Veronique Chachay, UQ Diamantina Institute

Red wine for fatty liver disease?

Non-alcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease in overweight people. It is characterized by fat accumulation in the liver, and increased risk of cardiovascular disease. There is currently no treatment for NAFLD other than weight-loss. Resveratrol is a compound found in the skin of grapes and red wine. In studies with obese rats, resveratrol was able to prevent fatty liver disease and mimic benefits of energy restricted diets. The present study investigated resveratrol as a possible treatment for NAFLD in humans. 20 obese men diagnosed with NAFLD were given resveratrol or placebo in capsules for 8 weeks. Numerous metabolic parameters were measured at the start and end of the study. Results showed no improvement in fatty liver disease with resveratrol, suggesting that high-dose resveratrol in capsules may not be an efficient treatment of obesity and fatty liver, even if it worked in animal studies.

Background: Non-alcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease in the obese population. NAFLD features hepatocyte triglyceride accumulation (steatosis), insulin resistance (IR) and dyslipidemia. NAFLD may progress to cirrhosis and hepatocellular carcinoma. It appears to be a precursor of type 2 diabetes, significantly increasing cardiovascular risk. Weight-loss is effective, but clinically challenging. Potential pharmacological treatment will ideally target both hepatic and cardiometabolic dysregulation. The polyphenol resveratrol found in grapes and other plants, has shown promising results in animal models of NAFLD. In over-fed animals, resveratrol prevented the development of steatosis, IR, inflammation, oxidative stress, and dyslipidemia.

Aims: To investigate the efficacy of resveratrol on hepatic and cardiometabolic dysregulation in patients with NAFLD.

Methods: Twenty obese non-diabetic men with NAFLD were randomized to 3000mg resveratrol (nutraceutical) or placebo daily for 8 weeks. Outcomes included: Peripheral IR measured by the euglycemic-hyperinsulinemic clamp; hepatic steatosis and abdominal fat distribution measured by magnetic

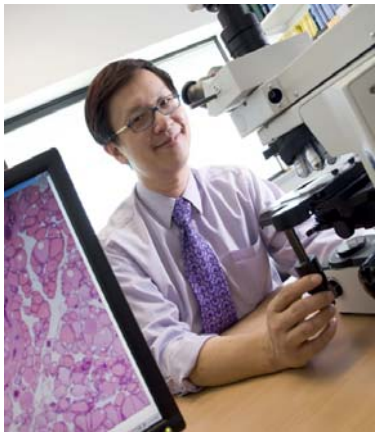
resonance spectroscopy and imaging; plasma biochemistry (liver function, inflammation, lipid profile, oxidative stress and antioxidant capacity); target gene transcription in peripheral mononuclear cells; resveratrol pharmacokinetics and safety.

Results: Resveratrol was well tolerated. Subjects presented with profound IR: glucose disposal rate (GDR)= 2.7±0.4mg/kg/min, and steatosis ranged from 6 to 54%. Resveratrol treatment did not result in change in GDR, steatosis, abdominal fat distribution, antioxidant capacity, lipid profile or gene transcription. Resveratrol was associated with significant increase in aminotransferases (ALT: 57±24 to 73±34U/L, p=0.03, AST: 36±9 to 45±15U/L, p=0.03), IL-10 (6.0±5.6 to 7.3±5.1pg/mL, p=0.03), and decreased IL-6 (12.5±15.4 to 8.6±11.3pg/mL, p=0.04).

Conclusion: Despite prevention of NAFLD in animals, nutraceutical resveratrol over 8 weeks did not demonstrate apparent clinical benefit in patients with established disease. Evidence of clinical efficacy and safety is required before high-dose nutraceuticals with purported claims of therapy for obesity-related complications, are available to the public.

Professor Alfred Lam, Griffith University

BRAF mutations in thyroid cancer



This research studied how mutations in a gene called BRAF affect the severity and outcomes of patients with thyroid cancer. We examined the characteristics and mortality of 123 patients with thyroid cancer and compared the prevalence of BRAF mutation in different groups. We also combined with international partners in 7 countries to expand the research population to 1849 patients. Finally, we also examined the activity of a gene called vascular endothelial growth factor (VEGF), which controls growth of blood vessels. We discovered a high prevalence of BRAF mutation in thyroid cancers, with mutation rates correlating to more aggressive cancers, production of VEGF and cancer mortality. Thyroid cancer is difficult to differentiate from benign thyroid diseases. Our results allow use of BRAF mutation to improve detection and treatment. Knowing the connection between BRAF and VEGF opens the way to using combined targeted therapies with improved results compared to current treatments.

This research aimed to study the prevalence of BRAF mutation in thyroid cancer and to evaluate its relationship to clinicopathological parameters of thyroid cancer in local and international patients. We also aimed to identify the relationship between BRAF mutation and vascular endothelial growth factor (VEGF) expression in thyroid cancer.

