LA TROBE UNIVERSITY

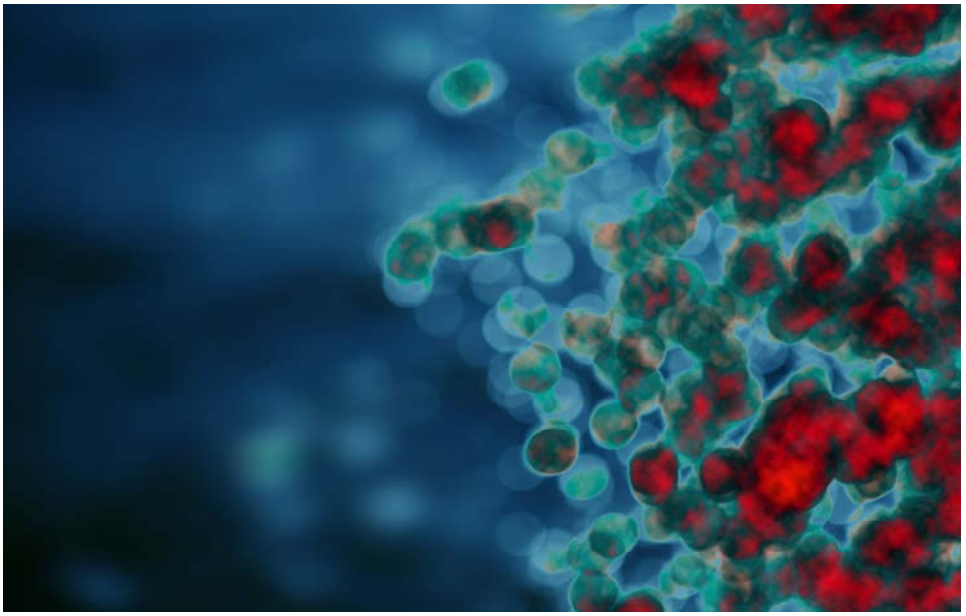
LA TROBE INSTITUTE FOR MOLECULAR SCIENCE

Olivia Newton-John Cancer Research Institute

2019 HONOURS RESEARCH PROJECTS

DEPARTMENT OF BIOCHEMISTRY & GENETICS

SCHOOL OF CANCER MEDICINE



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WHY DO HONOURS?

Honours in Biochemistry and Genetics will kick-start your career in science. The Biochemistry and Genetics Honours program equips graduates with technical, analytical, communication and time management skills demanded by employers in diverse scientific fields ranging from biotechnology to biomedicine. Our students work within teams to undertake novel research projects, under the supervision of leading scientists. Honours projects are carried out in state-of-the-art research facilities, either within the La Trobe Institute for Molecular Science (LIMS) on the La Trobe University Bundoora campus, or at the Olivia Newton John Cancer Research Institute (ONJCRI) in Heidelberg.

Objectives

- Extend knowledge of biochemistry, molecular genetics and medical biology
- Obtain an immersive scientific experience in an authentic research laboratory
- Plan and perform cutting edge experimental procedures
- Pursue an original research project to generate new knowledge

Skills you will learn

- Analyse and integrate research findings from numerous sources to formulate hypotheses
- Design and perform experiments using multiple advanced techniques to investigate complex scientific questions
- Critically interpret experimental data in the context of other results and prior literature
- Develop excellent time management skills
- Develop advanced scientific oral and written communication skills
- Work collaboratively as part of a cohesive and productive research team

Past student testimonials

- *Honours was an incredibly stimulating learning experience*
- *It was an amazing year which provided me with real research skills*
- *The lab experience was invaluable*
- *The independent research project was intellectually challenging*
- *Working in a biomedical research lab gave me a sense of contribution to society*
- *My honours project lead to a research publication, which set up my career*

COURSE STRUCTURE

Honours projects are available in the disciplines of Biochemistry, Genetics or Biomedical Science, and can be performed either at the **La Trobe Institute for Molecular Science (LIMS),** La Trobe University, (Bundoora campus) or within the **Olivia Newton-John Cancer Research Institute (ONJCRI),** Austin Hospital (Heidelberg) which is affiliated with the School of Cancer Medicine. All students, regardless of the location of their chosen laboratory, must attend compulsory sessions (e.g. training, assignments) on the Bundoora campus at La Trobe University.

The course will commence on **Thursday 7th February 2019**, with a series of compulsory induction lectures. After this, students will write a literature review that summarises relevant prior knowledge and present an introductory seminar to the department (accounting for 8% of the year's assessment). The majority of the year is dedicated to performing novel research, under the supervision of the laboratory head and other senior staff. Throughout the year, students will learn about diverse research topics through attending research seminars delivered by invited experts (1%). In May, Honours students will complete a module introducing them to advanced biotechnology techniques (10%) and in August they will create and present a poster outlining the goals of their project and their progress to that point (8%). In September, students will complete their experiments to focus exclusively on writing their theses, which will be submitted in mid-October (40%). Supervisors will continuously assess students' laboratory performance throughout the year (10%). The year culminates in late October, with students delivering seminars summarising their research findings to the department (8%) and undertaking oral examinations (15%).

At the end of the course we expect our students to be able to enter the work force, and perform competently at whatever tasks they are given. Indeed, graduates from our Honours program are highly sought after by universities (both nationally and internationally), research institutes, hospitals and biotech companies alike.

Choosing an Honours Project

There are a number of things to consider before deciding which supervisor/laboratory is best for you. These include:

Research topic: Which research questions and techniques do you find most interesting and appealing? To what extent will the background knowledge you gained through your undergraduate studies prepare you for each of the research topics?

Supervisor: What supervisory style suits you best? Would you prefer to receive day-to-day supervision and feedback regarding practice presentations and thesis drafts predominantly from the laboratory head, or from a team that may include postdoctoral fellows and research assistants?

Career opportunities: How will each of the possible projects equip you for your chosen career, including possible postgraduate research (if applicable).

These questions can be answered through discussions with prospective supervisors, postgraduate students and current Honours students. Don't be afraid to ask!

PROJECT ALLOCATION PROCESS

Eligibility: students must complete at least one of the following subjects MED3PRJ, MED3LAB or GEN3LAB. Some projects have more restricted pre-requisites, so check the project descriptions carefully. However, mere completion of a pre-requisite subject will not guarantee entry into the course, as there are a limited number of places available. Students **MUST** discuss projects with prospective supervisors.

To organise an Honours research project and supervisor:

1. Read this booklet carefully.
2. Attend the **Project Pitching** presentations in the **RL Reid Building Seminar Room (RLR-101)**: **10am – 12.30pm, Tuesday 11th September, 2018**, to hear a brief presentation about each of the projects being offered.
3. Email any prospective supervisors who have offered projects that you find interesting and would consider undertaking, to organise appointments to discuss the proposed projects and your suitability. You can explore the possibility of securing "select" entry into your preferred laboratory during these meetings.

***Note**: Students **MUST** meet with a supervisor in order to be placed in their laboratory. To maximise your chance of being accepted into the Honours program, we therefore recommend that you meet with **AT LEAST FIVE** supervisors.*

There are two allocation routes: "select" (by negotioation) and "ranked" (based on marks).

If your meeting with a supervisor leads him/her to offer you a "**select**" place in his/her laboratory, and you accept, ensure that the supervisor notifies the course co-ordinator (c.hawkins@latrobe.edu.au) (cc'ing you) by **Friday 26th October, 2018.**

If you do not arrange **"select"** entry by Friday 26th October, you can apply to gain **"ranked"** entry into the course, by ranking the supervisors you met with on the attached nomination form (page 60 of this booklet).

"Ranked" entry will be determined by your final average marks in third year biochemistry, molecular genetics or biomedical science subjects. This means students with better marks have a higher chance of being assigned to their preferred supervisor.

To apply for **"ranked"** entry into Honours, fill out the form on page 61 of this booklet, specifying your ranking for each supervisor with whom you have met.

Submit the form to c.hawkins@latrobe.edu.au by **5pm Friday 9th November, 2018**.

Provisional offers will be made via email in late November (subject to ratification by the University of semester 2 marks) so make sure you check your university email account during this period.

If you have any questions about the allocation process, of the Honours course, please e-mail c.hawkins@latrobe.edu.au to make an appointment.

THE DEPARTMENT OF BIOCHEMISTRY & GENETICS

The Department of Biochemistry and Genetics is engaged in both fundamental and commercially driven research. It has a strong record of attracting competitive funding from National (i.e. the Australian Research Council (ARC), the National Health and Medical Research Council (NHMRC)) and international (i.e. National Institutes of Health) funding agencies. The Department has published numerous papers in highly influential journals such as Cell, Science and Nature. Research conducted within the Department has led to the generation of valuable intellectual property. The Department currently houses three biotechnology companies involved in product development and marketing.

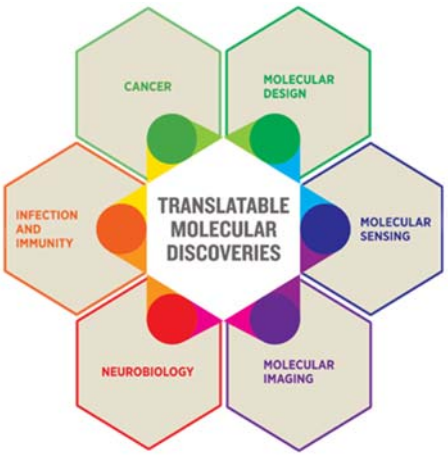
Many of our Honours students progress to undertake PhDs, and our students often receive awards in recognition of their research excellence. The following prizes were awarded in 2017 to previous Honours graduates who are currently PhD students:

- Alyce Mayfosh (Honours 2014) received the Best Student Poster at the Victorian Infection & Immunity Network Young Investigator Symposium
- Katie Owen (Honours 2014) was awarded the Best Overall Presenter Award at the 8th Annual Australian Society for Medical Research symposium (see photo)
- Georgia Atkin-Smith (Honours 2015) was awarded an ASBMB fellowship
- Linda Brain (Honours 2016) and Georgia Atkin-Smith (Honours 2015) were awarded ASBMB poster prizes at the ComBio2017 conference

Our graduates are highly employable, being sought after by other universities and research institutes, as well as biotechnology companies. Many of our Honours/PhD graduates undertake postdoctoral research, within Australia or overseas. Others, including those listed below, pursue diverse roles in academia, biotechnology or medicine:

- After occupying a number of senior roles within La Trobe University Damian Spencer (Honours 2001, PhD 2007) recently accepted a position as Dean (Teaching and Learning) at Cambridge International College, Australia.
- Dr Nicole van der Weerden (Honours 2003, PhD 2007) is now the Chief Executive Officer of Hexima (a successful biotechnology company).
- David Bloomer (Honours 2012, PhD 2017) is also working in the biotechnology industry, within the recombinant proteins sector at CSL.
- Mark Miles (Honours 2013, PhD 2018) is now employed as Associate Lecturer within the department and is currently coordinating MED2BMS.
- Stephanie Paone (Honours 2013, PhD 2018) works as Clinical Research Coordinator at Nucleus Network.

The **Department of Biochemistry and Genetics** contains **29 research laboratories**, teaching facilities and administration across two buildings (LIMS1 and 2). These buildings contain state-of-the-art facilities with 10,000 m^2 of usable space including 18 new research and support laboratories, an equipment barn, and ~ 3,000 m^2 of teaching facilities.



Research at LIMS is aimed at generating **Translatable Molecular Discoveries** and encompasses **research in six thematic areas:**

Cancer
Infection and Immunity
Neurobiology
Molecular Imaging
Molecular Sensing
& Molecular Design

Facilities include:

- Confocal and widefield microscopy (for imaging fluorescently labelled biomolecules in fixed and live cells)
- FACS/flow cytometry (analyses or sorting of cells based on surface markers or other features)
- Fluorescence spectroscopy (for sensitive detection of intrinsically and extrinsically labelled fluorescent biomolecules)
- Gel doc and Chemidoc systems (for stain free analysis and Western blotting)
- Gel electrophoresis equipment (for separating proteins, DNA and RNA)
- Histology preparation equipment (staining of biological tissue samples)
- Ion S5 next generation sequencer (for small RNA profiling and targeted DNA sequencing)
- Liquid chromatography systems (for protein purification)
- Mass spectrometry (for protein/peptide sequencing, and identification/quantification of proteins in complex samples)
- Protein interaction facility (including Analytical ultracentrifugation, Isothermal Titration microcalorimetry and Surface Plasmon Resonance) for measuring biomolecular interactions
- Microscale thermophoresis instrumentation (for quantification of protein-protein and protein-ligand interactions)
- Plate readers (for UV/Vis, luminescence and fluorescence assays in 96- and 384-well plate formats)
- qRT-PCR systems (for quantification of gene expression) and automated liquid handling instruments
- Tissue culture facilities (for bacterial, insect, mammalian, plant cell & viral culturing)
- UV/Vis spectroscopy (for quantifying DNA, RNA, peptides & proteins and performing enzyme kinetics assays)
- X-ray crystallography (for determining the three-dimensional structure of proteins and biomolecular complexes).

Professor Marilyn ANDERSON

ANTIFUNGAL AND INSECTICIDAL PROTEINS

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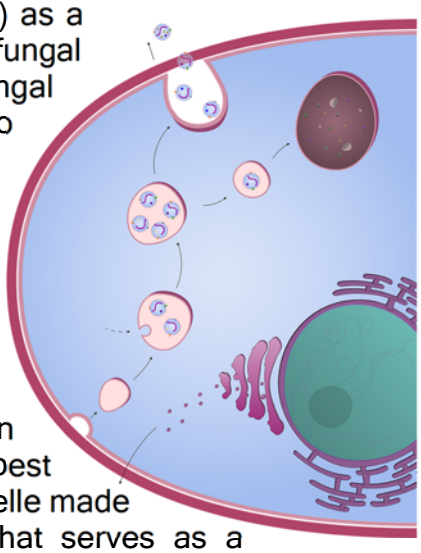
Subject prerequisites: MED3PRJ, MED3LAB or GEN3LAB **Theme:** Infection & Immunity

Project 1: The link between fungal EV release and cell wall stress.
Co-supervisor: Dr Mark Bleackley



Fungi, like all organisms, secrete extracellular vesicles (EVs) as a component of their communication systems. Once secreted, fungal EVs can be taken up by other fungi or, in the case of fungal pathogens, host organisms. EVs contain a variety of cargo including proteins, lipids, nucleic acids and small molecules. Upon uptake by target cells the EV cargo can induce a variety of changes in the physiology of the target cell. Much of the focus on the effect of fungal EV uptake has been on increasing virulence by sending signals between fungal cells or increasing host susceptibility as a result of uptake of virulence factors by host cells.

The fungal cell wall is a unique feature of fungal EV secretion that differentiates it from EV secretion in mammalian cells, the best characterized system. The fungal cell wall is a dynamic organelle made up of predominantly of carbohydrates and glycoproteins that serves as a physical and chemical barrier that protects against biotic and abiotic stresses. It also serves as a barrier for the release of EVs. However, the cell wall is able to locally remodel to allow secretion and uptake of EVs. We have demonstrated that chemically or genetically decreasing the thickness of the fungal cell wall leads to an increase in EV secretion (Fig. 1).

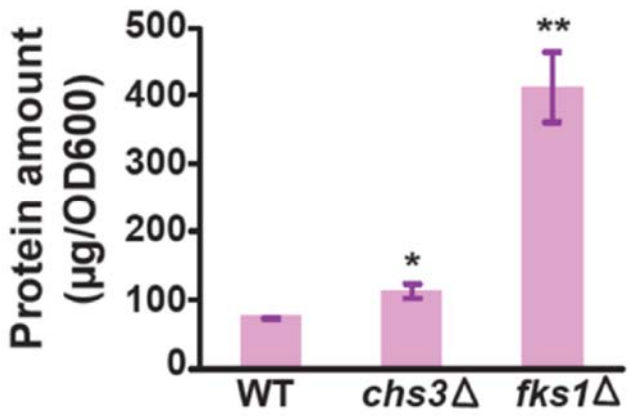


Fig. 1 | EV release is increased in two S. cerevisiae cell wall mutants.
EVs were isolated from WT yeast, two cell wall mutants (chs3Δ and fks1Δ), and then quantified based on protein content (* denotes $P \leq 0.05$, ** denotes $P \leq 0.01$; Error bar = ±SEM, n = 3).

