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TRI ANNUAL POSTER SYMPOSIUM 2014

Thursday 16th October

10.30am - 5pm TRI Level 2

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To collaborate you need to build relationships with researchers from different fields and institutes. This symposium will give you the opportunity to talk with your colleagues, and to share your scientific findings with each other.

Best wishes for a successful day.

Prof Ian Frazer
CEO and Director of Research

TRI

PROGRAM

9.00 am - 10.15 am
Registration - TRI Auditorium Foyer

10.30 am - 10.40 am
Welcome from Professor Ian Frazer - TRI Auditorium

10.40 am - 12.00 pm
Oral Presentations - TRI Auditorium

12.00 pm - 12.45 pm
Lunch - TRI Atrium

12.45 pm - 2.10 pm
Poster Session 1 - Seminar rooms 2003 & 2004

2.10 pm - 2.40 pm
Afternoon tea - TRI Atrium

2.40 pm - 4.00 pm
Poster Session 2 - Seminar rooms 2003 & 2004

4.00 pm - 4.45 pm
Keynote address and award presentations - TRI Auditorium

5.00 pm

Post-symposium social event - TRI Atrium

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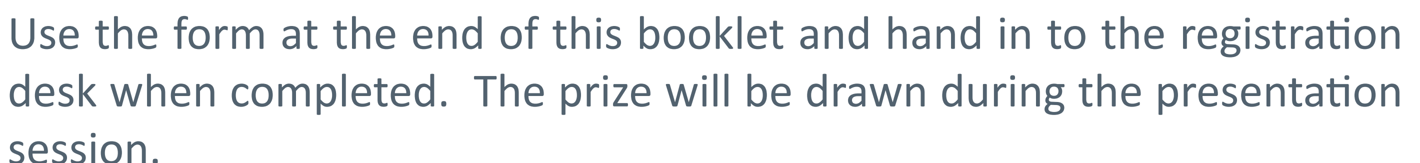

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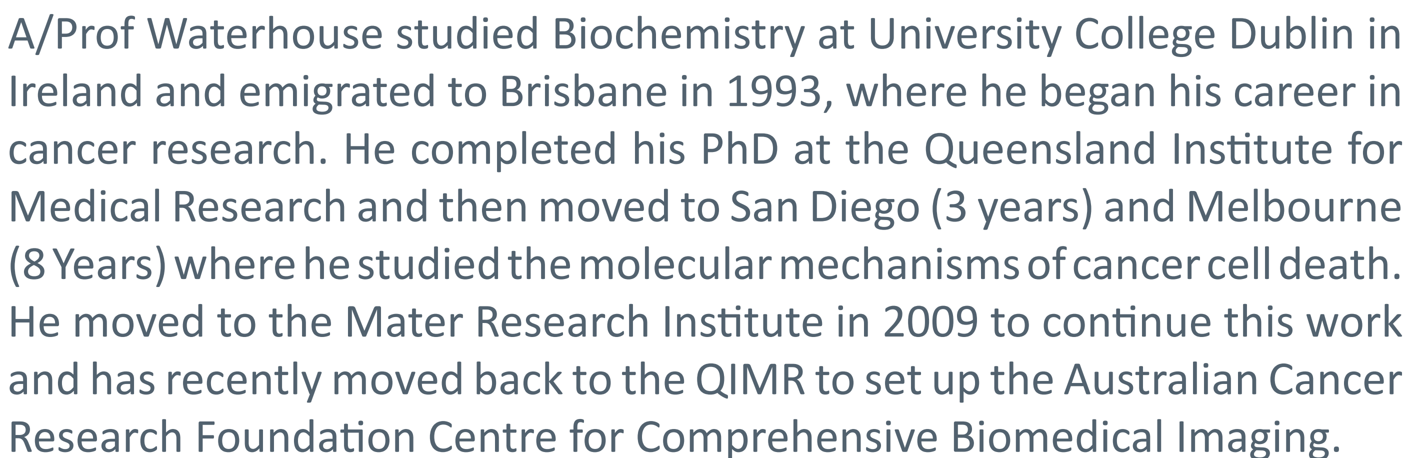
KEYNOTE SPEAKER

A/PROF. NIGEL WATERHOUSE

Nigel Waterhouse is currently Microscopy Facility manager at the QIMR Berghofer Medical Research Institute and Associate Professor at Mater Research Institute – UQ.



