

## Centre for Cancer Research



### Institute Director & Centre Director: Professor Bryan Williams

The Centre for Cancer Research was established in January 2006 by Centre Director and Institute Director, Professor Bryan Williams. Scientists working in the Centre for Cancer Research are dedicated to studying basic aspects of cancer biology, in an effort to learn more about the growth and development of different cancers, and to translate their findings into new approaches to cancer prognosis and therapy.

The Cancer Research team carries out its own research on a variety of tumours such as prostate, kidney, colon, lung, bladder and brain tumours.

CCR scientists also collaborate closely with scientists from MIMR's other centres and the Monash Health Research Precinct to share expertise, resources and knowledge into a broad range of cancers, including breast, endometrial, ovarian, and stomach cancers as well as leukaemia. CCR scientists are particularly interested in oncogenic and developmental signalling, metastasis, links between inflammatory processes and cancer, and the role of cancer stem cells. The CCR also has active collaborations with private industry pursuing drug discovery and improved cancer therapeutics.

### Functional analysis of relapse predictive genes in Wilms tumour

**Project Leader:** Prof. Bryan Williams  
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#### Project Description:

Wilms tumour (nephroblastoma) is a paediatric cancer of the kidney and is a classic model for the connection between normal development and cancer. Our lab has discovered a gene signature of which predicts tumours that are most likely to relapse. This project aims to characterize the role of one of these signature genes, CEBP $\beta$  (CAAT-enhancer binding protein beta) Wilms tumour progression. This project will study the effects of different isoforms of this protein on tumour development using a Wilms tumour cell line (WiT49) developed in our laboratory. The project will use a variety of techniques including: cell culture, siRNA (short interfering RNA) for downregulation of gene expression, cloning into lentiviral vectors and infection into cells for overexpression studies, FACS analysis, mutation analysis of luciferase reporter assays, quantitative (Real Time) RT-PCR, Western blotting, analysis of protein-protein and protein-DNA interactions by chromatin immunoprecipitation (ChIP) and tumour modelling using xenografts of tumour tissue in an animal model. The project is suitable for a PhD student or can be divided into smaller projects suitable for honours students.

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### RNA interference and innate immunity

**Project Leader:** Prof. Bryan Williams  
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**Project Description:**

We have shown that short interfering RNA (siRNA) can be robust activators of innate immunity. This can be attributed to the nature of the 3' ends of the siRNA and their interaction with a signalling RNA helicase RIG-I. This project will further explore the nature of this interaction using biophysical and structural biology techniques and characterize the different pathways activated in both immune and non-immune cells. Bi-functional siRNAs that have both gene silencing and innate immune activation activities are being developed and a project to test these both in vitro and in vivo in mouse cancer models is available.

### Regulation of intestinal inflammation by PKR

**Project Leader:** Dr. Tony Sadler  
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**Project Description:**

Inflammation is a major contributor to intestinal disease by both exacerbating the initiation and progression of a variety of pathologies. We have identified the protein kinase R (PKR) is protective in inflammatory conditions of the gut, and so, are interested in deciphering the mechanism(s) by which PKR alleviates pathologies associated with intestinal inflammation. A project exists in our laboratory to investigate how PKR-modulates this inflammation. The project will compare cellular and mouse models in which PKR is wild-type, ablated, or functional impaired to determine the mechanism by which PKR might moderate inflammation of the gut caused by chemical and microbial insult.

### Protective innate immune responses in autoimmunity

**Project Leader:** Dr. Tony Sadler  
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**Project Description:**

Our preliminary data has shown that the protein kinase R (PKR) decreases the degradation of cartilage in models of arthritis. The mechanism(s) by which PKR mediates this protection is unknown. However, our preliminary studies indicate a PKR-dependent difference in key interleukins known to play a role in inflammation and injury, and more particularly, in autoimmune disease. A project exists to verify if PKR modulates particular T-helper cells, and then to test a number of anticipated mechanisms by which PKR might mediate this action. The techniques to be used in the project include cell culture and manipulation, as well as experimentation in animal models.