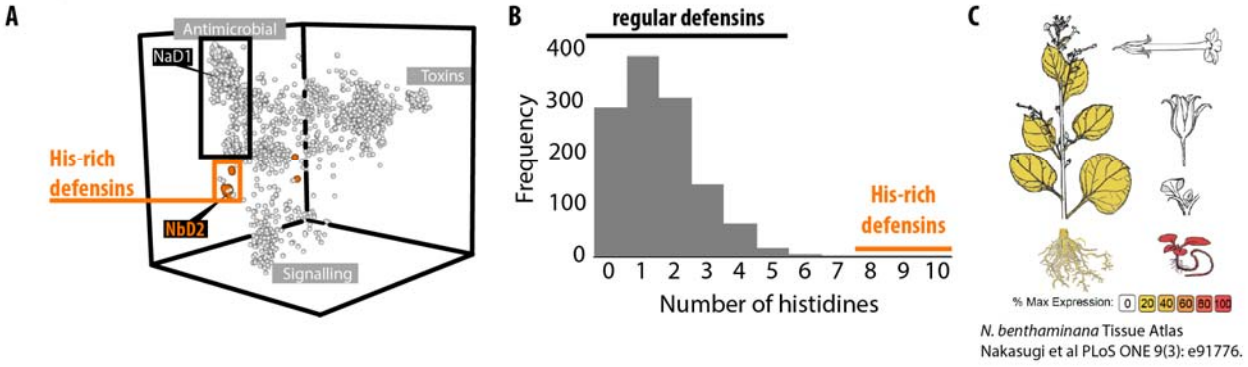
This project will focus on determining whether this is due to an upregulation of EV biogenesis and secretion as a component of the fungal cell wall stress response. A link between EV uptake and resistance to cell wall stress has also been demonstrated. Understanding the mechanism by which EVs protect against cell wall stress will also be a key aim of this project. The bulk of the work in this project will be performed using the model yeast *Saccharomyces cerevisiae*. We have a collection of *S. cerevisiae* deletion mutants available that will facilitate analysis of the link between cell wall stress and EV release/uptake.

Project 2: The role of histidine-rich defensins in the tolerance of metals by plants.
Co-supervisors: Drs Rohan Lowe and Mark Bleackley

Plant defensins are a family of proteins that are well known for their ability to protect plants against fungal diseases. These proteins have a conserved structural motif stabilised by four disulphide bonds but exhibit a massive amount of variation at the sequence level. This diversity has led to the evolution of additional functions, including roles in reproduction, enzyme inhibition and metal tolerance.

The Anderson Lab has recently identified a class of plant defensins that are enriched in histidine residues (Fig. 1). Histidine-rich proteins often bind metal ions. Recombinant expression and purification of the histidine-rich defensins by the Anderson lab has confirmed *in vitro* metal binding activity in this new class of defensins. However, it is unclear what the function of these defensins is *in planta*.

Fig.1 | Histidine rich defensins are exceptionally rare in nature. NbD2 is expressed in seedlings, adult leaves, and roots.



We have identified histidine-rich defensins in the genome of the Australian native tobacco *Nicotiana benthamiana*, a plant found in the Australian outback where soils are metal-rich, and the dry environment means there is little threat from microbes. We hypothesise that the histidine-rich defensins have evolved to deal with toxic levels of metals found in the outback soil, rather than defend against microbial threats. This kind of specialisation has been previously described for *N. benthamiana*, which sacrificed its viral defence system in favour of seedling vigour and increased survival in extreme environments. This project will examine the expression of histidine-rich defensins in *N. benthamiana* and compare the metal tolerance phenotype of *N. benthamiana* to closely related species that lack histidine-rich defensins.

Dr Suzanne CUTTS

MECHANISM OF ACTION OF ANTICANCER DRUGS

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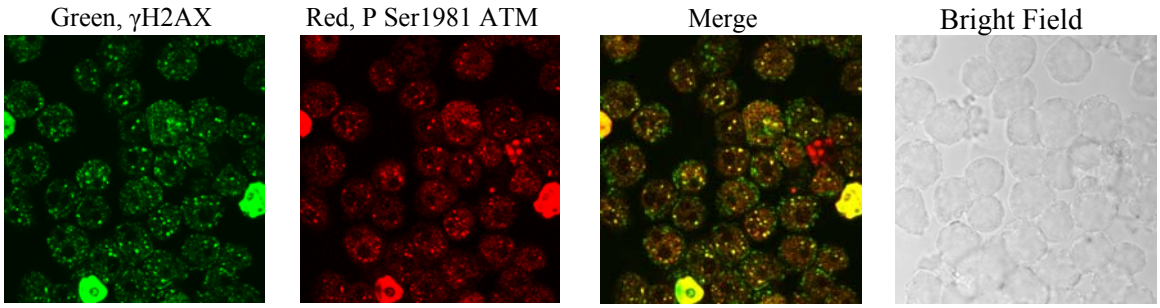
Subjects prerequisites: MED3LAB or MED3PRJ **Theme:** Cancer

The anticancer drugs doxorubicin (an "anthracycline") and mitoxantrone (an "anthracenedione") are widely used in cancer chemotherapy, and are classified as inhibitors of topoisomerase II. We strive to develop new therapeutic strategies for cancer treatment by understanding the specific mechanism of action of these currently used anticancer drugs, and building on this information to restrict their killing properties to cancerous cell types. In this way, the toxic side effects of these drugs can be minimised.

It is now well established that anthracyclines such as doxorubicin can bind covalently to DNA to form DNA adducts when activated by the simple molecule formaldehyde. We can activate doxorubicin to bind covalently to DNA in this manner by supplementing with low toxicity formaldehyde-releasing prodrugs. These lesions provide a more lethal death-inducing signal in cells than damage which occurs in the absence of formaldehyde (ie topoisomerase II-mediated damage). The combination of anthracyclines with formaldehyde-releasing agents may prove clinically beneficial.

Project example: Which classes of drugs are promising for killing highly metastatic cancer cells?

Mitoxantrone and doxorubicin are anticancer drugs that mainly function by poisoning the nuclear enzyme topoisomerase II. Poisoning of topoisomerase II leads to lethal double stranded DNA breaks and also depletion of cellular topoisomerase II levels, both of which can culminate in cell death. The DNA damage that is generated by topoisomerase II poisoning is measurable by γH2AX induction.



Mito 2 (12.5 nM) induces DNA damage (shown by γH2AX foci that colocalise with phosphorylated ATM) at growth inhibitory concentrations. Image courtesy of Dr Ben Evison

For over 80% of the patients who die of cancer, metastasis is the cause of death. The ability to effectively treat metastases is limited and new therapies are urgently needed. We have access to a panel of cell lines which vary in their capacity to undergo metastases in *in vivo* models. These human and mouse cell lines therefore range from non-metastatic to highly metastatic. We will examine the ability of different classes of drugs to induce DNA damage and subsequent apoptosis in each of these cell lines. Some of these drugs will be prepared in nanoparticle formulations. We will also examine if switching the mechanism of action from topoisomerase II inhibition to other forms of DNA damage alters the potency of the treatments. In the first instance these treatments will be investigated in cells in culture, and then finally selected cell lines and treatments will be analysed further in *in vivo* models including patient-derived xenografts.

The types of techniques to be utilised include mammalian cell culture, MTT cell viability assays, nanoparticle preparation & analysis, apoptosis assays, genomic DNA extraction & quantitation of drug-DNA complexes, Comet DNA damage assays, quantitative PCR, immunohistochemistry, westerns and use of a variety of drugs and chemical inhibitors. Equipment used includes microplate absorbance reader, FACS machine, fluorescence microscope, & scintillation counter. Training in the use of several software packages will also be required.

Dr David DOUGAN

PROTEOSTASIS IN HEALTH & DISEASE

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Subject prerequisites: MED3PRJ, MED3LAB or GEN3LAB **Theme:** Infection & Immunity

Proteostasis is the maintenance of a functional proteome within a cell. Our lab is particularly interested in the cellular machines that are responsible for maintaining "proteostasis". A major focus of the group is understanding how large ATP-dependent machines contribute to the overall "health" of a cell. We are interested in the biological function of these machines, their mechanism of action and their role in disease. We are also interested in the identification and development of novel compounds that dysregulate these machines as a means generate novel therapeutics or antibiotics.

We focus on the role of molecular chaperones and proteases in the maintenance of the cellular proteome, not only under normal conditions, but also under condition of cellular stress. An ultimate goal of the lab is to develop novel drugs, either chemical chaperones that can stabilise vulnerable proteins in the cellular proteome during times of stress or chemical dysregulators of these machines that serve as novel antibiotics to kill pathogenic bacteria.

Project 1: Investigating the role of the putative sporulation transcription factor (WhiA) in Mycobacterium smegmatis proteostasis.

Our lab uses *Mycobacterium smegmatis* (*Msm*) as a non-pathogenic model organism to understand the role of *M. tuberculosis* in Tuberculosis (TB) and identify any potential weaknesses this organism may have. The proteostasis network is an important target for the development of novel drugs against mycobacteria. Recently, we identified the transcriptional regulator that controls sporulation – WhiA as a putative interacting protein of a novel component of the Clp protease, and hence is a crucial component of the proteostasis network in *Msm.* We hypothesize that the metabolic stability of WhiA is regulated by its interaction with this novel proteolytic system and as such this proteolytic system may regulate *Msm* sporulation. This project will use a range on molecular, biochemical and genetic approaches to study the interaction of WhiA with the novel Clp protease and analyse the consequences of this interaction.

Reference: Alhuwaider AAH & Dougan DA (2017). **AAA+ Machines of Protein Destruction in Mycobacteria.** *Front Mol Biosci*. 4: 49 doi: 10.3389/fmolb.2017.00049.

Project 2: Identifying the physiological role on the N-end rule in Mycobacterium smegmatis.

Co-supervisor: Dr Kaye Truscott (LIMS)

Previously our lab identified the recognition component of the N-end rule pathway in *Escherichia coli* - ClpS (Erbse *et al*., 2006 *Nature* 439: 753-6) and the physiological substrates of this pathway (Ninnis *et al*., 2009, EMBOJ 28: 1732-44). This project aims to identify novel components of the N-end rule pathway in *Msm* and as a result elucidate the physiological role of this pathway in mycobacteria. The project will examine the metabolic stability of several ClpS-interacting proteins that we have previously identified in *M. smegmatis*. It will involve the use of several *Msm* gene deletion strains, which lack specific components of the putative N-end rule pathway. The student involved in this project will gain experience in molecular biology, biochemistry and genetics.

Reference: Kirstein et al., (2009) **Adapting the machine: adaptor proteins for Hsp100/Clp and AAA+ proteases** *Nature Rev Micro*. 7: 589-599.

Associate Professor Mick FOLEY

SHARK ANTIBODIES AS HUMAN THERAPIES

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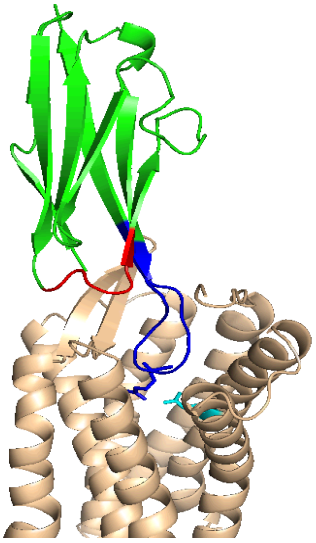
Subjects prerequisites: MED3LAB or MED3PRJ **Theme:** Infection & Immunity

Shark antibodies as therapeutic agents of disease

Co-supervisor: Dr Kate Griffiths (LIMS)



Shark antibodies (IgNARs) are a subset of antibodies found in sharks, rays and other cartilaginous fish. Some IgNARs have been shown to possess an elongated CDR3 loop, that is significantly larger those of human and murine antibodies. The IgNAR extended CDR3 loop is considered to be ideal for targeting cleft-type epitopes such as enzyme active sites and surface receptors which are otherwise inaccessible to conventional antibodies. Using Plasmodium falciparum as a model system we have identified peptides and shark antibodies that block invasion of malaria parasites into host erythrocytes. The structure of the complex of this IgNAR and its target revealed that the IgNARs penetrate a hydro- phobic trough on the malarial protein.

Recently we have created a humanized version of these antibodies and have identified antibodies from this library that bind to the chemokine receptor CXCR4. This molecule is up-regulated in many cancer cells and is an important target in fibrosis. We are therefore exploring the use of these antibodies in both cancer and fibrosis. These antibodies can bind to and block the growth of cancer cells as well as block inflammatory cells from migrating towards the site of inflammation. Moreover these antibodies can prevent the development of fibrosis in an animal model. This honours project will examine the mechanism of action of these antibodies in either cancer or fibrosis with a view to developing improved molecules to progress towards human clinical trials.

Dr David GREENING

EXTRACELLULAR VESICLES, EXOSOMES & IMPLANTATION

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Subjects prerequisites: MED3LAB or MED3PRJ **Theme:** Cancer

Extracellular vesicles in implantation

Extensive evidence suggests that the release of membrane enclosed compartments, more commonly known as extracellular vesicles (EVs), is a potent newly identified mechanism of cell-to-cell communication both in normal physiology and in pathological conditions. EVs contain diverse cargo including cell surface receptors, lipids, messenger RNAs (mRNAs), miRs, proteins and even DNA, and are identified by their size and the presence of cell surface markers such as the tetraspanins, CD9, CD81 and CD63. EVs are increasingly recognised as an important mode of cell-to-cell communication as they can transfer their contents to other cells thereby altering the recipient's behaviour.

Exosomes are a particular subtype of EVs that are secreted from a wide range of cells, including placental and endometrium cells. Exosomes are very stable vesicles that contain a broad spectrum of molecules, including proteins, mRNAs and miRNAs. Very little is known about this form of cell-to-cell communication in the context of ovarian follicular biology and implantation, but emerging data suggest that exosomes secreted by the blastocyst could influence gene expression and receptivity of endometrial cells thereby controlling its own implantation. Implantation involves intricate communication between embryos and the maternal endometrium. Increasing interest is centred on EVs and their contained cargo, particularly microRNAs (miRs), as important mediators of this dialogue. Recently, we have established a role for exosomes in cell-to-cell communication.

Project 1: Functional insights into EVs during human implantation

Co-supervisor: Dr Alin Rai (LIMS)

Actively released from cells, exosomes are tiny vesicles (50-150nm diameter) that transport cargo consisting of mRNA, miRNA and proteins, to recipient cells. These can be at a distance from the source of the exosomes which are taken up and their contents released to alter the behaviour of the recipient cells. Exosomes have been prepared from an human endometrial epithelial cell model and shown to contain select protein and miRNA cargo, some of which are not detectable in the cells of origin. We propose that these exosomes

play an important role in embryo-maternal communication at implantation. This project will utilise co-culture systems to further understand the important role that EVs play in this environment, how they target recipient cells, are internalised, and importantly alter the function of the recipient cell system to facilitate changes in implantation. EV (or EV specific cargo) is expected to functional modulate adhesive or invasive capacity of the trophectodermal cells

Project 2: Contribution of EV protein complexes to cell-cell communication

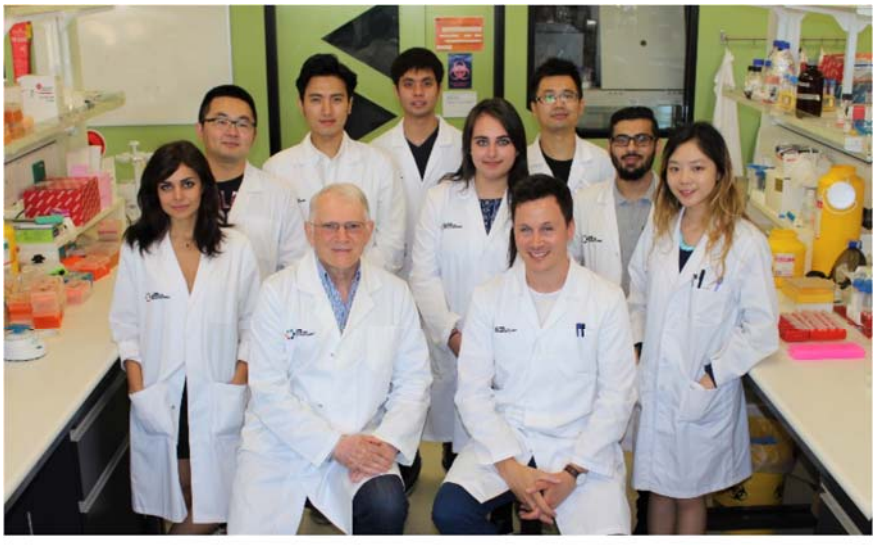
Co-supervisor: Dr Rong Xu, Prof Richard Simpson (LIMS)

Exosomes, small membrane vesicles of endocytic origin, are secreted by most cell types. Although functioning as powerful intercellular communicators, the identity of exosomal protein complexes (EPCs) and their specific components, together with the molecular mechanisms underlying their functions in recipient cells, remain unknown. This project focuses on the hypothesis that multiprotein complexes contained in cancer cell-derived exosomes play a crucial role in cell-cell communication and that perturbation of EPCs may affect the functionality of stromal target cells. This project will identify exosome protein complexes, their specific protein components and insights into their structural organization (i.e., core subunit interactions) and functionality in the context of cancer biology. Such complexes contained in cancer cell-derived exosomes play a crucial role in cell-cell communication and that perturbation of EPCs may affect target cell functionality and impact on exosome-targeted drug design. Use of innovative specialised techniques including immunoaffinity enrichment coupled to high-resolution mass spectrometry, cryo-lysis, nano-particle tracking, and high-resolution imaging will be employed.