A/Prof Waterhouse studied Biochemistry at University College Dublin in Ireland and emigrated to Brisbane in 1993, where he began his career in cancer research. He completed his PhD at the Queensland Institute for Medical Research and then moved to San Diego (3 years) and Melbourne (8 Years) where he studied the molecular mechanisms of cancer cell death. He moved to the Mater Research Institute in 2009 to continue this work and has recently moved back to the QIMR to set up the Australian Cancer Research Foundation Centre for Comprehensive Biomedical Imaging.

ABSTRACTS

ORAL PRESENTATIONS

TARGETED THERAPY TO REDUCE OSTEOSARCOMA METASTASIS

Eleni Topkas[1], Na Cai[1], Andrew Cumming[1], Nicholas Saunders[1], Liliana Endo-Munoz[1]

1. University of Queensland Diamantina Institute, Princess Alexandra Hospital, Woolloongabba, Queensland, Australia

Background: Osteosarcoma (OS) accounts for 56% of malignant bone cancers in children and adolescents. Pulmonary metastasis occurs in approximately 50% of patients and leads to a 5-year survival rate of only 20%. It is crucial to identify genes and pathways that drive the metastatic behaviour of OS for effective therapeutic targets.

Methods: Novel markers that define metastatic behaviour in OS, were identified using a comparative transcriptomic analysis of two highly metastatic (C1 and C6) and two poorly metastatic clonal variants (C4 and C5) isolated from an inherently metastatic cell line, KHOS. Two novel markers were therapeutically targeted in vitro and in vivo.

Results: DCBLD2 and TXNRD2 were identified as potential markers for OS metastasis with 2-4 fold increased expression in highly metastatic clonal variants. Gene expression of DCBLD2 and TXNRD2 was validated in a transcriptomic screen of non-malignant bone (NB), OS patient biopsies who developed metastatic disease (M-OS) and patients with localized disease (NM-OS). These markers were found to be highly expressed in 29-42% of M-OS with little to no expression seen in NB and NM-OS. Knockdown of DCBLD2 using shRNA reduced colony forming ability in vitro and significantly decreased pulmonary metastasis in vivo. Auranofin inhibits TXNRD2 activity which causes increased ROS and induced mitochondrial dysfunction resulting in apoptosis in vitro. Auranofin treatment in vivo significantly decreased pulmonary metastasis in a mouse model of spontaneous OS lung metastasis.

Conclusions: This transcriptomic screen was successful in identifying promising targets for the prevention and treatment of metastatic OS. By targeting these two novel markers; TXNRD2 and DCBLD2 we significantly reduce metastasis in vivo.

GENOME-WIDE ASSOCIATION STUDIES OF MIRSNPS IDENTIFY NOVEL PROSTATE CANCER RISK LOCI

Jyotsna Batra[1], Shane Stegeman[1], Kerenaftali Klein, Leire Moya[1], PRACTICAL Consortium, Amanda B. Spurdle[3], Judith A. Clements[1]

1. Institute of Health and Biomedical Innovation, Queensland University of Technology, Translational Research Institute, Brisbane, Australia