The pathology, clinical parameters, BRAF mutations and VEGF mRNA expression were studied in 123 patients with thyroid cancer. The results were part of a multi-centre study involving 7 countries and 1849 patients analysing the prognostic impact of BRAF mutations.

The prevalence of BRAF mutation was 45%. BRAF mutation was significantly more common in conventional papillary thyroid carcinoma than follicular variant (58% versus 31%, p=0.022). Also, the mutation was more often noted in high stage cancers (p=0.034) and in cancers with lymphocytic thyroiditis (p=0.006). BRAF mutation correlated with VEGF-A expression in

thyroid cancer ($p=0.05$). In multi-centre analysis, cancer mortality was 5.3% versus 1.1% ($p<0.001$) in BRAF–positive versus –negative patients.

Significance to health and medicine

Thyroid cancer is difficult to differentiate from benign thyroid lesions. Detection of the prevalence of BRAF mutation in thyroid cancer and its association with clinicopathological parameters make it possible to be used as both diagnostic and prognostic markers in clinical practice. We also explored the impacts of pathological factors such as histological variants and lymphocytic thyroiditis in the thyroid cancer which are novel findings. Some patients with thyroid cancer develop progressive disease which needs molecularly targeted drugs, with BRAF and VEGF (anti-angiogenesis) being common targets. In this study, we demonstrated in a large cohort of patients that these genes are linked and simultaneous blocking can be beneficial to patients with thyroid cancer. In addition, we demonstrated for the first time by an international study that BRAF mutation in thyroid cancer has significant impact on prognosis and mortality.

Dr Tara Roberts, Queensland Institute of Medical Research

Low levels of SMG1 protein increase cancer risk

Cancer is one of the greatest burdens to Australia's ageing population. In this study we have identified a gene, Smg1, which is involved in limiting cancer development. In an animal model decreased production of SMG1 protein from this gene resulted in an increased risk of developing lung cancer, lymphomas and chronic inflammation. Mutations in the Smg1 gene have also been found in human cancers. In humans chronic inflammation can increase the risk of developing cancer. Our study showed that the inflammation increased prior to the cancers developing suggesting that decreased SMG1 protein lead to inflammation and subsequent cancer development. Increasing SMG1 activation may therefore be a target for new cancer drug development or SMG1 loss could be used to identify cancers which could be successfully treated with anti-inflammatory drugs.

SMG1 is a member of the phosphoinositide kinase-like kinase family of proteins that includes ATM, ATR and DNA-PK, proteins with roles in DNA damage and cellular stress responses. SMG1 has a well-characterized role in nonsense-mediated decay as well as suggested roles in the DNA damage response, resistance to oxidative stress, regulation of hypoxic responses, and apoptosis. To understand the roles of SMG1 further, we generated aa Smg1 mouse model. Smg1 homozygous knockout mice were embryonic lethal, but Smg1 heterozygous mice showed a predisposition to a range of cancers, particularly lung and hematopoietic malignancies, as well as development of chronic inflammation. These mice did not display deficiencies in known roles of SMG1, including nonsense mediated decay. However, they did display increased pro-inflammatory cytokine production (interleukin (IL)-6, CSF-1 and IL-1β) in lung, heart, spleen and thymus as well as evidence of reactive oxygen/nitrogen species mediated damage to lymphoid rich tissues prior to the onset of disease. This was not due altered numbers of monocytes, granulocytes, neutrophils, B or T cells as these were equivalent to wildtype animals prior to disease. Our findings are supported by human data in the literature. Mutations in SMG1 have been associated with lung adenocarcinoma, breast, kidney and stomach cancer; these include mutations within the kinase domain and frameshift mutations which are likely inactivating mutations. SMG1 expression was also lost in a study of malignant lymphoma samples in contrast to strong SMG1 expression in normal lymphoid tissues. Combined with our findings this suggests that SMG1 may normally act to suppress tumour formation and that Smg1 deficiency leads to increased

risk of a number of cancers, particularly lung and haematopoietic cancers. Our data also indicates that SMG1 regulates inflammation prior to cancer development, possibly as a mechanism of tumour suppression.

Dr Kate Schroder, UQ Institute of Molecular Biosciences

Cellular players in inflammasome-dependent anti-microbial defence

The innate immune system is the body's front-line weapon against attack by microorganisms. My laboratory characterises the fundamental pathways by which the body senses microbial 'danger' and launches anti-microbial defence programs. In particular, we research the biology of a molecular machine called the 'inflammasome', which drives inflammation during infection. In this study, we identified new and surprising cell types that protect against infection through the inflammasome pathway, and in doing so, we made the important finding that inflammasomes trigger distinct inflammation-driving pathways in different cell types. There are 13 million deaths from infectious disease globally each year, and the sharp rise in bacterial antibiotic resistance makes the need for broad-acting anti-infective agents and vaccines increasingly urgent. Our research makes fundamental contributions to our understanding of the body's natural ability to fight infection and may ultimately lead to the development of new anti-infective agents or vaccine formulations to combat infectious disease.