References

[1] Extracellular Vesicles in Human Reproduction in Health and Disease. Endocrine Rev. (2018) doi: 10.1210/er.2017-00229

[2] Extracellular vesicles in cancer—implications for future improvements in cancer care Nature Reviews Clinical Oncology (2018) doi:10.1038/s41571-018-0036-9



Associate Professor Chris HAWKINS

APOPTOSIS & NECROPTOSIS RESEARCH

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Subjects prerequisites: MED3LAB, MED3PRJ or GEN3LAB **Theme:** Cancer

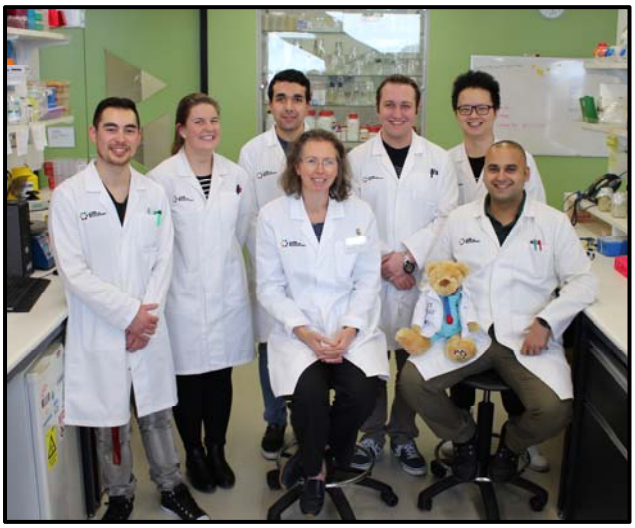
Our research focusses on two pathways through which surplus or dangerous cells can be eliminated: “apoptosis” and “necroptosis”. Research indicates that agents which selectively stimulate these pathways in malignant cells can effectively treat some cancers that do not respond to conventional therapies. Furthermore, direct engagement of apoptotic or necroptotic pathways may spare patients some unpleasant and sometimes lethal side effects associated with chemotherapy or radiotherapy. The Honours projects outlined below seek to enhance our understanding of cell death signalling pathways, and to exploit this knowledge to improve cancer treatment.

Project 1: Evaluating potential new drugs for sarcomas

We have recently obtained exciting data suggesting that members of two classes of drugs (“IAP antagonists” and “proteasome inhibitors”) may be useful for treating the bone cancer osteosarcoma. Experiments using cell culture techniques and mouse models will reveal whether these drugs may also be effective against other types of connective tissue cancers (sarcomas), that develop in the bones, muscle and neuronal tissues. Biochemical assays will examine the molecular mechanisms that determine sensitivity/resistance of sarcoma cells to these agents.

Project 2: Defining necroptotic signalling

Necroptosis is a relatively newly-identified form of programmed cell death, which can be triggered by immune cytokines or molecules derived from pathogens, and often occurs when apoptosis is blocked. Although the key effectors of this pathway have been identified, mechanisms regulating their activity are poorly understood. Microbiological and cell biology techniques will be used to define factors that determine necroptotic pathway activity.



Dr Begoña HERAS

BACTERIAL VIRULENCE FACTORS: STRUCTURE & FUNCTION

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Subjects prerequisites: MED3LAB or MED3PRJ **Theme:** Infection & Immunity

Bacterial virulence factors: structure and function

Bacterial resistance to antibiotics is increasing at an alarming pace with fears that we could return to the pre-antibiotic era where bacterial infections were virtually untreatable. There is an urgent need to increase our understanding of the mechanisms underlying bacterial pathogenesis to identify new targets for therapeutic intervention. In the Heras laboratory we are investigate the virulence mechanisms in Gram-negative bacteria in order to develop antibacterial drugs with novel modes of action.

Serine protease autotransporters of enterobacteriaceae (SPATEs): structure and function

Pathogens rely on an arsenal of virulence factors, to attach and infect their host. Autotransporter (AT) proteins are a major group of virulence proteins that bacteria use to establish highly persistent infectious diseases

In this project we will investigate a key AT subgroup, the Serine Protease Autotransporters of Enterobacteriaceae (SPATEs). These secreted trypsin-like serine proteases are associated with virulence functions such as colonisation, invasion and toxicity.

Crystal structure of the autotransporter adhesin Ag43

Two important SPATE proteins are SigA and Sat. Both are internalized cytotoxins present in pathogens like Shigella and *Escherichia coli* which cause diarrheal diseases and urinary tract infections and are among the most common infectious diseases of humans.

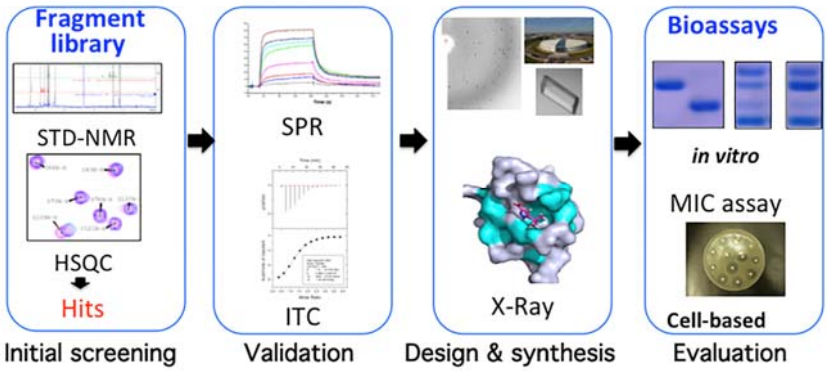
The outcomes of this research will contribute to a better understanding of the biology of Gram-negative pathogens. Furthermore, this work will provide valuable structure - function information that will be the all-important basis for the development of new and more effective antibacterial therapeutics, a subject of major significance given that these pathogens are becoming increasingly resistant to current antibiotics.

Application of a fragment based drug discovery approach for developing anti-neisserial agents

Collaborator: A/Prof Martin Scanlon (MIPS)

MDR pathogens include *Neisseria gonorrhoeae* the causative agent of sexually transmitted gonorrhoea, which was recently classified as one of the 'top urgent threats to global health, due to their increasing resistance to antimicrobials. The overall goal of this work is to develop narrow spectrum anti-neisserial therapeutics.

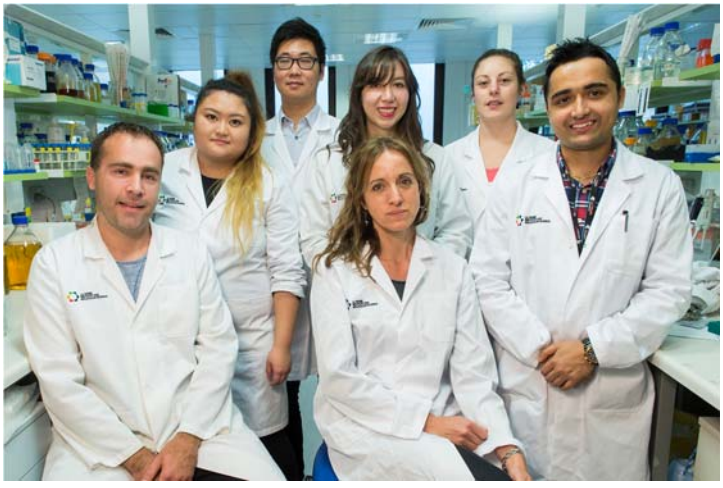
Bacteria contain periplasmic Disulfide Bond (Dsb) forming enzymes to catalyze the folding of many virulence proteins. These Dsb catalysts are are currently being investigated as potential targets for the development of antivirulence agents. DsbD,a member of the Dsb family is an essential enzyme for the viability of Neisserial pathogens.



Fragment-based drug discovery (FBDD) approach: Involves screening small fragments for the development of drugs

In this project a fragment-based drug design approach that combines NMR spectroscopy, X-ray crystallography and electron transfer assays will be employed to identify small molecules that bind to the catalytic domains of DsbD.

The binding mode and potency of these fragments will be investigated and their inhibitory activity tested using electron transfer in in vitro assays. Elaboration of these hit fragments will also be performed to obtain potent Neisserial DsbD inhibitors.



The ultimate outcome of this work is the development DsbD inhibitors as potential narrow spectrum antibiotics against Neisserial infections.

Professor Andrew HILL

NEURODEGENERATIVE DISEASES

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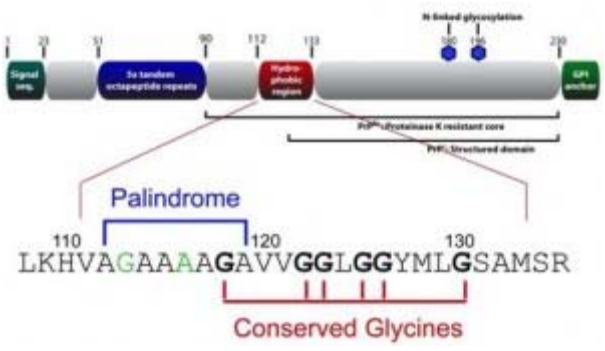
Subjects prerequisites: MED3LAB, MED3PRJ or GEN3LAB **Theme:** Neurobiology

Neurodegenerative diseases such as Alzheimer's and Parkinson's disease have an increasing prevalence amongst our ageing population. Recent estimates suggest the numbers of Australians suffering from dementia is set to double to 500,000 individuals by the year 2030. Many of these diseases are associated with the misfolding of certain proteins into aberrant forms that are found in the brain tissues of individuals with these diseases.

The Hill lab uses a combination of Biochemistry, Molecular and Cell Biology to investigate these diseases. We are interested in understanding the molecular mechanisms by which these proteins exert their neurodegenerative properties and in the case of prions, gain their infectious properties.

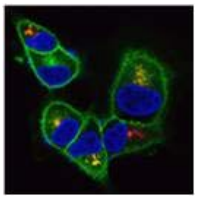
We are also interested in extracellular vesicles such as exosomes and microvesicles as vehicles for the transfer of misfolded proteins between cells. We also investigate the RNA content of these vesicles using next generation sequencing and have used this to develop potential diagnostics for prion and Alzheimer's diseases.

How do highly conserved regions of PrP control prion formation?



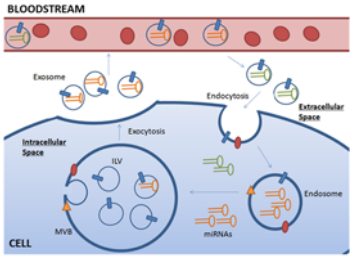
We are interested in understanding how the normal cellular isoform of the prion protein undergoes structural changes to become the disease associated form. We have developed several approaches to study this aspect of prion biology using cellular, molecular and biophysical techniques. We have refined a model based around a highly conserved region of the prion protein. This project will use a combination of structural and cell biological approaches to investigate the mechanism of prion inhibition through this conserved region of the prion protein.

Molecular mechanisms of Aβ toxicity in Alzheimer's disease - a role for PrP?



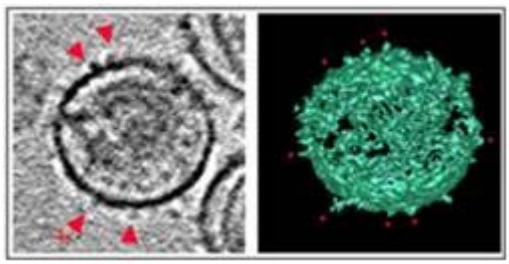
PrP can act as a receptor for Aβ oligomers which are implicated in the pathogenesis of Alzheimer's disease. Aβ is derived from a larger protein called the amyloid precursor protein (APP). We have well established cell lines expressing different forms of PrP and APP (which generate Aβ). These will be used to investigate the interactions of different forms of Aβ with PrP using a combination of biochemical and cell biological assays to determine the molecular mechanisms underlying this interaction.

Investigating the role of microRNA (miRNA) in neurodegenerative disorders



The role of microRNA in regulating the expression of key genes and pathways involved in neurodegenerative diseases are being investigated in both cell and in vivo models of these disorders. Using human clinical samples, we have identified a subset of serum microRNA biomarkers associated with various neurodegenerative diseases such as Alzheimer's disease. microRNA are enriched in exosomes and may regulate gene expression in target neuronal cells of the brain. These novel sequences are being tested for their functional effects and potential as disease biomarkers using cell models. This project will also determine whether exosomes assist in transporting microRNA through the tightly regulated blood-brain barrier to the peripheral system where they can be detected as a liquid biopsy. This project uses next-generation sequencing technologies, bioinformatics analyses, advanced molecular biology and cell biology techniques. These projects will be undertaken with co-supervision by Dr. Lesley Cheng, a Postdoctoral Researcher within Prof. Andrew Hill's Laboratory.

Extracellular vesicles in processing of proteins involved in neurodegenerative diseases



This project will investigate the mechanisms of exosome uptake by cells using modifiers of key cellular pathways and live cell imaging. This project will also investigate the role of modifiers in exosome biogenesis by altering their expression with RNAi, using a lentiviral delivery system in neuronal cells. We are also using high-resolution cryo-electron microscopy to study the structure of exosomes isolated from neuronal cells.

Are extracellular vesicles spreading toxicity in Parkinson's disease?

The synaptic protein α-synuclein is expressed throughout the brain, however genetic mutations in α-synuclein cause cell death beginning in dopamine-containing cells, which spread to other cell types late in the disease. This project will investigate if proteins, such as mutant α-synuclein, can be modified in one cell type, secreted in exosomes, and taken-up by other cells to spread toxicity in Parkinson's and other neurodegenerative diseases.

Dr Mark HULETT

INHIBITING TUMOUR PROGRESSION & INFLAMMATION

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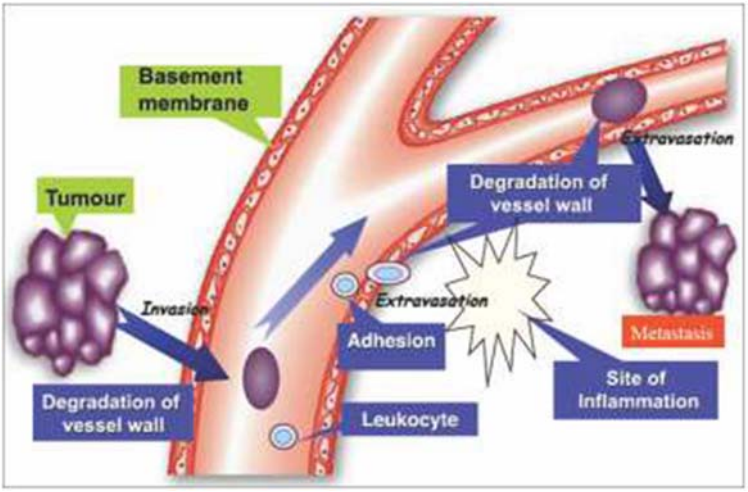
Subjects prerequisites: MED3LAB or MED3PRJ **Theme:** Cancer

Co-supervisors: Dr Ivan Poon, Dr Fung Lay, Dr Marc Kvansakul (LIMS)

Our research focuses on defining the molecular basis of tumour progression and inflammatory disease to develop novel anti-cancer and anti-inflammatory drugs. In consultation with prospective honours students we will "tailor" design projects that are appropriate to your interests under the following themes.