### Role of the IFN-induced helicase-1

**Project Leader:** Dr. Tony Sadler  
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**Project Description:**

A project exists to investigate mechanisms in the development of Juvenile diabetes (T1D). Although T1D disease is primarily considered a corollary of aberrant immune function, the mechanisms leading to immunological destruction of  $\beta$ -cells in the pancreas are unknown. The project is motivated by the recent realization that the ifih1 gene may be a causal locus in type 1 diabetes (T1D), and a novel observation made in our laboratory correlating polymorphisms in this gene product with different innate immune responses. This observation identifies a functional consequence for the reported genetic variance within ifih1 that may define an important mechanism in the development of T1D.

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### Structural basis of PKR activation and regulation

**Project Leaders:** Dr. Galina Polekhina, Prof. Bryan Williams & Dr. Tony Sadler  
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#### **Project Description:**

Protein kinase R (PKR) plays a key role in the innate immunity response to viral infection and cellular stress. PKR activity is regulated by various factors among which are binding to dsRNA, PACT, or heparin. While the structures of PKR's kinase domain, and separately, dsRNA-binding domains have been elucidated, the structure of the full length PKR in the active and inactive state is yet to be determined. Exactly how PKR is switched on by the activating molecules remains unknown and this information may be critical to understanding how exactly this kinase works and functions in these important biological processes. The primary goal of this project is to investigate the mechanistic/molecular details of PKR activation by elucidating the X-ray structures of the various intermediate states of PKR and its interaction with activators and binding partners.

### Molecular mediators of bladder cancer metastasis

**Project Leader:** Dr. Elizabeth Williams  
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#### **Project Description:**

The spread of cancer (metastasis) is the major cause of death in most cancer types, and new therapies to prevent and treat metastatic cancer are clearly needed. We have used gene expression microarray analysis to identify candidate molecules involved in this process in bladder cancer. This project will investigate the role of these molecules in metastasis using molecular biology and protein analysis techniques, cell culture, mouse models of bladder cancer and a cohort of human bladder cancer specimens.

### Structural and functional studies of cellular stress sensor

**Project Leader:** Dr. Galina Polekhina  
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#### **Project Description:**

Thioredoxin-interacting protein (TXNIP) is a cellular stress sensor that is induced by various stimuli altering cellular signalling and leading to oxidative stress. Oxidative stress has long been associated with many human pathological conditions including atherosclerosis, diabetes, carcinogenesis, ischemic reperfusion injury and neurodegenerative disorders as well as aging. Additionally TXNIP has recently been shown to activate the large protein complex involved in regulation of innate immunity, inflammasome. Given all these biological roles, TXNIP is considered to be an attractive drug target in several human diseases. This project involves structural and functional studies of TXNIP as well as its complexes with the binding partners. Techniques such as X-ray crystallography using synchrotron radiation, molecular modelling, biochemical techniques as well as several structure-based drug design approaches will be used.

### Prostate cancer bone metastasis

**Project Leader:** Dr. Elizabeth Williams  
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#### **Project Description:**

Prostate cancer is the most common cancer in Australian men. Upon disease progression prostate cancer typically spreads to lymph nodes and bone. Bone metastases are currently incurable and contribute significantly to disease-specific morbidity and mortality. This project aims to develop new strategies to treat and/or image bone metastatic prostate cancer. Tumour growth and interaction with bone will be imaged using a number of techniques including fluorescence and X-ray modalities.

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### Prostate cancer lymph node metastasis

**Project Leader:** Dr. Elizabeth Williams  
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#### **Project Description:**

Prostate cancer is the most common cancer in Australian men. The most frequent site of prostate cancer metastasis (spread) is to the lymph nodes. We have established a panel of systems to study the interaction of prostate cancer cells with the endothelial cells of prostatic lymphatic vessels. This project aims to identify key molecules involved in this process. This project will use a number of fluorescent imaging techniques to monitor tumour cell interactions in vitro and in vivo. Gene expression levels of important molecules will be manipulated using molecular biology techniques, and protein expression followed in a number of different formats (including Western blotting and ELISA).

### Genetic screening of patients with bladder exstrophy

**Project Leader:** Prof. Wei Cheng  
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#### **Project Description:**

Bladder exstrophy is a congenital disease whereby baby is born with its bladder exposed to the exterior. The affected babies leak urine constantly. Untreated, the exposed bladder develops cancer. This condition requires multiple operations, consultations and life-long follow-up, which is a challenging physically, psychologically and financially to both the patients, the parents and the society at large.