The past 15 years has witnessed a dramatic change in the landscape of immunological research. The discovery that families of innate immune receptors recognise microbial structures, and trigger potent inflammatory responses to control infection has revolutionised the field and thrust innate immune research into one of the most exciting topics of modern immunology. Recent findings that these same antimicrobial receptors also recognise host-derived molecules indicative of organismal 'danger' (e.g. cell injury/dysfunction, metabolic stress) have, for the first time, provided molecular mechanisms for sterile inflammation in diseases such as gout and diabetes. One such innate immune receptor pathway is a multiprotein signalling complex called the 'inflammasome'. The inflammasome is a molecular danger detection system that triggers caspase-1-dependent cytokine production and thereby launches both protective and pathogenic immune system activation. An important but neglected question in the field is which

cells are important for generating inflammasome-dependent cytokines during in vivo infection or inflammatory disease? Inflammasome function has been mainly characterised in macrophages and dendritic cells in vitro, which has led to an assumption that these cells are critically important to inflammasome function *in vivo*. In this study, we systematically profiled the suite of inflammasomes expressed in human and mouse cells of the innate and adaptive immune systems, and studied the contribution of individual cell types to inflammasome-mediated host defence in vitro and in vivo. Our data reveal surprising new cellular players and cell type-specific signals engaged by inflammasomes during infection, and suggest an elegant interplay between innate immune cells for co-ordinating inflammasome-dependent immune defence in vivo. Our study gives insight into the molecular and cellular mechanisms driving inflammation, which may ultimately lead to the development of new anti-microbial agents or vaccine formulations that are urgently needed to combat the 13 million deaths that occur from infectious disease globally each year.

Dr Trent Woodruff, University of Queensland

The C3a Receptor Mediates Protection from Ischemia-Reperfusion and Spinal Cord Injuries by Inhibiting Neutrophil Mobilization

The complement system is an integral component of the body's immune system, however, it can also lead to tissue injury if unchecked. A major component of this system is the complement factor C3a, which is involved in inflammatory diseases such as sepsis, trauma, and heart attacks. In this study, we used mice that are unable to react to C3a, and a novel drug developed by us, to explore the function of this receptor in acute tissue injuries to the intestine and spinal cord. We found, that C3a is essential to reducing the number of white blood cells available to attack damaged tissues, and that by administering a potent C3a drug mimic, these injuries were reduced. This research has therefore revealed a new therapeutic target, and novel drug candidates, which may be useful in treating traumatic injuries typically arising from car accidents, such as crush injuries and spinal cord injuries.

The complement system is a major component of the innate immune system, and is implicated in the pathology of acute tissue injury through the activation of leukocytes such as neutrophils. C3a is a key complement activation fragment, yet its neutrophil-expressed C3a receptor (C3aR) still has no known role. In this study, we utilised two neutrophil-dependent mouse models of acute tissue injuries to explore the role of C3aR: an intestinal ischemia-reperfusion (IR) injury model, and a traumatic spinal cord injury model. Mice lacking the C3aR ($C3aR^{-/-}$) had worsened intestinal and spinal cord injury, which corresponded with increased tissue-infiltrating neutrophils. Circulating neutrophils were significantly increased in $C3aR^{-/-}$ mice after intestinal ischemia, or spinal cord injury. $C3aR^{-/-}$ mice also mobilized more circulating neutrophils following granulocyte colony-stimulating factor (G-CSF) infusion compared to wild-type mice, indicating a specific role for C3aR in constraining neutrophil mobilization in response to tissue injury. In support of this, $C3aR^{-/-}$ mice, reconstituted with wild-type bone marrow, reversed IR and spinal cord pathology back to wild-type levels, demonstrating the central role of mobilization of bone marrow neutrophils in the pathogenesis of tissue injury. C5aR antagonism in $C3aR^{-/-}$ mice also rectified the worsened pathology

after intestinal IR, but had no effect on circulating neutrophils, highlighting the opposing roles for complement C3a and C5a in disease pathogenesis. These results support blocking neutrophil mobilization or egress from the bone marrow as a clear strategy for reducing tissue injury. To demonstrate this, we showed that using a potent C3a agonist to activate C3aR *in vivo*, reduced neutrophil mobilization and ameliorated intestinal IR pathology in wild-type, but not in $C3aR^{-/-}$ mice. This study therefore identifies a novel role for C3aR in regulating neutrophil mobilization following acute intestinal and spinal cord injuries and highlights C3aR agonism as a new and potential treatment option for acute, neutrophil-driven, pathologies.