Single nucleotide polymorphisms (SNPs) within a microRNA (miRNA) binding sites of its target gene, referred to as miRSNPs, are known to have functional consequences for cancer risk. We investigated the association between 2,169 putative miRSNPs and prostate cancer risk in a large population including 22,301 cases and 22,320 controls of European ancestry from 23 participating studies within the largest prostate cancer (PRACTICAL) Consortium. We identified 22 SNPs to be associated with risk of prostate cancer, seven of which has not been previously reported by GWAS studies. We compared the expression levels of the 16 genes harbouring 22 significant miRSNPs and found the expression of 7 genes to be deregulated in prostate cancer in a previously published dataset of 59 tumour and 28 non-tumour samples. We then validated the functional role of KLK3 rs1058205 (T>C) and MDM4 rs4245739 SNP (A>G) SNPs. We showed that miR-3162-5p has specific affinity for the KLK3 rs1058205 SNP T-allele. As KLK3 has been shown to induce anti-angiogenic effects limiting prostate cancer growth, decreased KLK3 expression induced by miR-3162-5p targeting of the T-allele represents a mechanism by which the T-allele may be associated with increased prostate cancer risk. We also found miR-191-5p and miR-887 downregulated MDM4 protein expression in C-allele containing PC3 cells but not in LNCaP cells homozygous for the A-allele. Both miRNAs also induced a decrease in metabolic activity in PC3 cells. This study is the first to demonstrate regulation of the MDM4 rs4245739 SNP C-allele by two miRNAs presenting a mechanism by which the un-targeted A-allele of the MDM4 rs4245739 SNP may be associated with increased prostate cancer risk.

NEUROLOGICAL HETEROTOPIC OSSIFICATION REQUIRES BOTH SPINAL CORD INJURY AND MACROPHAGE-DEPENDENT SOFT TISSUE INFLAMMATION

I Kulina[1], F Genet[1], C Vaquette[1], AR Pettit[1], LJ Raggat[1], S Millard[1], NA Sims[1], IG Winkler[1], DW Hutmacher[1], JP Levesque[1]

1. Mater Research, Translational Research Institute, Brisbane, Australia

Neurological heterotopic ossification (NHO) is a frequent complication of spinal cord and traumatic brain injuries and manifests as abnormal ossification of soft tissues near joints. NHO is debilitating, causing pain, joint deformation, ankylosis and vascular and nerve compression. The mechanisms leading to NHO are unknown with complicated and expensive surgical resection, the only effective treatment approach. To elucidate NHO pathophysiology we have developed the first animal model of NHO in genetically unmodified mice. Mice underwent a spinal cord transection (SCI) with muscular inflammation. The combination of SCI with muscular inflammation was necessary to induce NHO which is consistent with clinical observations (NHO incidence is higher in patients with severe trauma or concomitant infection). Abundant F4/80+ macrophages, which can provide pro-anabolic support in bone formation, were detected within the inflamed muscle and associated with areas of intramuscular bone formation (confirmed by von Kossa and collagen type 1 staining). In vivo depletion of macrophages with clodronate-loaded liposomes prevented NHO formation. This supports that macrophage-mediated inflammation is a key activator of NHO following SCI. SCI was necessary for NHO development suggesting that it causes release of systemic factors priming NHO. Also, blood plasma from mice with SCI and inflammation induced osteogenic differentiation of cultured muscle mesenchymal progenitor cells (mMPC) sorted from naïve mice. In conclusion, our model suggests that NHO is a 2-insult process with 1) SCI inducing the release of factors turning mMPC to osteogenic differentiation in muscles and 2) macrophages in inflamed muscles then triggering abnormal osteogenic differentiation of mMPC.

O4

HEPATIC FIBROSIS IS ASSOCIATED WITH SMALL INTESTINAL PERMEABILITY IN CHRONIC LIVER DISEASE WITHOUT ASCITES.

Ashok Raj[1,2], Gerald Holtmann[1,2], Purnima Bhat[1,2], Linda Fletcher[1,2], Cuong Tran[3], Marrianne Black[1], David Vesey[4], Graeme Macdonald[1,2]

1. Department of Gastroenterology and Hepatology, Princess Alexandra Hospital. 2. University of Queensland School of Medicine, Translational Research Institute. 3. University of Adelaide. 4. Centre for Kidney Disease Research, Translational Research Institute.