Heparanase function in tumour metastasis and inflammatory disease

The ability of malignant tumour cells to escape from primary tumour sites and spread through the circulation to other sites in the body (metastasis) is what makes cancer such a deadly disease. An essential process in metastasis is cell invasion - where tumour cells move into and out of the vasculature. Cell invasion is also a critical event in the migration of white blood cells of the immune system (leukocytes) to sites of inflammation to combat infections. The heparan-sulphate (HS)-degrading enzyme has been shown to play a key roll in the degradation of extracellular matrices and its activity strongly correlates with the metastatic capacity of tumour cells and the migratory capacity of leukocytes. We have shown that heparanase is the dominant HS-degrading enzyme in mammalian tissues, making it an attractive drug target. We are currently working towards (i) further understanding the molecular basis of heparanase function at the structural level, (ii) defining the dysregulation of heparanase gene expression in cancer and inflammatory disease, and (iii) using heparanase conditional knockout mice in disease models to define the precise role and contribution of heparanase in tumour progression and inflammation. Our overall goal is to better understand both the biology and



structure of heparanase to enable the development of specific inhibitors of the enzyme, which will lead to new drugs for the treatment of tumour metastasis and inflammatory diseases.

Innate defense molecules as anti-cancer agents

Defensins are innate immunity proteins involved in host protection against pathogens. We have identified a subfamily of defensins that show promise as anti-cancer agents and have an extensive programs dedicated to (i) defining the molecular basis of the anti-cancer activity of defensins using a range of biochemical and biophysical methods including live cell imaging, electron microscopy, X-ray crystallography and small-angle X-ray scattering; and (ii) in vivo testing and pharmacokinetic properties of defensins in mouse models of tumour growth and progression. See *Elife* (Cambridge). 2014; 3:e01808

Histidine-rich glycoprotein in necrotic cell/pathogen clearance and autoimmunity

Histidine-rich glycoprotein (HRG) is an abundant multi-functional plasma protein of vertebrates. We have shown HRG is a novel pattern recognition molecule that forms an adaptor complex with other innate immunity molecules to mediate the clearance of necrotic cells through phagocytes. Based on these observations we propose that HRG plays a key role in maintaining efficient clearance of necrotic cells from the circulation, a critical process of the innate immune system for the elimination of self-antigens to prevent autoimmune disease. In addition, we have observed striking similarities between the recognition of necrotic cells and pathogens by the innate immune system. We propose that the same molecular mechanisms are used to clear these potentially harmful materials and promote the resolution of tissue injury.

We are currently working on (i) defining the in vivo role of HRG in necrotic cell clearance using HRG deficient mice, (ii) investigating the role of HRG in pathogen recognition and clearance, (iii) defining the HRG complex for the recognition of necrotic cells and pathogens.

Professor Patrick HUMBERT

CELL POLARITY & TISSUE ARCHITECTURE

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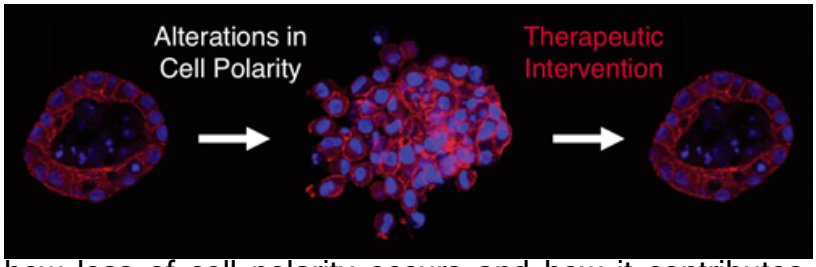
Subjects prerequisites: MED3LAB or MED3PRJ **Theme:** Cancer

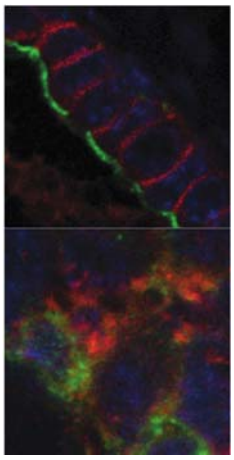
Topic 1: Cell Polarity and Cancer

In the Humbert Laboratory, researchers investigate the fundamental role of tissue organisation and asymmetry on cancer progression with the aim of identifying new therapeutic strategies.



Loss of the proper orientation of cells within a tissue, known as cell polarity, is one of the hallmarks of breast cancer and is correlated with more aggressive and invasive cancers. However how loss of cell polarity occurs and how it contributes at the molecular level to tumour formation remains unknown. Using a number of approaches such as RNAi screening, our laboratory has identified a network of cell polarity tumour suppressor genes that share a common function in modulating key oncogenic pathways.

Project Description: Cell Polarity Proteins and Cancer



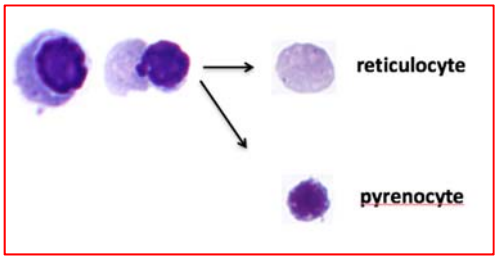
In this project, you will characterize how key polarity regulators from this gene network can supress oncogenic signalling at the proteome and phospho-proteome level. Using quantitative phospho-proteomics, you will reveal the transcriptional and phosphorylation events that are critical for tumour suppression by polarity genes. A variety of biochemical, cell biological and functional assays set up in our laboratory will allow you to delineate the precise molecular mechanisms by which these protein modifications can impact on cancer signalling and cell transformation. These studies include gene knockdown studies in 3D mammary organoid cultures and analysis of established genetically engineered mouse models of breast cancer.

The above experiments will provide essential information as to the requirement for intact

polarity signalling in breast cancer development and the signalling pathways regulated by the genes that control tissue organization to suppress invasion and tumour growth. Techniques: Cell culture, RNAi, Proteomics, Mass Spectrometry, 3D organotypic cultures, immunostaining and histological analysis.

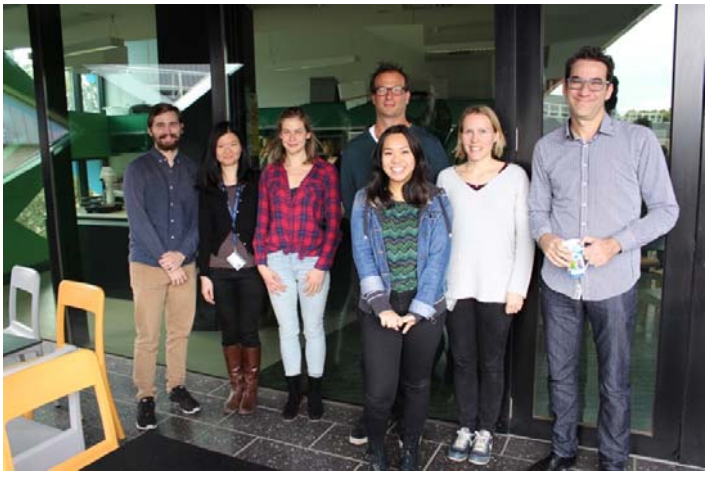
Topic 2: How the Red Blood Cell lost its Nucleus

Erythroid enucleation is the process by which the future red blood cell (RBC) disposes of its nucleus prior to entering the blood stream. Although the process of enucleation has been recognized for more than a century, the molecular and cellular programs governing it are still poorly understood. With a large proportion of cancer and surgical patients undergoing blood transfusions as part of their treatment, a major challenge for transfusion medicine is the constant difficulties in obtaining sufficient supplies of specific RBC subtypes. Despite exciting advances in the in vitro production of human red blood cells from hematopoietic, embryonic and induced pluripotent stem cells, the reduced ability of these cultured cells to fully enucleate remains a major hurdle. A better understanding of the enucleation process should lead to improved strategies for the efficient and rapid production of RBCs for autologous (i.e. self generated) patient transfusion.



Project Description: Exploring the role of CDK9 in erythroid enucleation

Our lab has identified a novel role for the serine/threonine kinase CDK9 in the enucleation process. How CDK9 regulates nuclear extrusion is completely unknown. This project aims to identify the phosphorylation targets and binding partners of CDK9 during erythroid enucleation using mass spectrometry approaches. You will initially use recombinant purified CDK9 protein to perform pull down experiments on protein lysates obtained from enucleating erythroblasts. Binding partners of CDK9 will be identified by mass spectrometry. In addition, phosphoprotein profiles will be established following inhibition and activation of CDK9 kinase activity in enucleating erythroblasts using mass spectrometry. Follow up work will include using confocal microscopy, ex vivo cell biology assays and biochemical techniques to validate potential CDK9 targets and binding partners. Together your studies will provide insights into how CDK9 regulates erythroid enucleation and help develop strategies to enhance the production of red blood cells in vitro for patient transplantation purposes.



Associate Professor Marc KVANSAKUL

HOST PATHOGEN INTERACTIONS

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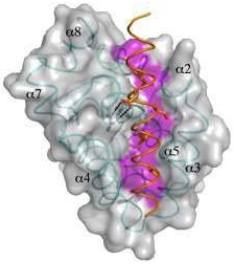
Subjects prerequisites: MED3LAB or MED3PRJ **Theme:** Molecular Imaging

Virus-mediated inhibition of programmed cell death



Programmed cell death or apoptosis is a critical process that allows removal of infected, damaged or otherwise unwanted cells. Failure to correctly control apoptosis plays an important role during pathogenic infections, autoimmune diseases and cancer. Using X-ray crystallography we aim to understand at the atomic level how certain viruses hijack the host cell's apoptotic machinery to ensure their own survival and proliferation. We are particularly interested in certain poxviruses as well as tumour viruses such as those from the herpesviridae. All projects in this area aim to express milligram quantities of these proteins and biochemically characterize them using isothermal calorimetry, surface plasmon resonance or additional functional assays. Once this is completed, we aim to crystallize these proteins in complex with ligands identified during the initial biochemical studies.

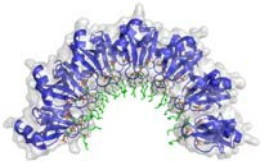
The role of LgL, a cell polarity gene, in tumour formation and metastasis

Collaborator: A/Prof Helena Richardson, Prof Patrick Humbert (LIMS)

Lethal giant larvae (Lgl) is a polarity gene that controls cell orientation and whose dysregulation contributes to the development of certain cancers such as prostate, lung, liver, colorectal, breast, ovarian, melanoma and glioblastoma tumours. Lgl works in concert with the three other proteins called Scribble (Scrib), Discs Large (Dlg) and Gukholder (GukH) as part of a multi-protein module called the Scribble module to define the polarity of a cell. Previous work by our collaborator A/Prof Richardson has shown that Lgl may function to control epithelial tissue formation. We now need to establish how Lgl and its partners contribute in this process and clarify their molecular mechanism of action. The Scribble polarity module has previously been shown to play a prominent role in tumour development, and an improved understanding of how the components of this module work together to control cell polarity may enable targeting of these proteins for therapeutic purposes. To achieve this, we aim to biochemically characterize the interactions between Lgl and Scrib, Dlg and GukH, and using X-ray crystallography, show in atomic detail how they perform their function.

Structural and functional studies defensin-mediated innate defence

Collaborator: Dr Mark Hulett (LIMS)



Defensins are small cationic proteins that are involved in innate immune processes in plants as well as humans. In plants, defensins have been shown to deliver significant resistance against plant pathogens such as fungi, however their precise molecular mechanism of action is currently not fully understood. We are interested in biomedical applications of these molecules, and together with the Hulett laboratory employ a strategy based on biophysical methods including X-ray crystallography, electron microscopy and small-angle X-ray scattering to understand their function.

Dr Mihwa LEE

GENE REGULATION & DNA DAMAGE

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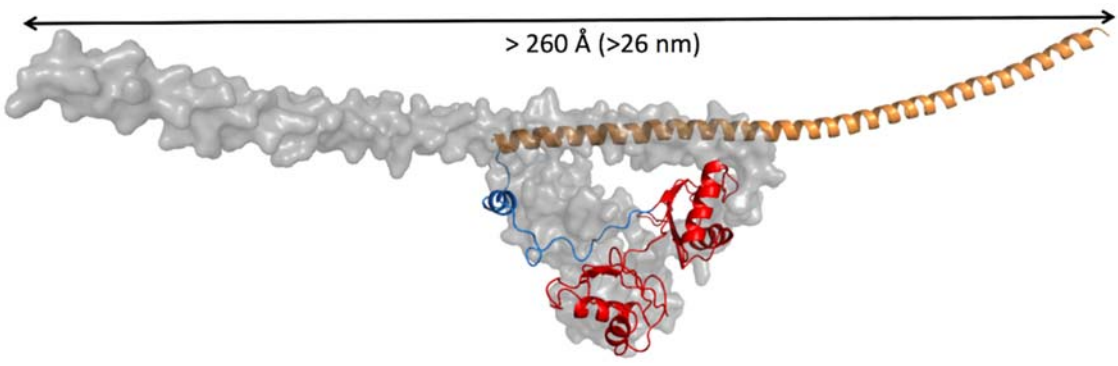
Subjects prerequisites: MED3LAB or MED3PRJ **Theme:** Molecular Imaging

How can we explain the complexity of the human species when we have a comparably similar number of genes as the simple roundworm, *C. elegans*? The attribute that distinguishes humans from the less complex organisms is not the number of genes itself but the regulation of gene expression. More complex gene regulation, therefore, is required for higher organisms and aberrant regulation of gene expression leads to various disorders such as developmental abnormalities, cancer and metabolic disorders.

My laboratory's research interests lie in the area of gene regulation and DNA damage repair pathway. We are particularly interested in characterising the macromolecular complexes (protein-protein and protein-nucleic acid complexes) in the nucleus to understand their roles in gene regulation and DNA damage repair pathway.

SFPQ, multifunctional nuclear protein

Splicing factor proline-glutamine rich (SFPQ) is an abundant and essential nuclear protein involved in various aspects of gene expression by interacting with DNA, RNA, and other protein partners. The resulting biological implications are wide ranging, including neural cell development, regulation of circadian rhythm, regulation of viral expression and tumour repression and progression. Capitalising on the recent success in determination of the structure of the core domain of human SFPQ (Figure below), the current research in our laboratory aims to understand the multifunctions of SFPQ by characterising the complexes of SFPQ-interaction partners.



Understanding dual nucleic acid specificities of SFPQ in reversible gene regulation

SFPQ has been reported to repress the transcription of several genes via direct binding to the promoter regions. Despite the presence of two RNA recognition motifs, the cognate RNA sequences of SFPQ are yet to be identified. However, several long noncoding RNAs (lncRNAs) that interact with SFPQ have been identified in the recent reports on the reversible regulatory roles of SFPQ. In the proposed mechanism of reversible gene regulation, the transcription repression exerted by SFPQ via direct binding to the promoter region can be reversed by its interactions with lncRNAs. This project aims to solve the structure of SFPQ in complex with nucleic acids to provide the structural basis of the reversible gene regulation.

Identification and characterisation of protein-interaction partners of SFPQ

SFPQ, first identified as a splicing factor, is a multifunctional nuclear protein that has been implicated in a variety of nuclear processes including RNA biogenesis (transcription coactivation, corepression, splicing and transcriptional termination) as well as DNA repair. How can SFPQ carry out all of these diverse nuclear functions? It appears that SFPQ has many different protein interaction partners directly and indirectly at different stages of gene regulation; although the list of interaction partners is extensive, most of these characterisations have been carried out at the cell-biology level, thus, the detailed molecular mechanisms of interaction and its consequences are yet to be characterised. This project aims to identify and characterize the interaction partners of SFPQ using yeast two-hybrid library screening, with the ultimate goal of determining the structure of SFPQ and its interaction partners in complex to unveil the action mechanisms of the complexes at the molecular level.

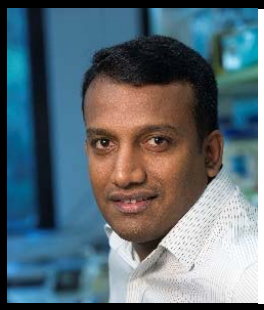
Understanding the roles of SFPQ in DNA damage repair pathway

SFPQ has been reported to be play a role in DNA repair by direct interaction with the homologous recombinase, Rad51 and modulates its homologous-pairing and strand-exchange activity. The recruitment of SFPQ to DNA damage sites has been shown *in vivo* and the attenuation of SFPQ expression sensitises the cells to ionising radiation. Interestingly, the region of Rad51 required for SFPQ binding overlaps with that required for interaction with BRCA2, the breast cancer susceptibility gene 2. This observation leads to the working hypothesis that SFPQ may compete with BRCA2 for interaction with Rad51 and that SFPQ may be an important regulator to modulate the interaction between Rad51 and BRCA2. This project will focus on the production of SFPQ-Rad51 and SFPQ-Rad51-BRCA2 complexes, and the biophysical and structural characterisation of these complexes

Techniques used in our laboratory

The main technique to be used in our laboratory is X-ray crystallography, complemented by molecular biology techniques (cloning and mutagenesis), protein overexpression and protein purification. Other biophysical techniques employed in the laboratory include small-angle X-ray scattering in collaboration with Dr. Andrew Whitten at Australian Nuclear Science and Technology Organisation (ANSTO), microscale thermophoresis (MST), and electrophoretic mobility shift assay (EMSA). *In vivo* macromolecular interaction is currently being explored using yeast two-hybrid system in collaboration with A/Prof. Christine Hawkins in the Department.