Dr Jyotsna Batra, QUT Institute of Health and Biomedical Innovation

Identification and characterization of a novel genetic signature at the 5p15region associated with risk of prostate cancer

Prostate cancer is the most common cancer in Caucasian men. However, early detection through screening programs has proven challenging, and about 30% of the 19,000 new cases diagnosed annually in Australia already have advanced disease. Discovery of novel early detection biomarkers is highly warranted. We replicated the association of a genetic variant on chromosome 5 with the risk of prostate cancer in Australian men, initially identified in a Japanese population. We also undertook the fine-mapping of this region to identify the precise genetic variant behind this association, and the analysis of the functional role of these novel gene/s and variants in prostate cancer pathology is now underway. Our results supplement the ongoing genetic research in prostate cancer, which aims to provide a foundation for the development of sensitive and readily applicable screening tools to be used clinically, and will also provide impetus for drug-targeted research by further our understanding on this multifactorial disease.

Prostate cancer (PCa) is the most frequently diagnosed cancer in males in developed countries. To date, genome-wide association studies (GWAS) have identified ~70 genetic loci that confer modest increased risk of PCa. Five additional novel risk loci were identified in a recent GWAS of Japanese individuals. We proposed that apart from linkage disequilibrium (LD) patterns, limitations of Stage1 GWAS power to prioritize single nucleotide polymorphisms (SNPs) for publication and/or study design could explain the missed reporting of these loci in previously conducted GWAS on Caucasians. In the absence of the knowledge of where the causative SNP may be located, we investigated the possible differences in LD patterns between Japanese and European populations for a +/-10kb region around the 5 SNPs. This extended analysis of Fst values suggests that heterogeneity may drive differences between Japanese and European populations only for two of the five SNPs. Thus, we undertook a replication study in 1,357 PCa patients and 1,403 normal Australian males of European descent. We found the rs12653946 SNP at *5p15* to be significantly associated with risk of PCa (OR=1.20, 95% CI=1.07-1.34,

p=0.002), with per-allele effect size similar to that reported in Japanese men. We then undertook fine-mapping of this region in 50,000 PCa cases and controls through 21 collaborative studies (PRACTICAL consortium), and identified rs10866528 to be the most significant risk SNP is this region (OR=1.09, 95% CI=1.06-1.12, $p=4.8 \times 10^{-10}$). On the basis of LD calculations, the rs10866528 SNP represents an independent locus, distinct from the previously identified *TERT-CLPTM1L* cancer nexus region. Further, our bioinformatic analysis indicates that rs10866528 falls in the intron of a testis-expressed gene predicted to translate a conceptual 8.1kDa protein named tojy.
Significance to health and medicine: Our findings indicate that follow-up of apparently ethnic-specific risk associations are warranted to highlight risk-associated loci for experimental studies, and for incorporation in future risk prediction models for PCa.

Dr Sumaira Hasnain, Mater Medical Research Institute

Improving insulin production in Type 2 Diabetes by modulating local cytokines

Obese individuals are prone to developing type 2 diabetes, characterized by high blood sugar. Insulin is a hormone that normally controls blood sugar. In diabetes, insulin production by beta cells in the pancreas fails to keep up with demand. We have found that insulin production fails because of immune factors that cause stress in the protein production machinery within beta cells. We have also found another immune factor which protects beta cells and maintains insulin production. In obese mice with diabetes, we have reversed the failure to produce insulin by interfering with the stress-inducing factors or by treating with the protective factor, pointing to new ways to treat diabetes.

The burgeoning global prevalence of obesity and type 2 diabetes (T2D) is associated with significant morbidity and mortality, burdening healthcare systems. In obesity, high nutrient intake and declining insulin sensitivity in peripheral tissues result in increased demand for insulin biosynthesis and secretion by pancreatic β-cells. T2D ensues when insulin secretion ultimately is insufficient to control blood glucose concentrations. Although β-cell dysfunction is associated with pancreatic inflammation and endoplasmic reticulum (ER) stress, these have not been directly mechanistically linked. We have the first evidence that a suite of cytokines, previously not identified as contributors to β-cell dysfunction, induce ER stress, thereby impairing insulin biosynthesis and secretion, whereas another cytokine, IL-22, suppresses ER stress and restores insulin production. In cultured β-cells and murine pancreatic islets, the innate cytokines, IL-23, IL-24 and IL-33, were the most potent inducers of β-cell ER stress, acting via distinct intracellular mechanisms. These cytokines were elevated concomitantly with ER stress in the pancreatic islets in murine T2D. In mice with high fat diet-induced diabetes, antibody neutralization of IL-23 or IL-24 lowered pancreatic ER stress, and improved insulin production and glucose tolerance. In contrast, IL-22 was identified as a cytokine that potently supresses ER stress and insulin secretory abnormalities in β-cells. Systemic administration of recombinant IL-

22 to diabetic mice eliminated pancreatic ER stress and decreased pancreatic inflammation, while reversing β-cell abnormalities. IL-22 promoted high quality insulin production, integrity and secretion, which restored glucose homeostasis without altering peripheral insulin sensitivity. Thus we have identified specific non cell-autonomous regulation of β-cell ER stress by immunity, and shown that therapeutic manipulation of these pathways provides efficacious treatment, reversing the hyperglycaemia that drives diabetes pathology.

