☐ 3-6 mths ☐ 6-12 mths ☐ 1-2 yr ☐ 2 yrs or more

2. In what capacities have you known the applicant?

☐ Lecturer ☐ Course coordinator ☐ Head of School /Department

☐ Dean of Faculty ☐ Coursework Master's supervisor ☐ Research degree supervisor

☐ Examiner of his/her thesis ☐ Other.....

3. How would you rate your knowledge of the applicant as the basis for providing a reference?

☐ Very Good ☐ Good ☐ Adequate ☐ Limited

4. Please indicate your ranking of the applicant relative to other students you have known and/or supervised:

☐ Top5% ☐ Top 8% ☐ Top 10% ☐ Top 15% ☐ Top 20%

5. How would you rate the applicant's writing skill?

☐ Average ☐ Above Average ☐ Very good ☐ Excellent

6. How would you rate the applicant's laboratory skills?

☐ Average ☐ Above Average ☐ Very good ☐ Excellent 5

7. How would you rate the applicant's knowledge of discipline?

☐ Average ☐ Above Average ☐ Very good ☐ Excellent

8. How would you rate the applicant's ability to plan?

☐ Average ☐ Above Average ☐ Very good ☐ Excellent

9. How would you rate the applicant's initiative and motivation?

☐ Average ☐ Above Average ☐ Very good ☐ Excellent

10. How would you rate the applicant's ability to meet deadlines?

☐ Average ☐ Above Average ☐ Very good ☐ Excellent

11. How would you rate the applicant's team work?

☐ Average ☐ Above Average ☐ Very good ☐ Excellent

REFEREE COMMENTS:

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Signature:..... Date:...../...../.....

GARVAN
INSTITUTE

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