Associate Professor Suresh MATHIVANAN



OVERCOMING DRUG RESISTANCE IN CANCER

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Subjects prerequisites: MED3LAB or MED3PRJ **Theme:** Cancer

Project 1: OVERCOMING DRUG RESISTANCE IN CANCER

Cancer is one of the leading causes of death worldwide. Currently, chemotherapy is the mainstay in the treatment of advanced cancer. However, cancer cells become resistant to the drugs by both known (Fig. 1) and unknown mechanisms severely limiting the use of chemotherapeutic drugs. This multidrug resistance mechanism affects patients with a variety of cancers and solid tumors, including colon, breast, prostate, ovarian and lung cancers. Recent studies reveal the association of drug resistance with epithelial-mesenchymal transition (EMT), a process by which adherent epithelial cells convert to motile mesenchymal cells. In this project, we will develop drug resistant colorectal cancer cell lines by continuously culturing them with 5-fluorouracil. Furthermore, we will perform functional analysis of the parental drug sensitive and resistant colorectal cancer cell lines to understand the molecular pathways that are regulated in the drug resistance process. Gene knockout of key genes will be performed to understand their role in conferring drug resistance. A range of molecular biology and biochemical techniques including CRISPR gene knockout assays, PCR, mass spectrometry, Western blotting, immunoaffinity assays, microscopy and luciferase assay will be employed.

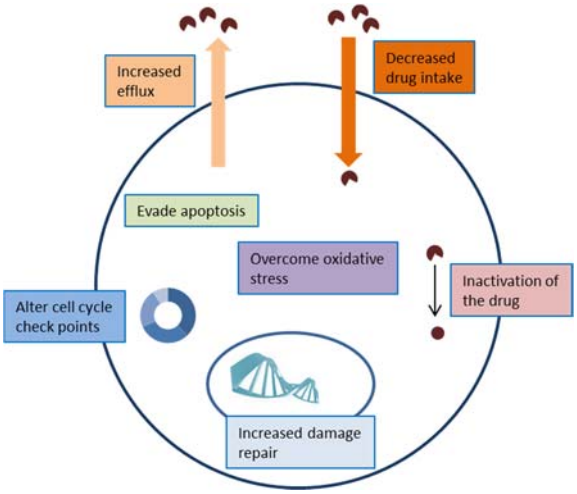

Fig. 1. Schematic representation of various mechanisms that regulate chemotherapeutic resistance in cancer cells.

Project 2: CANCER AND THE TUMOR MICROENVIRONMENT

Bowel cancer is the most common form of cancer in Australia with 12,000 people diagnosed each year. Of these, more than 4,000 people will die of their disease. Most often, bowel cancer is diagnosed at an advanced stage when the cancer has already spread to other parts of the body lowering the patient survival rate. For tumors to progress, bidirectional crosstalk between different cells occurs within the tumor and its surrounding supporting tissue. Stromal elements include the extracellular matrix (ECM) as well as other cell types that are activated and/or recruited to the tumor microenvironment such as fibroblasts, immune and inflammatory cells, fat cells and endothelial cells of the blood and lymphatic circulation. Recent literature indicated that all aspects of cellular tumorigenicity are profoundly influenced by reciprocal interactions between responding normal cells, their mediators, structural components of the ECM, and genetically altered neoplastic cells. In this project, the role of the secreted proteins in the progression of cancer will be studied. A range of molecular biology and biochemical techniques including gene knockdown assays, PCR, mass spectrometry, Western blotting, sucrose gradient centrifugation, immunoaffinity assays, microarray, microscopy and luciferase assay will be employed.

Associate Professor Robyn MURPHY

SKELETAL MUSCLE BIOCHEMISTRY

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Subjects prerequisites: MED3LAB, MED3PRJ or GEN3LAB **Theme:** Neurobiology

My overall research interest is in the area of skeletal muscle in health and disease. The research of my group focuses on various aspects of skeletal muscle biochemistry in health and disease, using exercise and disease models in humans, as well as animal models. In particular, we measure proteins in segments of individual fibres allowing issues with the heterogeneity of skeletal muscle to be overcome. We also examine movement of proteins following micro-dissection of fibres, allowing us to quantitatively assess the redistribution of proteins following various interventions.

Effect of age and fibre-type on proteins related to autophagy in skeletal muscle function
Co-supervisor: Dr Stefan Wette, Postdoctoral Researcher, Dept of Biochemistry and Genetics
Ageing is a natural process of life; which has inevitable consequences on the quality of both skeletal muscle mass (sarcopenia) and muscle strength/function (dynapenia). Both sarco/dynapenia impair gait and increase the likelihood of a fall by an older adult. Muscle is heterogenous in nature, and its fibre type composition is dependent on age, disease state or fitness of a person.

In the elderly, Type II fibres are substantially affected, whereby there is a reduction in the number of these fibres compared with Type I fibres, along with them being weaker than Type I fibres, leading to muscle atrophy. Further, many proteins important for muscle metabolism, contractility and growth are fibre-type specific. Therefore the analysis of proteins in single fibres allows a thorough investigation of these proteins.

In this project you will assess the abundance of various proteins related to autophagy in skeletal muscle obtained from both young and older healthy individuals. It is hypothesised that the most change will be in the Type II fibres of older adults but not in Type I.

This project will see you learning to dissect single fibres from human muscle samples, to use a new approach for rapid and efficient fibre typing of each individual fibre and then to use a specialised and highly sensitive quantitative Western blotting technique developed in the Murphy lab to determine the abundance of various autophagy-related proteins. This will be

in addition to possibly utilising whole muscle tissue preparation, cryosectioning of tissue and immunofluorescence techniques, which will be driven by the particular research question you wish to address. Time-dependent, you will develop an enzymatic assay for use in the single muscle fibres.

Other projects are possible by discussion with Robyn. There are many possibilities that fall under the overall research objectives of the group. These could include animal or human studies.

Dr Jacqueline ORIAN

NEURODEGENERATION AND NEUROREPAIR

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Subjects prerequisites: MED3LAB, MED3PRJ or GEN3LAB **Theme:** Neurobiology

The major focus of the laboratory is multiple sclerosis (MS), an autoimmune disease of the central nervous system (CNS) characterized by infiltration of immune cells, demyelination and neuronal and axonal loss. The cause of the disease is unknown and current treatments are of limited benefit. The projects below relate to the development of improved animal models for the disease and of imaging approaches to investigate the role of platelets, which are key elements in the development of the autoimmune process.

Project 1. Development of a B cell driven model for multiple sclerosis.

Emerging data from human and animal model studies show that B cells play an early and critical role in the autoimmune disease multiple sclerosis (MS). It is also evident that in this disease, B cells infiltrate the central nervous system (CNS) and survive in niches, thereby becoming resistant to treatment whilst continuing to proliferate, generate antibodies and cause tissue destruction. To gain an insight into when and how B cells enter the CNS and survive in this environment, we have generated a new variant of the experimental autoimmune encephalomyelitis (EAE) MS model, in which the autoimmune process is driven by B cells. Elevated B cell numbers were detected in blood and secondary lymphoid tissues at the peak of disease, together with lesions (or areas of focal damage) in multiple regions along the CNS. The most prominent site of B cell and Ig accumulation was along the central canal (Fig. 1). Also significant, was the observation of Ig producing cells in a major blood vessel running along the whole length of the spinal cord, suggesting a route of entry into the CNS. This model, therefore, may facilitate identification of mechanisms by which Ig production persists in MS despite apparently efficient B cell targeting therapeutics and may lead to the generation of improved MS treatments. The specific aims of the project are to further characterize the B cell driven EAE variant:

Aim 1. Identification of the timing of earliest entry of cells of the B lineage in the spinal cord.
Aim 2. Identification of a route of entry into the CNS for B cells.
Aim 3. Identification of potential niches for B cell survival and proliferation over the disease trajectory.

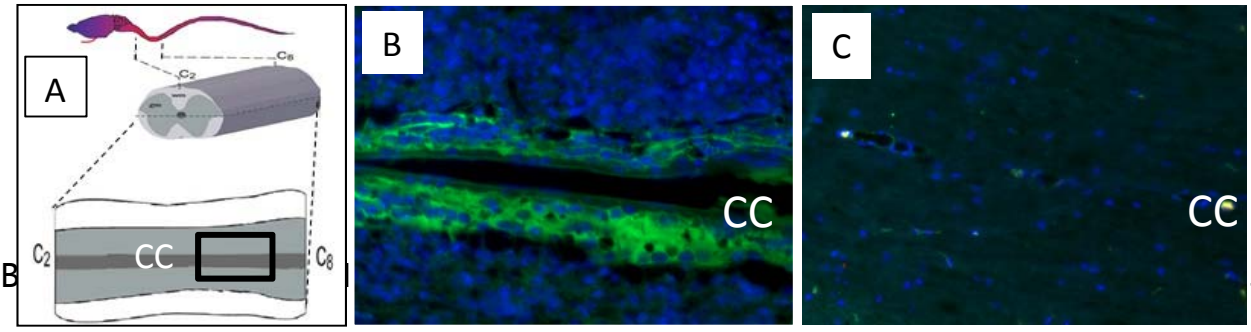

Fig. 1. Ig production along the central canal (cc): *A is a representation of the mouse CNS and dissection of the spinal cord; boxed area is shown in B and C. B shows immunodetection of Ig along the central canal by severe EAE and C absence of Ig in control tissue.*

Project 2. Detection and monitoring of neuroinflammation by FLECT technology. *(In collaboration with Prof. Karlheinz Peter, Baker Heart and Diabetes Institute, Melbourne).*

Molecular imaging facilitates the identification and targeting of cellular processes, as well as monitoring of disease progression. The Peter laboratory has demonstrated the potential of this approach with novel imaging agents with high specificity and sensitivity to platelets, generated by conjugation of an activation-specific anti-GPIIb/IIIa integrin single chain antibody (scFv GPIIb/IIIa) to a positron-emitting ^{64}Cu. These have been successfully applied to the detection of myocardial infarcts and inflammatory diseases such as rheumatoid arthritis, in animal models. A collaboration with the Orian laboratory, using in the EAE model demonstrated (a) platelet accumulation and its timing prior to inflammatory cell accumulation, (b) a cause-and-effect relationship between platelet accumulation and disease development and (c) preclinical detection of activated platelets by PET imaging in the eye (Fig. 2A) coincident with platelet leakage from retinal blood vessels (Fig. 2B, C). These findings represent a change in paradigm regarding the involvement of platelets in neuroinflammation, but importantly, suggest a potentially therapeutically targetable mechanism to battle multiple sclerosis, with broader implications for inflammatory diseases.

With advances in optical technology and fluorescence probe design, our unique agents have been developed as fluoroprobes providing higher stability and sensitivity, without radiation risks. This project will combine FLECT and immunopathology to test the hypothesis that early targeting of platelets will result in disease amelioration in EAE.

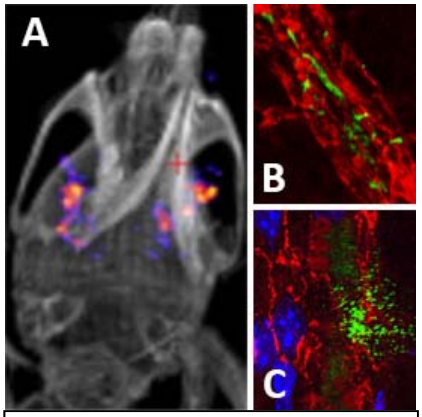

Fig. 2. Preclinical platelet detection: *(A) = PET imaging; B & C = immunodetection of platelets in sham-(B) and of platelet leakage in EAE (c).*

Aim1. A longitudinal study of lesion development by FLECT;
Aim2. Confirmation of the accuracy and sensitivity of FTECT imaging by quantitative immunopathological techniques;
Aim3. FLECT and immunopathological disease evaluation in the presence of anti-platelet therapy.

Dr Belinda PARKER

CANCER, MICROENVIRONMENT & IMMUNOTHERAPY

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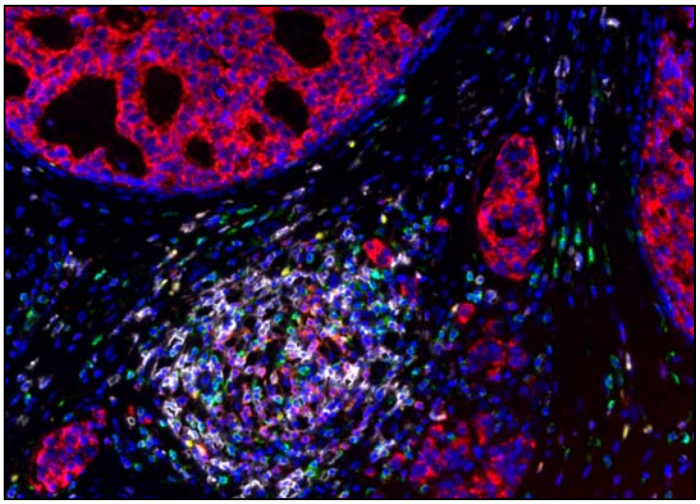
Subjects prerequisites: MED3LAB or MED3PRJ **Theme:** Cancer

Breast and prostate cancer are the 2nd leading causes of cancer-related death in women and men, respectively. Many aggressive cancer subtypes rely on untargeted therapies, such as chemotherapy. Our laboratory has identified key immune pathways in cancer cells that, when intact, predict response to such therapies and a much better patient survival. Recent work in our laboratory is centred around profiling the immune landscape in breast and prostate cancers to predict more aggressive cancer subtypes and to dissect the mechanisms by which cancer cells can go undetected in the body to form deadly metastases in other organs. We are also trialling new therapies aimed at switching the anti-cancer immune response back on to block this deadly spread.

We are offering 2 projects that aim to further our understanding on how the body's reaction to cancer cells can provide important information on how an individual cancer is likely to behave and what treatments are going to be most beneficial for blocking cancer spread.

Project 1: Visualising the immune landscape in metastatic cancers

This project will use a recently developed technology that allows visualisation of multiple immune cell markers on a single slide of cancer tissue to profile primary cancers and matched metastases in lung and bone to determine the immune cells that associate with metastatic cancers and if changes to the immune landscape in cancers can predict the risk of cancer spread. This will be combined with detailed flow cytometry profiling of immune cell numbers and activation in the blood, bone marrow and in primary tumour and metastases. We have already optimised this multicolour imaging technology in the laboratory (as illustrated in this image).



Project 2: Predicting immunotherapeutic response in breast cancer

Co-supervisors Dr Belinda Parker and Dr Damien Zanker

This project will expand on recent published work in the laboratory to develop immune markers to predict response to immune therapies. The use of immune checkpoint inhibitors has been very successful in some cancers (such as melanoma) but not in other cancers such as breast cancer. We have discovered that the expression of immune proteins called type I interferons (IFNs) in cancer cells is important for promoting an anti-cancer immune response. We have also recently published the benefit of combining IFN-inducing agents with checkpoint inhibitors to treat breast cancer. This project will dissect the key immune responses that are switched on by IFN-based therapies and how such responses can predict response to checkpoint inhibitors and conventional therapy (such as chemotherapy). This is an important project that aims to link in to future clinical trials that utilise novel IFN inducers in patients with aggressive types of breast cancer.

Techniques to be utilised:

Both projects will incorporate a wide range of techniques including histology, multiplex immunohistochemistry, flow cytometry, models of cancer, mouse dissections, tissue disaggregation, and other routine molecular biology techniques.

Related publications from the Parker Laboratory:

- Tumor inherent interferons: Impact on immune reactivity and immunotherapy. *Cytokine*, (2018).
- Neoadjuvant Interferons: Critical for effective PD-1 based immunotherapy in TNBC, *Cancer Immunology Research,* 5(10): 871-884, (2017).
- Antitumour actions of type I interferons: implications for cancer therapy, *Nat Rev. Cancer,* 16(3): 131-44, (2016).
- Silencing of Irf7 pathways in breast cancer cells promotes bone metastasis via immune escape, *Nat Med.,* 18(8): 1224-31, (2012).