Dr Kyle Upton, Mater Medical Research Institute

Revealing the Extensive Role of Mobile Genetic Elements in Genetic Disease

Mobile Genetic Elements (MGEs) account for more than 60% of the human genome sequence, and use a copy and paste mechanism to replicate. While most elements are inactive, a small number remain active and have the potential to alter or destroy gene function which can lead to genetic disease including cancer. Understanding the prevalence and effect of these insertions has been limited by technical difficulties in identifying rare insertion events. To address this issue, I have played a lead role in the development and application of a high throughput sequencing technique which produces an accurate high resolution map of MGE insertions in the human genome. I have produced first author publications in the highest impact journals Nature and Cell, describing MGE mobilisation in the brain and in liver cancer. Further collaborative projects are ongoing with promising results. This ground breaking research enhances understanding of the aetiology of genetic disease.

Retrotransposons are mobile genetic elements which use a copy and paste mechanism to replicate in the metazoan genome, and comprise more than 60% of the human genome. Of the millions of insertions in the genome, fewer than 0.1% remain active, with as few as six believed to drive the majority of new insertions. Nonetheless they remain a powerful and underappreciated source of insertional mutagenesis. Over 100 instances of diseases have been linked to retrotransposon insertions, however new insertions are difficult to identify and the true prevalence is likely to be much higher. To this end, I have played a lead role in the development of a novel high throughput sequencing technique to identify recent retrotransposon insertions in the human genome, Retrotransposon Capture Sequencing (RC-Seq). Using RC-Seq I have detailed a large level of retrotransposon mobilisation in human post-mortem brain samples. This finding was communicated as a first author publication in Nature, and recognised as the joint most important finding in 2011 by the National Institute of Mental Health, USA. Retrotransposon mobilisation is well characterised in the germline and occurs in somatic tissues at a lower rate. This was demonstrated in another first author publication in Cell where I have described multiple aetiologies of Hepatocellular Carcinoma resulting from retrotransposon mobilisation. RC-Seq provides base pair resolution of retrotransposon insertion sites. By intersecting this data with gene function knowledge bases we can identify both risk factor alleles, and disease causing somatic mutations. This research has broad applications for any disease with a genetic component. I am currently pursuing multiple collaborations to

understand the broader role of retrotransposons in normal development, disease susceptibility, spontaneous disease initiation, cancer initiation and chemotherapy resistance.

Kerina Denny, University of Queensland

A Role for the Immune System in Neural Tube Defects

Neural tube defects are one of the most common birth defects, affecting approximately 1 in 1000 pregnancies worldwide. The most frequent neural tube defect is spina bifida, a condition that can cause bladder and bowel incontinence, severe infections, and paralysis. At present, our most effective weapon to prevent neural tube defects is dietary supplementation with folic acid prior to pregnancy. However, despite folic acid food fortification programs, neural tube defects continue to be a significant physical, emotional, and financial burden to individuals, families, and the healthcare system. Recent research in our laboratories has revealed a role for a key component of the immune system, the complement system, in the formation of the neural tube in both mice and humans. It is hoped that this research will provide a novel platform on which to develop new strategies to prevent neural tube defects.

Background: Preconceptional folic acid markedly reduces the incidence of neural tube defects (NTDs). However, despite food fortification programs, NTDs remain a leading cause of perinatal morbidity and mortality. There is thus a pressing need to investigate novel risk factors for, and mechanisms of, abnormal neurulation. This study addressed the hypothesis that the complement factor C5a, a key component of innate immunity, is involved in the pathogenesis of NTDs.

Aims: To determine (1) the expression and localisation of complement factors in the developing mouse and human neural tube; and (2) whether pharmacological blockade and/or genetic ablation of the receptor for C5a, C5aR, affects neural tube development in mice under both folate-replete and folate-deficient conditions.