From the inheritance pattern, we speculate that there is a high possibility of genetic abnormality. We are sequencing the DNA of the patients, searching for a specific gene mutation, based on our previous mouse knock-out model. We hope our translational work will help patients in genetic counselling and pave the way for future therapies.

### Structural basis of resistance to chemotherapeutic agent methotrexate

**Project Leader:** Dr. Galina Polekhina  
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#### **Project Description:**

Acquired drug resistance is often a limiting factor in successful chemotherapy of cancer. Methotrexate, an inhibitor of dihydrofolate reductase (DHFR) enzymatic activity, is widely used in treatment of cancer. DHFR is known to regulate its own translation by binding its cognate mRNA and suppressing translation. Exposure to methotrexate, however, leads to a disruption of the DHFR-mRNA interaction thus leading to increased levels of DHFR protein synthesis. This partially (and paradoxically) counteracts the catalytic inhibition by this drug. This project aims to elucidate the detailed molecular structure of DHFR in complex with its cognate mRNA by means of X-ray crystallography using synchrotron radiation. This information will allow us to understand how methotrexate interferes with DHFR regulatory control and will make possible the discovery and development of better DHFR inhibitors.

### Role of Hedgehog (Hh) in gut regeneration

**Project Leader:** Prof. Wei Cheng  
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#### **Project Description:**

Hh is a group of genes involved in mammalian embryogenesis and cancers. In gut, both Sonic hedgehog (Shh) and Indian hedgehog (Ihh) are expressed. It has been shown that Ihh interacts with gut stem cells. We are investigating their roles in gut regeneration using murine knock-in and knock-out models. We have established 5 fluoro-uracil model of gut injury. This project may potentially help gut healing, tissue engineering and stem cell therapy.

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### The role of Integrin-Linked Kinase and Hedgehog signalling in growth of Rhabdomyosarcoma (RMS) cells

**Project Leader:** A/Prof. Greg Hannigan  
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#### Project Description:

RMS is an aggressive muscle tumour of childhood, for which effective therapies are largely lacking. Hedgehog (Hh) is a signalling pathway that regulates precursor and stem cell growth, differentiation, and patterning during mammalian development. Genetic activation of the Hedgehog signalling pathway in mice causes RMS, indicating that Hh signalling is sufficient to induce this cancer in an experimental model. We are interested in the role of a serine/threonine kinase, ILK, in Hedgehog signalling in RMS cells, as it we find it is critical for Hh signalling in normal cells. We have recently published that ILK has differential growth effects on genetically distinct RMS subtypes, promoting growth of one while suppressing growth of the other (Durbin et al., *J Clin Invest* **119**:1558, 2009). This project will analyse the requirement for ILK activity in regulating growth of Hh-activated RMS cell lines which show differential growth response to ILK. The ILK pathway is selectively inhibited by a small molecule or using siRNA, and in this project the student will analyse signalling molecules downstream of ILK and Hh (using western blot and RT-PCR analyses), to determine the role these targets play in RMS cell growth.

### The role of the primary cilium in regulating Hedgehog signalling and growth of Rhabdomyosarcoma (RMS) cells

**Project Leader:** A/Prof. Greg Hannigan  
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#### Project Description:

The primary cilium is a microtubule-based organelle that is present on the cell surface and acts as a signalling 'antenna' in most vertebrate cells. Intraflagellar transport (IFT) proteins selectively regulate the entry and activity of signalling molecules into the primary cilium. The cilium is required for signalling by the Hedgehog (Hh) developmental pathway, which can be aberrantly activated, by genetic or epigenetic mechanisms, to cause cancers such as RMS. It is not clear whether cancer cells with activated Hh require the primary cilium for Hh signalling, or whether aberrant signalling is independent of this structure. We have discovered that the serine/threonine kinase, ILK, localizes in the primary cilium and is, moreover, required for Hh signalling in normal (i.e. non-cancer) cells. This student project will involve analysis of RMS cell lines for the presence of primary cilia, using immunocytochemistry for specific ciliary markers, and for the presence of ILK. Confocal imaging of the markers acetylated tubulin, and ArlB13, will be used to determine the presence of primary cilia, and whether genetic knockdown of IFT protein blocks Hh signalling in RMS cells.