Associate Professor Helena RICHARDSON

TISSUE ARCHITECTURE & COOPERATIVE TUMOURIGENESIS

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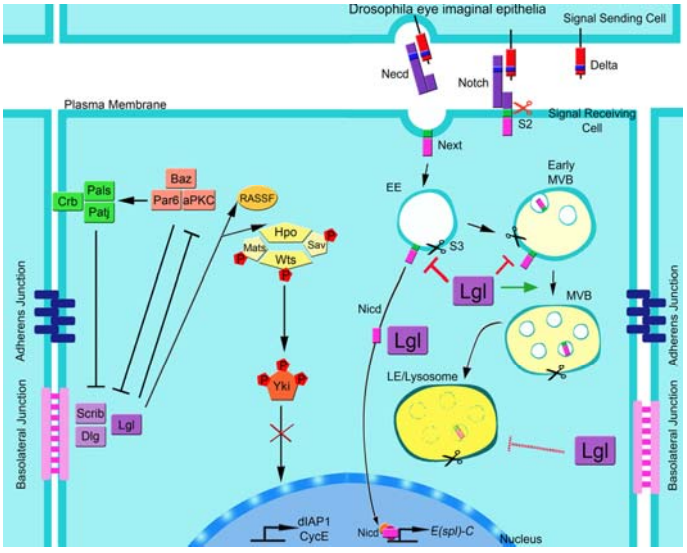
Subjects prerequisites: MED3LAB or MED3PRJ **Theme:** Cancer

Our lab uses sophisticated genetic and cell biological analysis of the vinegar fly, *Drosophila,* model, to address the fundamental questions of how cell shape regulators control signalling pathways, cell proliferation, survival and differentiation, and how their deregulation leads to cancer in epithelial tissues. We are also interested in the interaction of the tumour with the surrounding normal cells (the tumour microenvironment), how the innate immune system interacts with the mutant tissue in either eliminating the mutant cells or promoting tumourigenesis.

The regulation of tissue architecture and growth:



The correct structure of epithelial tissues (including the skin, and linings of internal organs, such as the ducts of the mammary gland, prostate gland and pancreas) is critical for cell function. The structure of cells in these epithelial layers is regulated by the evolutionarily conserved apico-basal cell polarity complexes, the Scrib, Par and Crumbs modules. These complexes act in a mutually antagonistic manner to define apical and basal-lateral membrane domains and position the adherens junction (AJ) and tight junction (TJ), which are important for cell-cell adhesion and communication. Mutants in the Scrib module (comprised of Scrib (Scribble), Dlg (Discs-large) and Lgl (Lethal-giant-larvae) were original discovered in *Drosophila* as neoplastic tumour suppressor genes, which regulate tissue architecture and growth. We have discovered that mutants of Scrib, Dlg or Lgl, affect cell proliferation and survival by modulating signalling pathways. Lgl regulates the Hippo negative tissue growth pathway by controlling the localisation of the pathway components, Hpo and Rassf, and also regulates Notch signalling by affecting the acidification of endoctyic vesicles, which is required for ·-secretase-mediated activation of Notch (S3 cleavage) (**Figure 1**). By contrast, Scrib and Dlg control Ras-MAPK signalling. In order to investigate how Scrib and Dlg control cell polarity and signalling, we are collaborating with Drs Marc Kvansakul (LIMS) and Patrick Humbert (LIMS) to investigate the Scrib protein network using biochemical and genetic approaches.

Figure 1 – Regulation of the Hippo and Notch pathways by Lgl

Cooperative tumourigenesis: When Scrib, Dlg or Lgl are mutated in patches (clones) of cells within *Drosophila* epithelial tissues, despite increased proliferation, they do not overgrow as cells at the clonal borders are removed by apoptosis mediated by recruitment of innate immune response macrophage-like cells (hemocytes) and induction of the TNF-JNK stress response pathway, a process termed "cell-competition". Various signalling pathways are involved in initiating cell competition, but precisely how this occurs in *scrib, dlg* or *lgl* mutant tissue is unclear. When an oncogene, such as Ras^{V12}, is co-expressed in *scrib, dlg* or *lgl* mutant cells, apoptosis is blocked and massive invasive tumours (marked by green fluorescent protein, GFP) are induced. In this context, the innate immune response acts to promote tumourigenesis. In collaboration with Drs Marc Kvansakul (LIMS) and Patrick Humbert (LIMS) we are using this cooperative tumourigenesis model to screen drug libraries to identify novel bioavailable, tumour-specific anticancer compounds and investigate their mode of action. Moreover, in order to understand the mechanism of tumourigenesis, we have undertaken loss- or gain-of-function genetic screens to identify novel genes that act similarly to mutants in the *scrib* module to promote Ras^{V12}-driven tumourigenesis (**Figure 2**). Amongst the genes identified as contributing to tumourigenesis in these screens, were those involved in actin cytoskeleton, signalling, protein trafficking, metabolism, and epigenetic regulation. We now seek to investigate the mechanism by which these genes promote tumourigenesis and how they are involved in the function of the Scrib module.

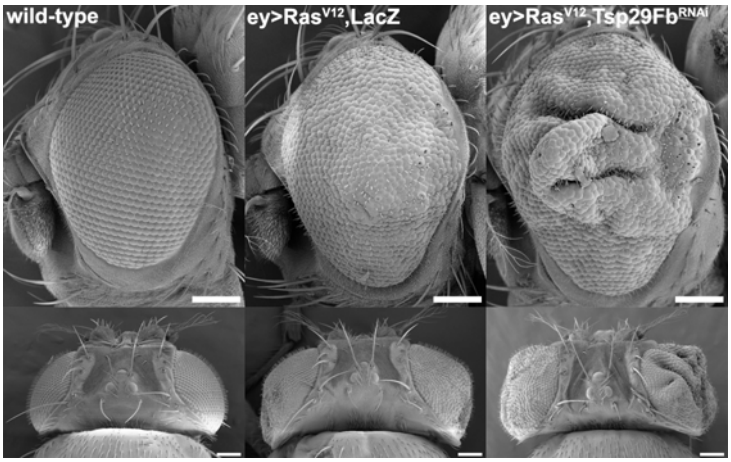


Figure 2 – An example of a gene that acts as tumour suppressors in cooperation with RasV12, identified in our genetic screen

Project 1: How cell polarity regulators affect signalling pathways

The project will investigate the question of how Lgl affects the Notch and Hippo signalling pathways in *Drosophila.* From our Mass Spec analysis of *Drosophila* Lgl protein interactors, several endocytosis regulators have been identified. This project will investigate the importance of these proteins in Lgl's regulation of these signalling pathways, by utilizing *in vitro* physical interaction and *in vivo* genetic-cell biology approaches.

Project 2: The role of novel tumour suppressors in Ras-driven tumourigenesis

This project extends from our genetic screen for novel tumour suppressors that cooperate with Ras^{V12} to promote invasive overgrowth of neural-epithelial tissue. It will utilize sophisticated *Drosophila* genetics, and molecular, cell biology and biochemical approaches to determine how one of these novel tumour suppressors affects the hallmarks of cancer in *Drosophila*. The project will include analysis of how the tumour suppressor affects signalling pathways, cell proliferation, apoptosis, differentiation and recruitment of the cellular innate immune response, and whether it genetically and physically interacts with the Scrib module.

Both projects will suit those who enjoy working on genetically-tractable model organisms and are interested in a holistic understanding of cancer. These projects will encompass a wide range of cell biology (microscopy), biochemistry, genetics and molecular biology techniques.

Professor Richard SIMPSON

CANCER SECRETOME & EXTRACELLULAR COMMUNICATION

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Subjects prerequisites: MED3LAB or MED3PRJ **Theme: Cancer**

Extracellular vesicles in cancer

The sustained growth, invasion, and metastasis of cancer cells depend upon bidirectional cell-cell communication within complex tissue environments. Such communication predominantly involves the secretion of soluble factors by cancer cells and/or stromal cells within the tumour microenvironment (TME), although these cell types have also been shown to export membrane- encapsulated particles containing regulatory molecules that contribute to cell-cell communication. These particles are known as extracellular vesicles (EVs) and include species of exosomes and shed microvesicles. EVs carry molecules such as oncoproteins and oncopeptides, RNA species (for example, microRNAs, mRNAs, and long non- coding RNAs), lipids, and DNA fragments from donor to recipient cells, initiating profound phenotypic changes in the TME. Emerging evidence suggests that EVs have crucial roles in cancer development, including premetastatic niche formation and metastasis. Cancer cells are now recognized to secrete more EVs than their nonmalignant counterparts, and these particles can be isolated from bodily fluids. Thus, EVs have strong potential as blood- based or urine- based biomarkers for the diagnosis, prognostication, and surveillance of cancer

PROJECT 1 Defining extracellular vesicles from blood plasma & other biofluids

Co-supervisor: Dr David Greening, Dr Rong Xu

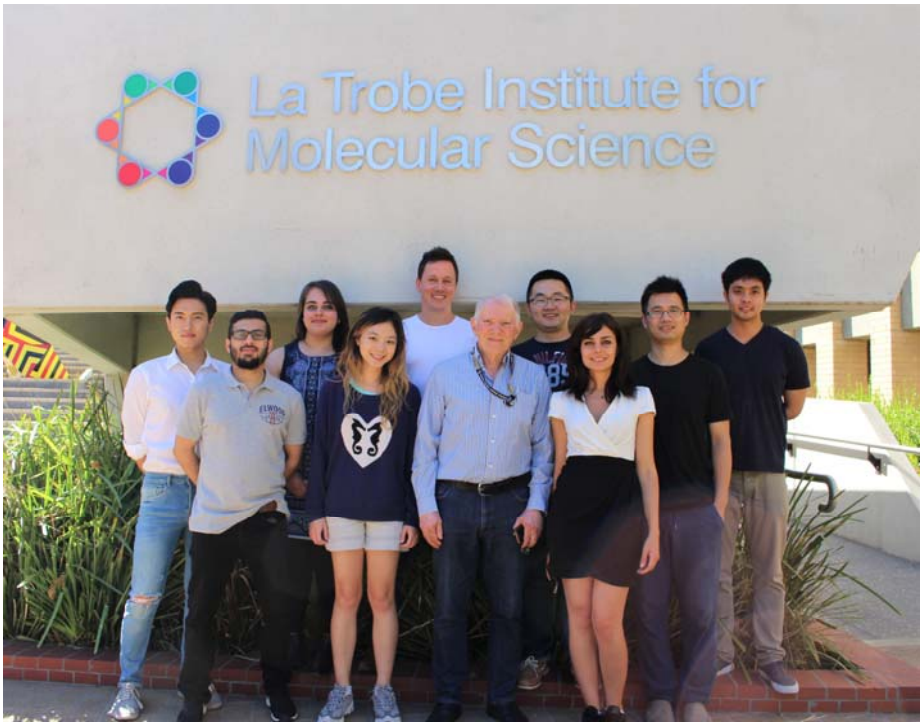
Plasma and other body fluids contain diverse membranous extracellular vesicles (EVs) populations. EVs participate in physiological and pathological processes and have potential applications in diagnostics or therapeutics. Knowledge on biofluid EVs is, however, limited, mainly due to their sub-micrometer size and to intrinsic limitations in methods applied for their characterization. The aim of this project is to establish a comprehensive description of EVs from plasma, utilising distinct methods for isolation, characterisation to define unique differences in distinct EV subpopulations. Use of innovative specialised techniques including immunoaffinity enrichment coupled to high-resolution mass spectrometry, nano-particle tracking, fluorescent labelling and high-resolution imaging will be employed. Discrimination

between subpopulations of EVs will therefore be of great importance for studies on EV biology and function, and assist in the development of exosome-based diagnostics and therapeutics.

PROJECT 2 Understanding the contribution of extracellular vesicles (EVs) in the context of epithelial-mesenchymal transition

Co-supervisor: Dr Alin Rai, Dr David Greening (LIMS)

The metastatic cascade describes the process by which tumour cells escape their primary site and colonize secondary locations. Tumour angiogenesis facilitates passage, and cells at the leading edge of the primary tumour are thought to undergo epithelial-mesenchymal transition (EMT) to acquire increased motility and invasiveness. Whether oncogenic cells that have undergone EMT directly promotes endothelial cell recruitment remains largely unknown, and the role of extracellular vesicles (EVs) (30-1,000 nm diameter) in this process has not yet been definitively explored. Conventional biological assays for cell proliferation, motility, migration, and invasion are already established in our lab. Using global profiling approaches, including proteomic (mass-spectrometry based profiling) and genomic (miR/mRNA profiling) analyses, we intend to catalogue and identify the contribution of EVs during EMT, especially induced expression of signalling pathway receptors/ modulators in recipient cells to modify their function. We currently employ a multi-omics and multidiscipline approach integrating cancer biology, molecular biology, genomics, proteomics, and bioinformatics to explore EVs and their functions. Preliminary results demonstrate that oncogenic cells undergoing EMT can communicate with endothelial cells via specific EVs, and establish angiogenic promoters that may function during the initial stages of metastasis.



Dr Tatiana SOARES DA COSTA

ANTIBIOTIC & HERBICIDE DISCOVERY

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Subjects prerequisites: MED3LAB or MED3PRJ **Theme:** Infection & Immunity

Overview of research: The overall focus of our laboratory is to examine the structure and function of essential proteins in bacteria and plants to guide the development of new classes of antibiotics and herbicides, respectively.

Project 1: Development of new classes of antibiotics against multi-drug resistant Gram-negative bacteria.

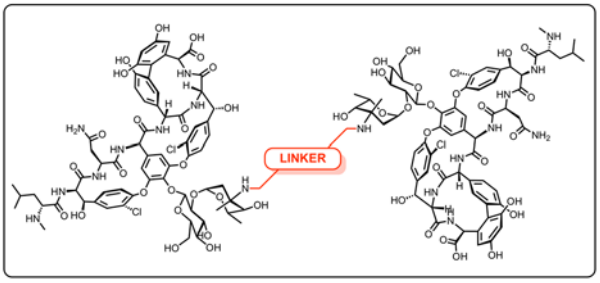
Co-supervisors: A/Prof Matthew Perugini (LIMS) and Dr Santosh Panjikar (Australian Synchrotron)

This project focuses on dihydrodipolinate reductase (DHDPR, pictured), which is an essential bacterial enzyme involved in cell wall and protein syntheses. Specifically, the project involves (i) determining the 3-dimensional structure of DHDPR from the superbug *Acinetobacter baumannii* using X-ray crystallography, (ii) screening for drug-like compounds targeting the active site of DHDPR *in silico*, and (iii) testing the *in vitro* and *in vivo* efficacy of compounds.

Project 2: Reengineering vancomycin to combat bacterial resistance

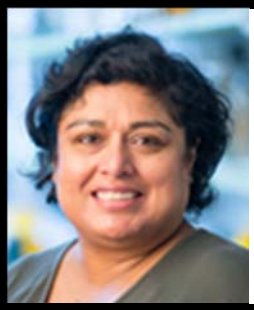
Co-supervisor: Dr Andrew Barrow (Chemistry, LIMS)

This project focuses on characterising the antibiotic properties of a library of vancomycin dimers that have been developed using innovative click chemistry. Specifically, the project involves (i) determining the minimum inhibitory concentrations of compounds against methicillin-resistant *Staphylococcus aureus* and vancomycin resistant *Enterococci*, (ii) examining the mode of action using time-kill assays, (iii) assessing their toxicity in mammalian cells, and (iv) defining the potential and frequency for resistance to emerge with the dimers compared to vancomycin.



The projects in our laboratory encompass a wide range of biochemical and biophysical techniques, including recombinant protein expression & purification, enzymology, analytical ultracentrifugation, circular dichroism spectroscopy, microscale thermophoresis, structural biology, surface plasmon resonance, *in silico* docking, rational drug design, plant growth and antibacterial assays.

Dr Lakshmi WIJEYEWICKREMA



A Structure Function Study of Pineapple Cysteine Proteases

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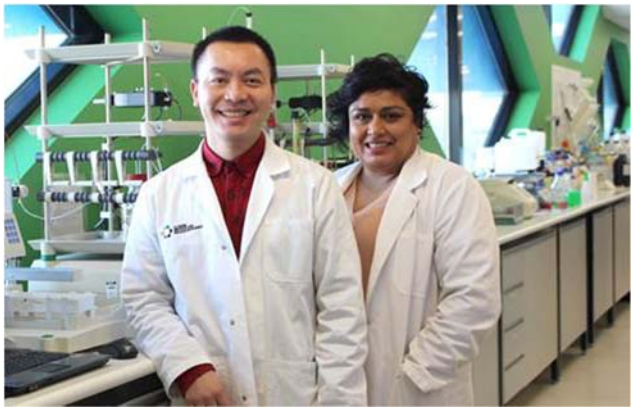
Subjects prerequisites: MED3LAB or MED3PRJ **Theme:** Infection & Immunity

Pineapple cysteine proteases: magic bullet for diarrhoea-related diseases?

