Research Student Projects in Pathology 2021

Honours

Master in Biomedical Science

PhD

Department of Microbiology & Immunology

School of Biomedical Sciences

MDHS

University of Melbourne

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Identifying immunosuppressive mechanisms in brain cancer

Primary supervisor: Dr Theo Mantamadiotis theom@unimelb.edu.au

Melbourne Brain Centre, Kenneth Myer Building, on Parkville campus

Determining the best immunotherapy which could be applied to a specific cancer type requires a deep understanding of the immunobiology of the tumour tissue. This project aims to understand the types and distribution of specific cells and histopathological properties in brain tumour tissue, and how the tumour microenvironment, including immune cells, contribute to oncogenesis. Unique brain cancer cell lines and brain tumour tissue from animal models and patients will be used to investigate the tumour microenvironment using state-of-the-art multiplex immunohistochemistry and computational analysis. This project would suit students interested in learning and applying computational analysis toward analysis of the tumour microenvironment.

Investigating the cellular and molecular nature of the blood brain barrier in brain cancer

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Unlike all other blood vessels in the body, the brain vasculature system has evolved a highly specialized protective system, the blood-brain barrier (BBB). Under normal conditions the BBB regulates selective transport of biomolecules needed to support the functions of the central nervous system (CNS) while preventing the entry of many factors that might be potentially harmful to the brain. Unfortunately, the presence of the BBB also poses a significant barrier to therapeutic delivery to treat diseases within the CNS; a problem especially important for the treatment of brain cancer. The precise nature of the cellular, sub-cellular and molecular characteristics of the BBB in brain cancer, compared to the healthy brain remains unclear. This project will focus on analyzing the expression of drug transporters and other factors in the BBB, in mouse and human brain tissue, both in pathological conditions, including cancer, as well as the healthy brain. The techniques used will be immunohistochemistry (IHC), including multiplex IHC.

Investigating the role of novel signalling-transcriptional mechanisms in tumour cells

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This project will use molecular and cell-based techniques to investigate the role of key factors involved in regulating brain tumour growth, drug resistance and cancer stem cell

biology. We recently reported a novel role of the cAMP pathway in triggering death in some brain cancer cell lines (1). The sensitivity or resistance of these cells to cAMP-induced death correlates with the MAPK pathway and CD44 expression. We have since developed a hypothesis that the transcription factors, Ets1 and Myb form a signalling-transcription network, cooperating with the cAMP, MAPK and PI3K pathways to enhance brain tumour cell malignancy and drug resistance. Human and animal cell lines, patient-derived brain tumour cells and patient specimens will be used for experiments. PCR, IHC and other molecular techniques will be used to investigate how upstream signalling pathways cooperate with the Ets1 and Myb transcription factors to regulate malignant brain cancer tumour cell function.

The role of the prion protein in brain cancer

Primary supervisor: A/Prof Vicki Lawson v.lawson@unimelb.edu.au

Location: Peter Doherty Institute & Melbourne Brain Centre, Kenneth Myer Building, on Parkville campus

Glioblastoma (GBM) is the most malignant and invasive tumour originating within the brain. Recent studies implicate the prion protein (PrP), a glycosylated, GPI-anchored signalling protein, has a role in cancer pathogenesis, including GBM.

This project will examine PrP isoform expression in GBM.

Techniques to be used include cell culture, western blotting, and immunohistochemistry.

Molecular basis for natural killer cell surveillance of cancers expressing PDGF-D

Primary supervisor: Dr Alexander Barrow alexanderdav@unimelb.edu.au

Location: Peter Doherty Institute

Many tumours secrete platelet-derived growth factor (PDGF)-D to promote tumour growth. Natural killer (NK) cells evolved the activating receptor NKp44 to sense tumour cell expression of PDGF-D and trigger the secretion of cytokines that halt tumour growth (Barrow et al. Cell, 2018). An alternatively spliced isoform of NKp44 is predicted to be inhibitory and associated with poor cancer prognosis. Cancers may induce the inhibitory NKp44 isoform to dampen NK cell function as a form of immune evasion. Using monoclonal antibodies specific for the different NKp44 isoforms, this project will use confocal microscopy to determine the functions of the different NKp44 isoforms and how they impact NK cell surveillance of cancers expressing PDGF-D.