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### An in vivo model of acquired chemoresistance in small cell lung cancer (SCLC)

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#### **Project Description:**

Small cell lung cancer (SCLC) is a common, highly metastatic cancer with an extremely poor prognosis. When first diagnosed, SCLC is usually extremely sensitive to platinum-based cytotoxic chemotherapy, with the majority of patients achieving a partial or complete clinical response. Remarkably, little is known about the mechanisms of acquired chemoresistance in SCLC, or for that matter in most adult solid tumours. In part, this is due to the reliance on conventional cell line models of SCLC that may not reflect the responses of these tumours to chemotherapeutic agents in vivo. This project uses a novel in vivo model of SCLC to investigate the mechanisms of acquired drug resistance in SCLC. In this model, we have generated a series of chemosensitive primary SCLC xenograft lines derived from chemo-naïve patients and passaged exclusively in immunodeficient mice. We will ask whether the process of acquiring chemo-resistance in SCLC is associated with a common set of genomic, epigenetic or transcriptional adaptations in vivo by tracking these changes during the acquisition of chemoresistance by serially sampling the same tumour.

### Roles for the tumour suppressor gene Hic1 in development, differentiation and cancer

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#### **Project Description:**

Hypermethylated in Cancer-1 (Hic1) is a unique tumour suppressor gene in that it is inactivated through deletion or epigenetic gene silencing, but not by mutation. It functions as a tumour suppressor by repressing expression of genes that are normally expressed in embryonic development, but not in adult tissues. Using mouse models, this project examines how Hic1 regulates gene expression during development, repair and tumour suppression. In addition, our Hic1 knockout is the only available mouse model to study epigenetic gene silencing in cancer. The potential projects would be the study of Hic1 in mouse development, targeted knockout of Hic1 in embryos and adult mice, and the study of how epigenetic silencing of Hic1 is triggered during tumour formation. At present, we are developing projects to study the role of Hic1 in mediating differentiation in neural development. With respect to cancer, we are now studying the role of Hic1 in malignant brain tumours, osteosarcoma and lung cancer.

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### Development of Hedgehog (Hh) inhibitors as cancer therapeutics

**Project Leaders:** Prof. Neil Watkins & Dr. Luciano Martelotto  
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#### Project Description:

Aberrant activation of Hh signalling has been identified in a several important and common forms of cancer. In the last 5 years, intense interest in this area has lead to the development of small molecule inhibitors of Hh signalling as potential cancer therapeutic agents. This project covers the preclinical and clinical development of these agents in early phase clinical trials, and includes collaborations with the biotechnology industry, laboratory groups across Australia and in the USA, and clinicians involved in cancer research. Cancers of interest include lung cancer, breast cancer, osteosarcoma and brain tumours.

### Interactions between Hedgehog and Ras signalling in lung adenocarcinoma

**Project Leaders:** Prof. Neil Watkins & Dr. Anette Szczepny  
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#### Project Description:

The Hedgehog (Hh) pathway is a highly conserved system for regulating cell fate and self-renewal in metazoans. Studies from my laboratory have also shown that this pathway plays an important role in cancer by sustaining the growth of tumour stem cells. To understand how the pathway initiates cancer, we have developed a series of mouse models in which we can conditionally activate expression of the Hh pathway in the lung of adult mice. In addition, we can also activate expression of the KRas oncogene in the same cells. Since activation of KRas initiates lung

cancers in mice, we can use this model to understand how activation of Hh signalling in the lung cooperates with KRas to initiate tumour formation in lung cancer. Studies in this project also include the understanding of the role of Hedgehog signalling in human cancers by studying primary human cancer tissue and cell lines.

### Receptor tyrosine kinase mutations involved in Glioblastoma Multiforme

**Project Leaders:** A/Prof. Terry Johns & Dr. Sameer Greenall  
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#### Project Description:

Glioblastoma multiforme (GBM) is a highly invasive and aggressive cancer of the brain, which has an extremely poor prognosis. Cancerous cells within the GBM often contain genetic mutations and/or deletions in surface receptor tyrosine kinases (RTK), which can lead to abnormal cell signalling. This irregular signalling is critical to the development and progression of GBM. Recent studies have shown that different RTK's, such as c-Met and de2-7 EGFR, can activate each other ("cross-talk") leading to increased abnormal signalling. This project will use a wide range of techniques including tissue culture, FACS, Western Blotting and protein array technology to study cross-talk between RTK's in GBM. The ultimate aim of these studies is to identify key RTK's that could serve as targets for the development of novel therapeutics.