Background: Intestinal permeability may play a role in the progression of hepatic fibrosis.

Aim: To assess the relationship between hepatic fibrosis and small intestinal permeability in chronic liver disease, in a prospective cohort study.

Methods: 113 subjects with chronic liver disease (CLD) caused by hepatitis C (CHC, n=42), B (n=32) and non-alcoholic fatty liver disease (NAFLD, n=39) were compared to 30 healthy volunteers (HV). Subjects were excluded if they drank alcohol within 24 hours of testing or had gastrointestinal pathology. Small intestinal permeability was assessed by the plasma lactulose:rhamnose ratio, 90 minutes after oral ingestion of 5g lactulose and 1g rhamnose. Hepatic fibrosis was measured by Transient Elastography (kPa). The limulus-amebocyte lysate assay was used to detect endotoxaemia in peripheral blood. Statistical analysis was performed utilising SPSS.

Results: 84 subjects without ascites completed evaluation of small intestinal permeability and hepatic fibrosis (54 with CLD, 30 HV). In these subjects there was a significant positive correlation between hepatic fibrosis and small intestinal permeability (Spearman rank test, r=0.22, $p < 0.05$). All 143 subjects (113 with CLD, 44 with cirrhosis, and 30 HV), were tested for endotoxaemia. In the 44 with cirrhosis (defined as LSM>13kPa or clinical diagnosis in those with ascites), the proportion of endotoxin-positive subjects was significantly higher (7/44) compared to CLD without cirrhosis (3/69), $p<0.05$ (Fisher's Exact).

Conclusion: In chronic liver disease due to CHC, CHB and NAFLD, hepatic fibrosis is associated with small intestinal permeability in the absence of ascites. CLD with cirrhosis is associated with peripheral endotoxaemia.

TRANSGENIC EXPRESSION OF PROINSULIN OVERCOMES TOLERANCE DEFECTS IN NOD MICE

Peta LS Reeves[1], F Susan Wong[1], Emma E Hamilton-Williams[1], Raymond J Steptoe[1]

1. Diamantina Institute, University of Queensland, Translational Research Institute, Brisbane, Australia

Type 1 diabetes (T1D) results from autoimmune destruction of pancreatic beta cells. CD8+ and CD4+ T cells are critical to T1D progression and CD8+ T cells directly destroy β cells. For an effective immunotherapy, diabetogenic CD8+ T cells must therefore be inactivated. Targeting antigen expression to antigen-presenting cells (APC) inactivates cognate CD8+ T cells and has therapeutic potential. To investigate this, we determined whether expressing proinsulin in APC is tolerogenic in the non-obese diabetic (NOD) mouse, a model where spontaneous autoimmune diabetes is associated with tolerance defects. Insulin-specific CD8+ (G9) T cells transferred to non-transgenic (non-Tg) mice proliferated in pancreatic lymph nodes (LN), where insulin is normally presented, but not in other lymphoid sites. G9 cells accumulated in pancreatic LN where they predominantly exhibited an effector phenotype. In contrast, in proinsulin-transgenic recipients, G9 cells proliferated in spleen, skin-draining LN and pancreatic LN indicating widespread expression of potentially tolerogenic proinsulin. Despite this, the number of G9 cells recovered was low relative to non-Tg recipients and decreased over time. The few G9 cells remaining had reduced TCR expression and a phenotype consistent with tolerance. These data suggest G9 cells undergo deletion in proinsulin-transgenic recipients and remaining G9 cells are inactivated. Overall, an important observation here is that insulin-specific CD8+ T cells are amenable to peripheral tolerance induction when antigen is over-expressed despite the demonstrated tolerance defects in NOD mice. This has potential implications in developing immunotherapeutic approaches to T1D and other T cell-mediated autoimmune diseases.

SALIVARY DNA METHYLATION OF DAPK-1, RASSF1a AND MICRO RNA 9 AS BIOMARKERS FOR HEAD AND NECK CANCER.