The role of growth factor immunosurveillance in human cancers

Primary supervisor: Dr Alexander Barrow alexanderdav@unimelb.edu.au

Location: Peter Doherty Institute

Growth factor surveillance is a new mode of cancer immunosurveillance in which the immune system responds to growth factors overexpressed by cancer cells. However, definitive proof that growth factor surveillance plays a role in immune responses to human cancers remains to be determined. Our transcriptional analysis has implicated natural killer (NK) cell recognition of platelet-derived growth factor (PDGF)-D in the control of GBM. In collaboration with the Mantamadiotis group, the Barrow group is developing novel immunohistochemical methods to assess the role of NK cells and PDGF-D in overall survival of glioma patients in addition to other cancers that express PDGF-D.

Mining The Cancer Genome Atlas (TCGA) to identify novel tumour surveillance pathways and targets for immune checkpoint blockade.

Primary supervisor: Dr Alexander Barrow alexanderdav@unimelb.edu.au

Location: Peter Doherty Institute

The Cancer Genome Atlas (TCGA) is a cancer genomics program that has molecularly characterized over 20,000 primary cancers and matched normal samples spanning 33 cancer types. However, immune surveillance pathways associated with a favourable anti-cancer immunity for the many different cancers remains unknown. Moreover, immune pathways that are detrimental to patient survival that could be potentially blocked to promote anti-cancer immunity and exploited for cancer immunotherapy remain to be identified. This project will use in silico methods to identify novel immune surveillance pathways and molecular targets that can be exploited for immune checkpoint blockade in cancer.

Topical wound healing formulations in the context of diabetic complications

Primary supervisor: Dr Tom Karagiannis karat@unimelb.edu.au
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Cutaneous wound healing is a physiological response to a stimulus which results in injury to the skin, such as from cuts, abrasions and burns. It is essential to maintain skin integrity, as compromised skin leaves the body susceptible to external elements such as pathogenic microorganisms. This multicellular process of repair involves the replacement and restoration of lost or damaged tissue with less functional connective tissue. This results in the formation of a scar which continues to remodel and develop for various amounts of time, depending on the severity of the initial injury. The cutaneous wound healing process involves three overlapping phases: acute inflammation, proliferation and remodelling. The process involves different cell types, including cells of the immune system, keratinocytes, fibroblasts and endothelial cells, as well as many different molecular factors and cytokines. Immediately following skin injury, damage signals, both mechanical and chemicals, are sent out from damaged cells and blood vessels to other cells in the area. Damaged cells activate stress pathways, and also leak molecular signals such as damage-associated molecular patterns which indicate stress to other cells. Chromic ulcers which display delayed wound healing are a common complication of diabetes.

The aim of this BSc Honours project is investigate the effects of various dietary antioxidants and chromatin modifying compunds in various cell lines including human keratinocytes, endothelial cells and fibroblasts in models simulating diabetes. Further, we will utlize EpiDerm full thickness 3D models of human skin containing keratinocytes, fibroblasts and intact basement membrane for ex-vivo evaluations of topical formulations in response to high glucose induced stress.

Identification of novel dietary chromatin-modifying using molecular dynamic simulations

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Chemical modification of histones represents an important epigenetic mechanism critical for DNA metabolism including, transcription, replication and repair. A well-known example is maintenance of histone acetylation status by the opposing actions of histone acetyltransferase and histone deacetylase enzymes (HDACi) which add and remove acetyl groups on lysine residues on histone tails, respectively.

Although numerous compounds have been developed to specifically alter the function of chromatin modifying enzymes (for example, histone deacetylase inhibitors are relatively well-investigated), we are only at the early stages of understanding the long-term epigenetic effects of dietary biomolecules. In this project the student will utilise in silico molecular modelling approaches combined with known experimental affinities for controls, to identify potential dietary chromatin modifying compounds. Acetate and sodium butyrate, which are

well known dietary HDACi will be used as starting points for simulations. The student will have access to all of the software (including, Autodock Vina in PyRx, Swissdock and Hex-Server) and expert tuition to complete the relevant simulations.

DNA-targeted photoimmunotherapy for cutaneous T-cell lymphoma

Primary supervisor: Dr Tom Karagiannis karat@unimelb.edu.au
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The overall aim of this research area is to develop a platform technology for receptor-targeted isotope radiotherapy / diagnostic imaging and for UVA phototherapy. For the UVA phototherapy component of the project, we have developed, UVASens, an ultraviolet light (UV) photosensitiser, which is ~1000 times more potent than compounds used clinically for the treatment of cutaneous T-cell lymphoma.