Methods: *In situ* hybridisation was employed to determine the expression pattern of C5aR mRNA in the mouse embryo throughout the period of neurulation. Expression of C5aR protein in both the mouse and human neural tube was confirmed with fluorescent immunohistochemistry. To investigate whether C5aR was involved in neural tube closure *in vivo*, folate-replete and folate-deficient pregnant mice were administered a specific C5aR peptide

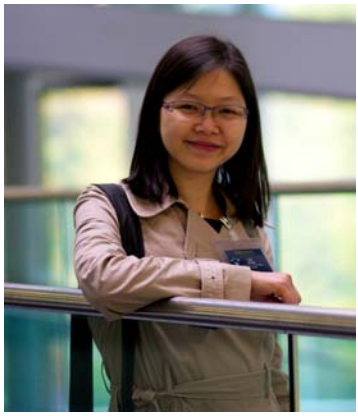
antagonist in early gestation. C5ar1$^{-/-}$ mice were additionally employed to confirm results observed with the C5aR antagonist.

Results: C5aR mRNA and protein were expressed in the developing mouse neuroepithelium throughout the period of neurulation. C5aR was also found to be expressed in the neuroepithelium of early human embryos. Ablation of the C5ar1 gene or the administration of a C5aR antagonist to folate-deficient pregnant mice resulted in a high prevalence of NTDs compared to mice with a functional C5aR.

Conclusions: This study demonstrates, for the first time, an interaction between dietary folate deficiency and the immune system in the pathogenesis of NTDs, and provides a novel platform on which to develop new strategies to prevent NTDs.

Tam Duong, UQ Institute of Molecular Bioscience

Genetic ablation of SOX18 function suppresses tumour lymphangiogenesis and metastasis of melanoma in mice



Cancer metastasis is responsible for 90% of deaths in cancer patients with solid tumours. Therefore, it is important to understand the environment within the body, that helps cancer cells to escape their primary site and move to distant organs. My work focuses on the lymphatic vessels that surround the tumour. These vessels are normally in charge of trafficking immune cells and reach most part of the body, therefore they can also serve as efficient ways for cancer to spread. Using a melanoma cancer model, I identified that the protein SOX18 was turned on and triggered new vessels to form around tumour tissue allowing the cancer spread. When SOX18 was absent, there was less new lymphatic vessel formation, and less cancer metastasis. My research provides new approaches to block cancer metastasis by disrupting SOX18 activity and thereby inhibiting lymphatic vessel formation.

Previous work has identified the transcription factor SOX18 as a molecular switch to induce lymphangiogenesis in the mouse embryo. My recent work (Duong et al, 2012, *Cancer Research*) demonstrated that SOX18 was re-expressed in tumour lymphatic vessels and played a key role to induce tumour lymphangiogenesis. Using *in vivo* live imaging approach, I showed that genetic disruption of SOX18 function interfered with tumour lymphangiogenesis, kinetics of tumour lymphatic drainage and, remarkably, cancer metastasis to regional lymph node in a melanoma mouse model. In a second study, I have investigated further the molecular mode of action of SOX18 and uncovered previously unknown genetic interaction between SOX18 and VEGF-D during early vascular development (Duong et al, 2013, under review) (3). In particular, VEGF-D can enhance SOX18 transcriptional activity *in vitro* and *in vivo* by inducing SOX18 concentration in the nucleus. This work suggests that VEGF-D mediated cancer metastasis include or involve an underlying dysregulation of SOX18-mediated transcriptional networks.

Outcomes and Significance: My discovery has provided the first evidence of SOX18 function during tumour lymphangiogenesis and metastasis.

These finding are particularly congruent with recent clinical study from Eom et al (2012), revealing that SOX18 is a key indicator to stage gastric tumour progression and can be considered as a prognostic marker for gastric cancer patients. This study has opened novel therapeutic approaches targeting SOX18 to inhibit tumour neo-lymphangiogenesis, and hence cancer metastasis, without compromising existing lymphatic vessels, which do not express SOX18 in normal condition.

Future directions: Following up this study, our current work is focused on developing a small molecule inhibitor targeting SOX18 activity to block the formation of tumour neo-lymphangiogenesis and lymphatic spread.

This strategy is particularly important as a complementary therapy for anti-cancer metastasis since the evasive resistance is emerging in current anti-angiogenic therapies based on VEGF blockage

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Yi Lu, Queensland Institute of Medical Research

Successful application of endophenotype approach in understanding genetics of complex eye diseases

We conducted the world's biggest study into the genetics of eye disease, which has made discoveries with major implications for prevention and treatment of keratoconus. Keratoconus is a progressive abnormality of the cornea. It changes from its normal curve to a more conical shape, distorting vision. It affects about 10,000 Australians, most of them young. Owing to the limited availability of medical treatments, keratoconus is the leading causes of corneal transplantation worldwide. The genome-wide study of 20,000 people, including 1000 Queensland twins, has identified 16 genes that influence corneal thickness, six of them greatly increasing the chances of having keratoconus. One of the new genes identified was also linked to open angle glaucoma, the most common type of glaucoma. These findings mean that specialists can now screen people for keratoconus, to identify individuals at highest risk, and to ensure their eyesight being closely monitored.