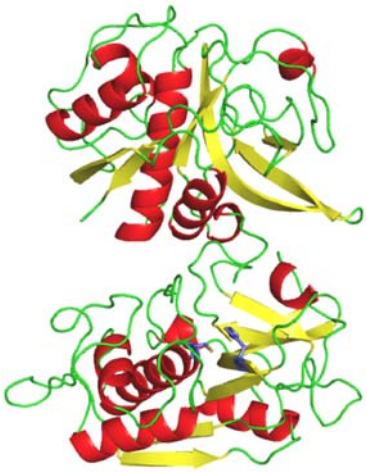
The honours student will use a broad range of techniques on many aspects of protein biochemistry, including gene cloning, site directed mutagenesis, recombinant protein expression and purification, enzyme characterization, crystallization and structure solving. This project will be co-supervised by SIEF/CSIRO Industry Research Fellow, Tang Yongqing.



Dr

Diarrhoea is responsible for over a million deaths per annum in humans with a prevalence of 1.7~5 billion cases annually. Diarrhoea in young farm animals (scour) also causes millions of dollars lost every year in Australia's livestock industry. For hundreds of years, pineapple (*Ananas Comosus*) has been used by the tropical natives as part of folk medicine to improve the health of their digestive system. It was later discovered that bromelain, a crude extract from the stem of pineapple, contributes to a wide range of therapeutic benefits, which led to an immediate success of the commercial market for bromelain.

It is recently emerged that a group of cysteine proteases, including ananain, stem bromelain and canizain, are the key ingredients responsible for the anti-diarrhoeal properties of bromelain. In this study, we will express and purify recombinant proteins of pineapple cysteine proteases using *E. coli*. We will study both the zymogen and the active form of these enzymes to reveal how these proteases are activated. The substrate specificity of the active proteases will be further characterized by using a tripeptide library. We will also enter the exciting world of protein crystallography and work in the Australian Synchrotron to examine the relationship between protein structure and function.



OLIVIA NEWTON-JOHN CANCER RESEARCH INSTITUTE SCHOOL OF CANCER MEDICINE

Level 5, Olivia Newton-John Cancer & Wellness Centre (ONJCWC)

145 Studley Road, Heidelberg, VIC 3084

www.onjcri.org.au

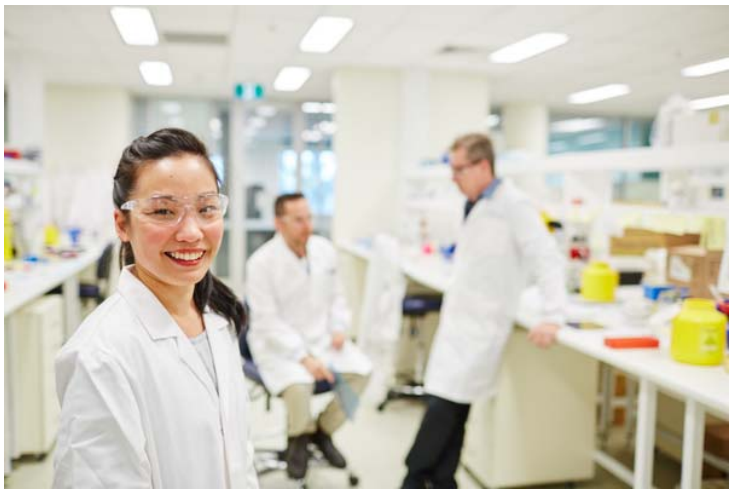
The **Olivia Newton-John Cancer Research Institute (ONJCRI)** at the Austin Hospital in Heidelberg is an independent medical research institute **with strong collaborative links to LIMS**. Honours projects in Biochemistry, Genetics or Biomedical science may be carried out within some ONJCRI laboratories as part of the LIMS program. In addition to meeting the attendance, training and assessment requirements of the LIMS program, students completing projects in ONJCRI laboratories will also be required to undergo safety training at the Institute, and where appropriate, animal handling training.

The ONJCRI is Australia's newest Cancer Research Institute, established to be a world leader in research and discovery regarding cancer, its origins, and developing new treatments for the care of people affected by cancer.

The Institute occupies three floors of state-of-the-art laboratories at the Olivia Newton-John Cancer and Wellness Centre (pictured) and is the successor to the internationally renowned Ludwig Institute for Cancer Research which established a collaborative clinical research programme with Austin Health in 1990. Embedded within the comprehensive cancer centre, the Institute is uniquely placed to bring together basic and translational cancer research in a co-ordinated and successful manner.

The Institute entered into a research collaboration agreement with La Trobe University in 2014, which led to the creation of the School of Cancer Medicine. The relationship fosters collaborative research and the joint training and scholarship support of research students enrolled at the University. Professor Matthias Ernst is the inaugural Head of School of Cancer Medicine, and Scientific Director, Olivia Newton-John Cancer Research Institute.

With a strong track record in attracting grant support from Australian and International sources and successful industry collaborations, our research teams are currently investigating melanoma, lung, breast, brain and gastrointestinal tumours. All research activities are enhanced and supported by outstanding platform technologies, infrastructure, facilities, and technical expertise including:



- ACRF Centre for Translational Cancer Therapeutics and Imaging
- Cell biology
- Mammalian Protein Expression and Purification Facility
- Protein chemistry
- Bioresource Facility
- Therapeutic modelling
- Flow cytometry and cell sorting
- Cell line repository and tissue banking
- Genomics including molecular pathology
- Radiochemistry and PET Solid Targetry
- Next generation sequencing and digital PCR
- Clinical trials centre to facilitate the clinical translation of laboratory discoveries.

The Institute invites applications from highly motivated and creative individuals to undertake PhD, and BSc (Hons), degrees. Students will enrol in the School of Cancer Medicine. The Institute attracts students from a wide range of disciplines biochemistry, genetics, immunology, medicine, microbiology, pathology and physiology. The Institute maintains a rigorous student mentoring and support program which has contributed to the Institute's outstanding track record of student success.

We are committed to providing post-graduate students with an environment in which to excel in cancer research and make original discoveries that will improve the understanding and treatment of cancer. The Institute provides state-of-the-art facilities and world-class scientists and medical specialists to guide young researchers.

Dr Andreas BEHREN

TUMOUR IMMUNOLOGY LABORATORY

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Subjects prerequisites: MED3LAB, MED3PRJ or GEN3LAB **Theme:** Cancer

Project 1: Deciphering the role of EMT, NK cells and T_{RM}-T cells in melanoma

Lab Co-Supervisor: Dr Jessica Duarte

Background: Recent data suggest that in multiple cancers, including melanoma, the presence of dendritic cells (DCs) that drive the efficient priming of anti-tumour responsive T cells is governed and partially regulated by the preceding influx of Natural killer (NK) cells (Barry 2018; Boettcher 2018). Work from our group and others have shown that the presence of NK cells in tumour tissue is often associated with a T cell signature, epithelial-to-mesenchymal transition (EMT)-like gene profiles, and improved disease outcome (manuscript submitted). Similarly, the presence of $CD103^{+}CD39^{+}$ tumour-reactive T cells with a tissue resident memory phenotype (T_{RM}) is associated with increased anti-tumour immunity and improved responsiveness to immunotherapy. These T_{RM} cells are the predominant subset of tumour-antigen reactive T cells within tumours which can be re-activated with immune checkpoint therapy (Duhen 2018). How these cell types are linked, what role EMT plays and in what sequence these cells appear in the tumour microenvironment remains elusive so far.

Methods: Using multicolour Immunohistochemistry we aim to explore the relationship and correlation of NK, DC, and T cell subsets and EMT in melanoma tumours from various stages of disease to understand the timing and correlation between these. This technology allows for the simultaneous staining of up to 6 markers of interest within a single tissue slides (Halse, 2018). As the role of EMT-like processes in the context of melanoma is still controversial, we will also profile markers and mediators of immunosuppression and immune escape within the same tumour slides. We will apply 3 different -multi-colour antibody panels to comprehensively stain the melanoma samples and to analyse correlation and co-localization of markers and cellular subsets within the tumour. Retrospective melanoma tissue is available from the Austin Pathology and human ethical approval has been granted. We aim to analyse at least 25 slides from Stage I/II, 25 slide from Stage III and 25 slides from Stage IV melanoma.

References:

*Barry et al.**Nat Med.** 2018 Jun 25. doi: 10.1038/s41591-018-0085-8. [Epub ahead of print]*
*Böttcher et al. **Cell.** 2018 Feb 22;172(5):1022-1037.*
*Duhen T et al.. **Nat Commun**. 2018 Jul 13;9(1):2724.*
*Halse et al.. **Sci Rep**. 2018 Jul 24;8(1):11158.*

Honours project 2: Novel diagnostic biomarkers for prostate cancer

Laboratory Co-Supervisors: Dr. Jessica Duarte (ONJCRI) and Dr. Jyotsna Batra (Queensland University of Technology).

Background: Prostate cancer is one of the most common cancers in men worldwide, with over 1 million cases and 300 000 deaths reported in 2012 (Ferlay *et al.*, 2015). Current screening approaches include the routine testing of serological levels of prostate-specific antigen (PSA), albeit limited by low sensitivity (Cooperberg, Broering and Carroll, 2010). Diagnosis is often only confirmed by invasive tissue biopsies, which may result in sampling-related false negatives (Marks, Young and Natarajan, 2013). Hence, improved diagnostic biomarkers are of great need to ensure adequate clinical intervention and patient management. We have developed a custom cancer-specific protein microarray platform which enables the antibody profiling against over 100 cancer-specific targets using blood (Beeton-Kempen *et al.*, 2014). Preliminary data using this array on a cohort of 20 prostate cancer patients showed that it can be used to identify novel potential diagnostic biomarkers with high sensitivity, while distinguishing prostate cancer from benign prostate hyperplasia and healthy individuals (Adeola *et al.*, 2016).

To ensure that this technology can be accessible to all, including those located in remote areas, novel "blood cards" are an attractive option. These enable patient blood to be collected from anywhere using a simple finger prick. A few drops of blood are placed directly onto these cards, and fractionated according to their content. These cards can then be sent via regular post to a clinical laboratory for testing, and bypass the need for blood collection via venepuncture.

Methods: We aim to screen retrospective serum from 100 prostate cancer patients for the presence of antibodies against cancer antigens using our custom array. This will enable us to determine whether we can we use the array to readily detect prostate cancer, thus validating the preliminary findings. Patient samples will include all stages of disease, and are accessible via collaborative access to the Australian Prostate Cancer BioResource (APCB). Findings will be compared with measured PSA levels and long-term clinical outcomes.

In order to combine the "blood card" approach with our cancer array test, we aim to test the use of novel "blood cards" on a subset of the above samples as a means to adequately collect, store and extract patient serum for downstream array assays.

References:

*Adeola, H. A., et al (2016) **Oncotarget,** 7(12): 13945-64. doi: 10.18632/oncotarget.7359.*
*Beeton-Kempen, N., Duarte, J., et al (2014) **Int J Cancer,** 135(8): 1842-51.*
*Cooperberg, M. R., et al. (2010) **J Clin Onc,** 28(7): 1117-1123..*
*Ferlay, J., et al (2015) **Int J Cancer.** 136(5): E359-86.*
Marks, L., et al (2013) 23(1): 43-50.

Dr Ashwini CHAND

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Subjects prerequisites: MED3LAB or MED3PRJ **Theme:** Cancer

Identifying novel treatments for advanced gastrointestinal cancers

Lab Co-Supervisor: Prof Matthias Ernst

Gastrointestinal (GI) cancer, comprising those of the stomach and colon, collectively comprise the third most common cause of cancer mortality and adding annually more than 18,000 new patients in Australia alone. The major clinical challenge for treating GI cancers is the late diagnosis and consequently limited opportunity for therapeutic intervention. Our previous studies show that the FDA-approved drug *bazedoxifene,* has significant anti-tumour effects in preclinical models of gastric and colon cancers. Mechanistically, we have shown that *bazedoxifene* interferes with binding of the inflammatory cytokine interleukin (IL)11 to its receptor gp130 thereby reducing the activity of the transcription factor Signal Transducer and Activator of Transcription 3 (STAT3). In turn, there is extensive evidence defining the importance of STAT3 in the cancers to promote proliferation, cell survival, angiogenesis and metastasis.

This project(s) will focus on understanding cytokine-dependent pathways in cancer progression and metastasis. It will involve *in vitro* assays that measure the effects of *bazedoxifene* and other novel inhibitors of the IL11/gp130/STAT3 pathway to assess effects on the metastatic potential of cancer cells. The project will also provide the student(s) with the opportunity to examine the effects of these drugs using *in vivo* metastasis models. The project will provide the student(s) with a detailed understanding of cancer biology and experience in a range of techniques including cell culture based proliferation and invasion assays, mRNA and protein expression analysis (qPCR, FACS, Western blot, immunohistochemistry), and the use of preclinical animal models.

The work at ONJCRI/La Trobe School of Cancer Medicine will take place in laboratory setting that has access to patient material and focuses on clinically relevant aspects in the treatment of cancer patients.

Professor Matthias ERNST

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Subjects prerequisites: MED3LAB or MED3PRJ **Theme:** Cancer

Investigating novel cytokines in cancer development

Lab Co-Supervisor: Dr Jennifer Huynh

Gastric cancer is the 2nd most common cause of cancer-related death worldwide. There is a pressing need to identify novel targets and develop therapies to overcome the morbidity and mortality associated with gastric cancer. There has been a longstanding link between inflammation and cancer which was first described over 100 years ago. This link has gained traction in recent years, and accumulating evidence point towards how secreted factors, known as cytokines, promote cancer growth. Cytokines are molecules which enable cells to communicate with one another and help co-ordinate the immune response. Cytokines can also be exploited during cancer to foster tumour growth. We have exciting preliminary data demonstrating that the novel cytokine, IL-36G, is highly up-regulated in stomach tumours indicating a possible role IL-36G in the development of gastric cancer. Indeed, consolidation of data from The Cancer Genome Atlas (TCGA) indicates IL-36G expression correlates with poorer patient survival and disease-free survival.

The aim of this project is to characterise the functional importance of IL-36G signalling in tumourigenesis and interrogate whether IL-36G could serve as a potential therapeutic strategy for the treatment of gastric cancer. The prospective Honours student will be exposed to a wide array of *in vitro* techniques including cell culture, qPCR, Western blot, ELISA, FACs and immunohistochemistry. The project will also provide the student access to a clinically relevant mouse model of gastric cancer which has been established by our laboratory.

The proposed study will be executed at ONJCRI and La Trobe School of Cancer Medicine, and will provide the prospective student access to comprehensive cutting edge support facilities in a multidisciplinary environment and access to clinical patient data.

Dr Doug FAIRLIE

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Subjects prerequisites: MED3LAB or MED3PRJ **Theme:** Cancer

Targeting cell death pathways in biliary tract cancers

Co-supervisors: Prof. John Mariadason, Dr Erinna Lee

Background

Biliary tract cancers (BTC) are cancers which arise in the bile ducts, the vessels which drain bile produced in the liver for storage into the gallbladder and subsequent release into small intestine. There are a number of risk factors associated with the disease including chronic inflammation, as well as liver fluke infections. Most BTC patients present with advanced disease and the prognosis is generally poor with median survival of about 12 months. Chemotherapy is the standard treatment though this only has a modest effect on patient outcomes.

One of the key hallmarks of most, if not all, cancers is defective apoptosis signalling. This is often due to over-expression of the pro-survival members of the Bcl-2 family of proteins. As a consequence, a new class of drugs ("BH3-mimetics") that directly target these proteins has been developed, and is showing significant promise for the treatment of certain cancers. However, there have been only very limited studies using these drugs in BTC.

Project outline

This project will examine a large panel of BTC cell lines for their sensitivity to a range of BH3-mimetic drugs, both as single agents as well as in combination with each other. This will enable us to precisely dissect the critical survival "factors" in the tumours and establish whether those from distinct locations within the biliary tree behave similarly. As we have detailed genomic information from these cell lines, we can also design potential drug combinations with BH3-mimetics that might provide enhanced cell killing activity. The mechanism-of-action of BH3-mimetics in BTC will also be examined.

Students undertaking this project will use a variety of techniques including cell culture, *in vitro* cell assays, CRISPR/Cas9 gene editing, FACS, Western blotting, immunohistochemistry as well as basic molecular biology techniques.