Our approach involves the use of a DNA minor groove binding ligand to target the radioisotope (radiotherapy / imaging) or iodine atom (phototherapy) to DNA. The DNA binding ligands used for these projects are based on the structure of Hoechst 33342, a well known DNA stain.

The aim of this project is to develop appropriate vehicles for the delivery of the DNA ligand to specific target cells. This will be performed by encapsulation in antibody-coated nanoparticles. Given the safety concerns with the use of radioisotopes, unlabelled DNA ligands will be used for the initial proof-of-concept experiments in this project. In this context the fact that these DNA ligands are intrinsically fluorescent is an advantage. It will enable the determination of the efficiency of targeting by fluorescence microscopy / flow cytometry (fluorescence yield of DNA bound ligand increases by ~30-fold).

The project will involve preparing the antibody-coated nanoparticle-based formulations and targeting specific receptors on cancer cells. Receptor systems that will be evaluated will be the IL-2 antigen and malignant MyLa cells overexpressing the receptor, and epidermal growth factor receptors and A431 cells. Evaluation of the efficacy of the constructs will involve flow cytometry (FACS), cell viability and apoptosis assays.

Investigating the link between phenotype change and treatment resistance in prostate cancer

Primary supervisors: A/Prof Niall Corcoran niallmcorcoran@gmail.com
Prof Chris Hovens chovens@unimelb.edu.au

Location: Department of Surgery, Clinical Sciences Building, Royal Melbourne Hospital, Parkville

The development of resistance to androgen (male sex hormone) deprivation therapy (ADT), the primary treatment for aggressive prostate cancer, is not clearly understood. Our phylogenetic analyses of resistant tumours demonstrate no significant tumour evolution or clonal/subclonal selection with therapy, supporting the concept that resistant tumours are "hardwired" to survive in the castrate environment. We have previously found no mutation or structural variant consistently shared between resistant tumours at any of the gene/pathway/ontology levels, and no evidence of previously characterised genomic drivers of resistance. We have performed whole genome and RNA sequencing on paired pre- and post-treatment tumour samples obtained from high-risk patients undergoing profound androgen suppression for 6 months before prostatectomy, in whom clinical responses ranged from complete involution to no effect. Transcriptional profiling indicated that resistant cells undergo a phenotypic reprogramming in response to therapy that may be important for cellular survival and suggests that these changes are regulated by alterations in post-translational histone modifications. This raises the possibility that hardwired resistance is epigenetically, and not genomically mediated. Our data from patient-derived tumours grown in androgen-deprived conditions support the concept that cancer cells adapt to castration though histone mediated transcriptional reprogramming and development of a stem cell–like phenotype. This project will involve establishing an organoid model of prostate cancer and investigating the effect of perturbing key nodes in this adaptive process.

The Genomic Drivers of High-Risk Prostate cancer

Primary supervisor: Prof Chris Hovens chovens@unimelb.edu.au

Location: Victorian Comprehensive Cancer Centre, Grattan St, Melbourne

The issue of prostate cancer (CaP) in the Western world represents a major clinical problem with the prostate being the most cancer prone internal organ, but only an unpredictable 10% of these cases progress to lethality. Nearly all lethal cases are linked with metastasis and subsequent emergence of therapy resistant disease. Multiple genomic studies have now attempted to ascertain molecular subtypes of prostate cancer; however, these studies have been hampered by a lack of matching clinical follow up data and lacked the power to detect low level but clinically meaningful aberrations. So far none of the defined molecular subtypes could be linked with any clinically relevant patient outcomes. We have now performed an intensive whole genomic analysis of the largest cohort of patients with prostate cancer ever assembled to date. This data spans the spectrum of high-risk disease, enriched with metastatic events, combined with an expanded low-intermediate risk cohort and our analysis is yielding new prognostic biomarkers which can discriminate between low and high-risk disease. We have already amassed and whole genome sequenced this cohort of 550 tumours, all with comprehensive and ongoing clinical follow up. Our aim is now to take these differential signature sets and design targeted capture probes and interrogate non-invasive liquid biopsies

harvested from prostate cancer patients. We will then formally determine whether these signatures can quantifiably measure ctDNA in plasma and blood in a 3 staged approach on independent cohorts of patients and derive proof of principle evidence that this approach can screen early stage patients with high risk disease features.