Dissecting the genetics of key risk factors may provide insights into the associated disease etiology. We started a large meta-analysis of genome-wide association studies on corneal thickness from over 20,000 individuals, in order to identify variants that not only influence corneal thickness but may also shed light on the genetic cause of complex eye diseases including keratoconus and glaucoma.

We identified 16 new genetic variants associated with corneal thickness, which lead to multiple biological and clinical insights. First, we showed that these variants converge on collagen and extracellular matrix pathways, which regulate corneal thickness in both European and Asian populations. Second, despite thinner corneas in glaucoma patients, there was significant overlap in genetic loci identified from individuals affected and unaffected with glaucoma, suggesting similar pathways regulating corneal thickness regardless of disease status. Third, we showed that genes harboring rare variants causing Mendelian disorders with clinical feature of extreme cornea thinning (for example, Brittle

Corneal Syndrome), also harbor common variants that influence corneal thickness in the general population.

The last but not least, we found that, despite the modest effect on corneal thickness, six genetic variants convey large risks on keratoconus and one linked to glaucoma. These variants are common in the population, thus may provide great importance in terms of overall public health. A risk profile based on these risk variants yielded reasonable risk prediction for keratoconus. For example, individuals carrying these risk variants are at 7.2-fold increased risk of keratoconus relative to individuals not carrying any. Therefore, further evaluation of the clinical application of these variants is merited.

This study showcased the endophenotype approach yielding disease relevant loci and showed that at least part of the genetic predisposition to complex eye diseases is mediated through the genes underlying corneal thickness.

Bridget Maher, Griffith University

Identification of X-linked genes in migraine susceptibility

Migraine is a common neurological disorder characterised by debilitating head pain and associated temporary neurological disturbances. The disorder affects 18% of women and 6% of men yet effective treatments and laboratory-based diagnostics are limited. Migraine is known to run in families and has a significant genetic basis. Previous studies in our laboratory have implicated the X-chromosome in migraine susceptibility. In this study we identified 2 additional migraine pedigrees that show linkage to a known susceptibility region on the X-chromosome. Subsequent studies using an unrelated case-control cohort identified a migraine risk haplotype in a gene involved in cholesterol biosynthesis. Further investigation of the X-chromosome in the Norfolk Island population also supported these findings and identified a new susceptibility region with association observed at a neuronally expressed gene involved in iron homeostasis. This research provides new evidence to support an X-chromosomal contributor to migraine susceptibility and novel avenues for migraine research.

Migraine is a debilitating neurovascular disorder with limited effective treatments or lab-based diagnostics. The disorder affects an estimated 12% of the population with a significant female preponderance of 3:1. Combined with a strong tendency for migraine to run in families, this evidence suggests that an X-linked genetic variation(s) may be contributing to the disorder and may explain some of the observed epidemiological trends.

To investigate this hypothesis my PhD research employed pedigrees, case-control cohorts and the genetic isolate of Norfolk Island to analyse the X-chromosome. Initially the research focused on a known susceptibility region at Xq24-q28. Using microsatellite and SNP analysis we identified 2 new migraine pedigrees that also showed linkage to the Xq27-q28 region with haplotype analysis refining the susceptibility locus to a 2.4Mb region. Subsequent candidate gene studies using an unrelated case-control cohort identified a risk haplotype (P=0.009) in the NSDHL gene involved in cholesterol biosynthesis (Maher et.al, 2011, Neurogenetics).

An X-chromosome scan was also undertaken using a large core-pedigree (n=288) from the genetic isolate of Norfolk Island. Results showed that of the top 25 SNPs (ranked by P-value) 13 localised to the Xq27-q28 region and 10 mapped to a new locus at Xq12. Two major haplotype blocks were identified at Xq12 each containing a haplotype significantly over-represented in migraineurs (P<0.0005). Follow-up analysis showed evidence of association in

the large WGHS cohort with a significant association at rs1028348, located within the 5'UTR of the HEPH gene - a neuronally expressed gene involved in iron homeostasis (Maher et.al, 2012, PLoS One).

Overall, this research provides compelling evidence for involvement of the Xq27-q28 region and a novel susceptibility locus on Xq12. Follow-up analysis in the migraine pedigrees is underway using NGS to determine causal genetic variants within these loci that will further the understanding of the molecular mechanisms underlying migraine.