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Subjects prerequisites: MED3LAB or MED3PRJ **Theme:** Cancer

Role of autophagy in tumourigenesis and chemoresistance

Lab Co-Supervisor: Dr Doug Fairlie

Cells possess distinct pathways that promote their survival or death. These pathways are tightly regulated but when this regulation goes awry, disease such as cancer ensue.

Autophagy is one mechanism of cell survival. It is an evolutionarily conserved process of cellular self-cannibalism. The toxic accumulation of damaged or unnecessary cellular components is prevented when proteins or organelles are sequestered into autophagic vesicles that are subsequently degraded following fusion with lysosomes. In response to stress or nutrient deprivation, the autophagic degradation of these damaged components also serves to provide the cell with molecular building blocks, hence, sustaining metabolic homeostasis.

One of the most extensively studied autophagy inducers is Beclin 1. Beclin 1 provided the first genetic evidence that pro-autophagy genes represent a novel class of tumour suppressors. Furthermore, autophagy has also been implicated as a protective mechanism that mediates the acquired resistance displayed by some cancer cells during chemotherapy.

The aim of this project is two-fold:

(1) To determine whether the mechanisms by which Beclin 1 exerts its tumour suppressive function is through its ability to interact with components of the cell death pathway known as apoptosis. As such this project will provide important insights into how autophagy contributes to tumourigenesis.

(2) To investigate the role of autophagy in cellular resistance to chemotherapy. This project could inform how autophagy can be targeted for the development of new treatment strategies.

Professor John MARIADSON

ONCOGENIC TRANSCRIPTION LABORATORY

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Subjects prerequisites: MED3LAB or MED3PRJ **Theme:** Cancer

Role of the EHF transcription factor in prostate cancer
Co-supervisors: Prof. Patrick Humbert, Dr. Ian Luk

Background
Each year in Australia, approximately 17,000 men are diagnosed with prostate cancer and ~3,400 men will die from metastatic disease. There is therefore an urgent need to identify the factors which drive the metastasis of prostate cancer cells so that new treatments which prevent this process can be developed.

Ets homologous factor (EHF) is a member of the Ets family of transcription factors. EHF is highly expressed in the normal prostate epithelium and is frequently lost in prostate cancer. Prostate cancer's which lose EHF are more likely to metastasize, and downregulation of EHF in prostate cancer cell lines drives epithelial to mesenchymal transition (EMT). While these findings suggest that EHF loss promotes prostate cancer metastasis, direct *in vivo* evidence for such a role is currently lacking.

Project outline
The goal of this project is to determine whether the targeted deletion of *Ehf* in the prostate epithelium increases the rate of metastasis in a mouse model of prostate cancer that accurately recapitulates human disease.

To address this, we will utilize the PB-Cre $Pten^{\Delta Pr}$ model of prostate cancer, where the *Pten* tumour suppressor gene is specifically deleted in the prostate epithelium. These mice mice develop high grade prostatic intraepithelial neoplasia beginning at 6 weeks, adenocarcinomas at 9 weeks and metastases to the lymph nodes and lung at 12 weeks. PB-$Pten^{\Delta Pr}$ mice will then be crossed to $Ehf^{Lox/Lox}$ mice to determine the effect of *Ehf* loss on the rate of development of PIN, prostate adenocarcinomas and metastasis. We expect *Ehf* deletion to increase tumour formation metastasis.

Direct demonstration of a role for EHF loss in prostate cancer metastasis will justify the exploration and testing of therapeutic strategies aimed at retaining EHF expression in prostate cancer cells as a means of preventing metastatic spread.

Students undertaking this project will learn the fundamental concepts of cancer biology and use a variety of techniques including mouse models of cancer, Western blotting, immunohistochemistry as well as basic molecular biology techniques.

Dr Delphine MERINO

TUMOUR PROGRESSION AND HETEROGENEITY LABORATORY

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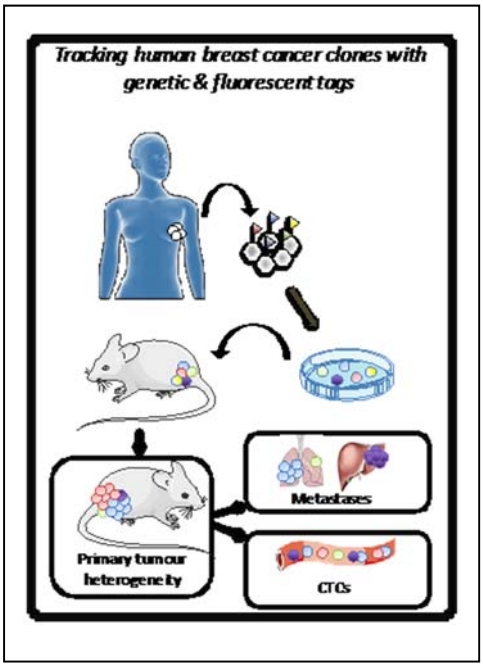
Subjects prerequisites: MED3LAB or MED3PRJ **Theme:** Cancer

Characterising the 'seeds' of breast cancer metastasis

Lab Co-Supervisor: Dr Belinda Yeo

Breast cancer is a highly heterogeneous disease. Many studies have shown that each cell in a given tumour has a specific genomic background. This high level of heterogeneity represents a major obstacle for patient biopsy and diagnostic, as all the clones responsible for tumour recurrence will need to be taken into consideration for a complete eradication of the disease.

To colonize distant organs, tumour cells must intravasate into blood vessels as circulating tumour cells (CTCs), extravasate and survive in their new environment as distant tumour cells (DTCs). Only a minor proportion of cells present in the primary tumour will survive and form clinically-relevant macro-metastases. Breast cancers are known to preferentially colonize lungs, bones, lymph nodes, liver, brain and ovaries, and how the different microenvironments of these organs impact on clonal selection is unclear.



Our laboratory aims at understanding the biologic properties of metastatic clones. We would like to understand how they interact with their microenvironment, whether they differ between different metastatic sites, and ultimately, to ***track and treat*** clones responsible for the relapse of the disease. Therefore, we are using genetic or fluorescent barcodes (or tags) to label cells from patient samples, and follow their behaviour after transplantation into mice. Using sequencing at bulk or single cell level, we are planning to identify the pathways involved in metastatic spread, organ specificity and cell survival, in order to stop disease progression.

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Subjects prerequisites: MED3LAB, MED3PRJ or GEN3LAB **Theme:** Cancer

Australia has one of the highest incidence rates of colorectal cancer (CRC) in the world and CRC accounted for the second most cancer deaths in Australia in 2017 (Cancer Australia). Infiltration of $CD8^+$ T cells that produce interferon (IFN)γ and granzyme B are known as Tc1 cells and predict good prognosis in CRC. On the contrary, IL-17-producing $CD8^+$ T (Tc17) cells are less cytotoxic and associate with poor patient survival in CRC and gastric cancer (GC). The regulatory networks required for the differentiation of Tc1 vs Tc17 and function of Tc17 cells during tumour progression is unknown. A better understanding of the factors controlling T cell differentiation will pave the way for new immunotherapies to induce favourable Tc1 differentiation and limit Tc17 cells. Investigating lymphocyte differentiation in CRC will lead to better tumour infiltrating lymphocyte (TIL) scoring and immunoscore protocols to predict patient prognosis and response to immunotherapies.

Hypothesis: The tumour microenvironment produces cytokines to skew $CD8^+$ T cell differentiation towards a Tc17 cell response that drives progression of CRC, therefore redirecting T cell differentiation to a beneficial Tc1 response will improve immunity in CRC.

This project aims to (i) uncover the mechanisms controlling Tc17 cell development in humans and their function in CRC progression and patient survival. (ii) Identify novel biomarkers of Tc17 cells to predict patient survival or potential targets for new immunotherapies to treat CRC.

This will be achieved using blood and tissue samples from healthy humans and CRC patients using flow cytometry, RNA Sequencing and immunofluorescent staining techniques.

Dr Bhupinder PAL

CANCER AND SINGLE CELL BIOLOGY LABORATORY
TRANSLATIONAL BREAST CANCER PROGRAM

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Subjects prerequisites: MED3LAB or MED3PRJ **Theme:** Cancer

Characterising the 'seeds' of breast cancer metastasis

Lab Co-Supervisor: Dr Delphine Merino

Targeting breast cancer cells is proving difficult due to the molecular heterogeneity that may arise from different factors including the cell of origin, somatic mutations in breast cancer susceptibility genes, and genetic alterations including mutations, deletions, fusions or amplifications of key genes. Furthermore, it is thought that the tumour microenvironment has a strong impact on cancer cell survival, and plays an important role in drug resistance and cancer metastasis. Finally, cancer cells that escape and seed at new sites throughout the body undergo global changes in their genetic and epigenetic landscape.

The technological advancements in the field of single cell biology has allowed the study of molecular heterogeneity in mixed cell populations and shed light on cell lineage relationships. Recent studies have revealed heterogeneity in breast cancer cells and novel cell clusters in normal mammary gland. The primary objective of our laboratory is to gain novel molecular insights into aggressive and drug resistant breast cancer subtypes, including BRCA associated tumours. We will use high-throughput single cell transcriptomic/genomic analysis approach to study breast tissues from normal and tumour microenvironment. By sequencing primary tumours, metastatic breast cancer and surrounding normal cells we aim to identify and characterise cell subsets, reveal molecular perturbations in rare cell populations that cause metastasis, and analyse host cells in the tumours. Novel biomarkers will be evaluated through further laboratory studies using breast cancer patient derived xenograft (PDX). Our laboratory is also involved in the development of new single cell assays and analytical tools for single-cell data.

Dr Normand POULIOT

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Subjects prerequisites: MED3LAB or MED3PRJ **Theme:** Cancer

Project 1: Stromal matrix regulation of immune infiltration in breast cancer metastasis

Lab co-Supervisor: Dr Andreas Behren

Changes in the matrix microenvironment of tumours contribute to cancer progression in part by modulating anti-tumour immune responses. However, the mechanisms involved remain poorly understood. This project will test the hypothesis that expression of the matrix protein laminin-511 (LM-511) in metastatic breast cancer regulates the recruitment of tumourinfiltrating immune cells and protects tumours cells from immune recognition. Using an immunocompetent mouse model of breast cancer metastasis we will test if knockdown of LM-511 in tumours alters the kinetic of tumour infiltration by immune cells. Changes in tumour-infiltrating macrophages, natural killer, granulocytes and lymphocyte subpopulations will be quantitated by immunohistochemical detection of specific immune cell markers in breast tumour tissues and by flow cytometric analysis of immune cell infiltrates in disaggregated tumours. Cytotoxic activity of tumour-derived NK or CD8+ lymphocytes against breast cancer cells expressing or not LM-511 will be tested in vitro. Immune suppressive effect of tumour-isolated myeloid-derived suppressor cells will be tested in a T cell suppression assay. The project will make use of a broad variety of techniques including cell culture and in vitro assays, mRNA and protein expression analysis (FACS, western blot, immunohistochemistry), basic molecular biology techniques and in vivo animal models of metastasis.

Project 2: Targeting tumour-vascular adhesive interactions to reduce the burden of disease in brain metastatic breast cancer

Lab co-supervisor: Dr Delphine Denoyer

This project seeks to address one of the most pressing issues for breast cancer patients - the lack of effective targeted therapies to treat or prevent the development of brain metastases that affect over 30% of advanced breast cancer patients. The mechanisms by which tumour cells overcome the protective function of the blood-brain barrier to home to, invade and colonise the brain remain poorly understood. A better understanding of these mechanisms will reveal new molecular targets to identify and treat high risk patients. This project will explore a new strategy of targeting adhesion receptors to disrupt tumour cell interaction with the blood brain barrier and colonisation of the brain. We now have evidence

that a new adhesion receptor called limitrin is involved in the migration of brain-metastatic breast tumour cells through interaction with integrins, implying that therapeutic targeting of limitrin or integrins could reduce the ability of tumour cells to cross the blood brain barrier and colonise the brain. The project will test the hypothesis that interfering with limitrin/integrin function will prevent or delay the outgrowth of brain metastases in clinically relevant mouse models of breast cancer brain metastasis. The project will involve assessing the relationship between limitrin and integrins and potential interaction in breast cancer cell lines or tumours of varying metastatic abilities. The function of limitrin and integrins will be assessed in in vitro functional assays and in preclinical models of brain metastatic breast cancer. Finally, some targeted therapies will be tested in the preclinical models to see if they can reduce the extent of brain metastasis.

Professor Andrew SCOTT

TARGETING AND MOLECULAR IMAGING OF TUMOURS

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Subjects prerequisites: MED3LAB or MED3PRJ **Theme:** Cancer

Our lab focuses on the targeting and molecular imaging of tumours and exploring receptor-based signaling pathways responsible for cancer cell growth. Through the development of innovative strategies for molecular imaging of cancer, and identifying cellular targets suitable for antibody therapy, we are developing new cancer therapies, with a particular focus on targeting of cancer cells with novel recombinant antibodies. In addition to pursuing clinical trials of novel antibodies, small molecules and imaging ligands, we are exploring mechanisms of clinical resistance to tumour targeting agents.

Project: Imaging metabolic changes in cancer cachexia

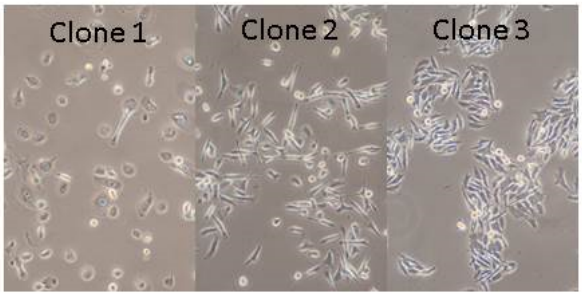
Lab Co-supervisors: Dr. Ingrid Burvenich (Tumour Targeting Laboratory, ONJCRI) and Dr. Laura Osellame (Department of Biochemistry, La Trobe)

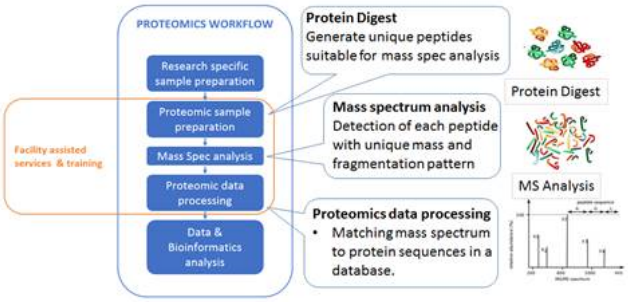
PET, MRI and SPECT are molecular imaging technologies which allow researchers to see whether treatments are effectively targeting a tumour. An exciting development in molecular imaging of cancer is the identification of critical biochemical pathways that are responsible for tumour growth and metastasis, which can be imaged with novel single photon emission computed tomography (SPECT) and positron emission tomography (PET) tracers. Extending the laboratory discoveries of novel metabolic tracers into clinical trials is a major focus of our molecular imaging/PET research program.

Through a collaboration with the department of Biochemistry at La Trobe, we are currently studying the metabolic changes occurring in tumour-bearing mice during cachexia, a wasting syndrome typically presenting symptoms such as severe body weight loss and muscle weakening at the end stages of cancer. In mice, we were able to demonstrate metabolic changes in cachectic versus non-cachectic mice, and cachexia treated versus untreated mice (see Figure). This project aims to elucidate the pathways involved in metabolic changes. In addition, several clones have been derived from a cachectic prostate cancer cell line that harbour different morphological and cachectic properties. In this study we will explore fundamental differences between these clones in vitro as well as in mice. Studies include responsiveness to an anti-cachectic drug as well as metastatic potential of the clones. Clones will be characterized by examining mRNA and protein expression.

In addition, clones will be barcoded, to allow fluorescent imaging in vivo. PET imaging will be used to characterize metabolic differences of clones through imaging.

IN VITRO CHARACTERIZATION





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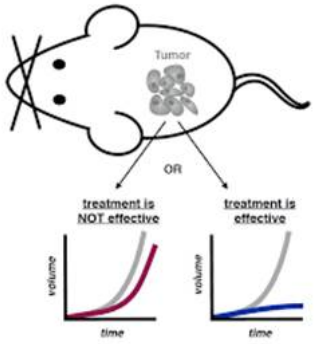

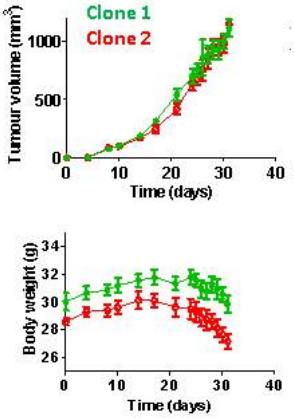

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