Michelle Neller, Queensland Institute of Medical Research

Broader is better: the number of targeted antigens correlates with clinical outcome following immunotherapy

Clinical trials of immune-based therapies for advanced melanoma have produced variable clinical outcomes. Although partial remission (PR) or complete remission (CR) occasionally results, most patients have progressive disease (PD). Our group reviewed the outcomes of 75 published clinical trials and identified higher response rates for patients who received therapies with the potential to generate immune responses against a wide range of tumour proteins. We also conducted clinical trials of one such immune therapy, which resulted in 15-33% of patients experiencing CR or PR. We assessed whether breadth of immune response correlated with clinical outcome by studying "T-cells" (immune effector cells) isolated from the blood of eleven patients. We found that T-cells from patients with good clinical outcomes recognised a much wider range of known tumour proteins than PD patients. Our data suggest that future immune therapies should generate T-cell responses against a broad range of tumour proteins.

Clinical trials of immunotherapies for advanced melanoma have produced variable clinical outcomes. Although successful clinical responses have been reported, i.e. partial remission (PR) or complete remission (CR), the majority of patients have had progressive disease (PD). Indeed, when our group assessed the outcomes of 1601 melanoma patients from published clinical trials, we found that only 10.2% of patients experienced CR or PR. Interestingly, the objective clinical response rate was significantly higher in trials of vaccines using antigens derived from tumour cells (11.2%), compared with trials of defined antigen vaccines, such as peptide or protein-based formulations (6.3%; $p<0.001$). It is possible that these contrasting response rates are due to the breadth of immune response generated by vaccines using different antigen sources. Our group has conducted clinical trials in which advanced melanoma patients received a fully autologous immunotherapy based on an undefined antigen source – irradiated tumour cells. Of the patients who completed these trials, 15-33% had CR or PR. To explore

whether breadth of immune response correlated with clinical outcome in our trials, we assessed the targets of anti-tumour T-cells from patients across three clinical response groups. Peripheral blood mononuclear cells from eleven patients were stimulated with irradiated autologous melanoma cells, then enriched for CD8+ T-cells – the key immune effector cell subset. These CD8+ enriched mixed lymphocyte-tumour cell cultures (CD8+MLTC) were then screened against a panel of 39 known melanoma antigens in functional assays. Strikingly, the number of targets recognised by CD8+MLTC from CR and PR patients was found to be significantly higher than those recognised by CD8+MLTC from PD patients. Therefore, the breadth of targets of anti-tumour T-cells is potentially a key factor in determining clinical outcome. These results suggest that the efficacy of immunotherapies could be improved by generating T-cell responses against a broad range of tumour antigens.

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A comprehensive investigation of the risky driving behaviour of young novice drivers

This thesis examined the factors contributing to the increased crash risk of young drivers. These factors include individual characteristics like anxiety and sensation-seeking; driving behaviours such as speeding; the structural environment of Queensland's graduated driver licensing program; and social factors including the influence of parents, friends, and the Police. The research has provided substantial insight into the behaviours of young novice drivers, such as speeding, through the use of a purpose-built scale and application of psychological theory. The impact of the July-2007 changes to Queensland's graduated driver licensing program was also identified through a comparison to the behaviours of novice drivers who progressed through Queensland's pre-2007 licensing program. In addition, the research has increased our understanding of how the broad range of personal, social and structural influences can impact upon the behaviour of the young novice driver whilst they drive with a Learner and then a Provisional 1 driver's licence.

Background: In Australia in 2011, young drivers aged 16-25 years comprised 13% of the population but contributed 22% of the crash fatalities. Research was undertaken to explore the young driver's experiences as a Learner and Provisional 1 driver, and to identify the nature and mechanisms of social (parent, peers, Police) and structural (graduated driver licensing, GDL) influences upon driver behaviour to inform the design of more effective countermeasures.

Methods: Research comprised small group interviews (n = 21) and Queensland-wide surveys ($n1$ = 761, $n2$ = 1170, $n3$ = 390); surveys two and three part of a six-month longitudinal study.

Results: The experiences of young drivers in the post-July 2007 GDL program were compared to those of the former program, with significantly greater, and less difficulty gaining, practice reported by current-GDL Learners. The Behaviour of Young Novice Drivers Scale improved the understanding of young driver behaviours; behaviours pertaining to transient violations, fixed violations, misjudgement, risky driving exposure, and driver mood. Parental, peer, and Police influences were evident across pre-Licence, Learner and

Provisional periods, with a lack of punishment in particular perceived as rewarding by the young driver.

Implications: Interventions enhancing the positive influence of parents and peers are likely to improve road safety outcomes not only for young drivers, but for all persons who share the road with them. Parent-targeted interventions warranting further development and evaluation include modelling safe driving behaviour; monitoring of driving during the pre-Licence, Learner and Provisional periods; and sharing the family vehicle during the first six months of independent licensure. Peer-targeted interventions include minimisation of social reinforcement and promotion of social sanctions for risky driving behaviour in particular. Random deployment of Police enforcement operations are also recommended, as is the consideration of additional sanctions for supervisors complicit in the risky and illegal behaviour of the Learner driver.