Yenkai Lim[1], Yunxia Wan[1], Chris Perry[2], William Coman[3], Chamindie Punyadeera[1]

1. Institute of Health and Biomedical Innovation, Queensland University of Technology, Translational Research Institute, Brisbane, Australia. 2. Princess Alexandra Hospital, Brisbane, Australia. 3. The University of Queensland, Brisbane, Australia

With an increasing recognition of the link between oral and systemic disease, attention has turned to saliva as an alternative diagnostic medium for a diverse array of health conditions. It is well established that tumour cells secrete biomolecules into saliva. Head and neck squamous cell carcinoma (HNSCC) encompasses a diverse group of aggressive tumours. HNSCC patients, particularly those with a history of smoking, often develop secondary tumours. Currently, there are no diagnostic tests to detect these cancers at an early stage; as such, most patients present with metastatic disease at the time of diagnosis, leading to 5-year survival rates of less than 40%. DNA methylation and microRNAs (miRNAs) are the most extensively studied epigenetic biomarkers in HNSCC. We collected saliva from HNSCC patients (n=60) and healthy controls (n=50) and interrogated CpG hypermethylation events in tumour suppressor genes using a sensitive methylation-specific PCR (MSP) assay. RASSF1a, DAPK1 and p16, showed an overall specificity of 87% and sensitivity of 80%. The test panel performed extremely well in the detection of the early stages of HNSCCs, with sensitivity of 94% and specificity of 87%, and high κ value of 0.8, indicating an excellent overall agreement between the presence of HNSCC and a positive MSP panel result. In addition, miR-9 and miR-191 provided a good discriminative ability with AUC values of 0.85 and 0.74 respectively ($p<0.001$) for discriminating HNSCC patients from healthy controls. In conclusion, we demonstrate that salivary DNA methylation and miRNA biomarkers are clinically useful in detecting HNSCC in a non-invasive manner.

PRENATAL ETHANOL EXPOSURE IS ASSOCIATED WITH ALTERED TRANSCRIPTION AND EPIGENETIC MARKS AT A GLUTAMATE TRANSPORTER

Christine RC Zhang[1,2], Nyoman Kurniawan[3], Graham Galloway[3], Mei-Fong Ho[1], Suyinn Chong[1,2]

1. Mater Medical Research Institute, Brisbane, Australia. 2. School of Medicine, The University of Queensland, Brisbane, Australia. 3. Centre for Advance Imaging, The University of Queensland, Brisbane, Australia.

Maternal alcohol consumption can result in a continuum of abnormalities which are covered by the umbrella term foetal alcohol spectrum disorders (FASDs). Foetal alcohol syndrome (FAS) sits at the most severe end of FASDs and is characterized by growth restriction, craniofacial abnormalities and brain deficits. Its pathogenesis is poorly understood. To investigate the impact of voluntary administration of 10% ethanol from fertilisation to mid-gestation on the adult brain, behavioural tests and magnetic resonance imaging were applied to a mouse model of prenatal ethanol exposure that had previously replicated the postnatal growth restriction and craniofacial abnormalities of FAS. The results revealed that ethanol-exposed offspring were hypoactive and had disproportionally smaller hippocampi. Genome-wide transcriptional analysis in the hippocampus identified several dysregulated genes following ethanol exposure. Elevated transcription of a glutamate transporter gene, *Slc17a6*, was validated in adult ethanol-exposed male-offspring, implicating altered hippocampal glutamatergic signalling. The increase in transcription at *Slc17a6* was associated with decreased DNA methylation and an enrichment of an active chromatin mark, H3K4m3, at the gene promoter. Interestingly, *Slc17a6* protein levels were decreased in the hippocampi of ethanol-exposed male-offspring, suggesting ethanol-associated changes in post-transcriptional control, possibly through microRNAs. Genome-wide transcriptional analysis of microRNAs revealed a number of ethanol-sensitive microRNAs. Subsequent qPCRs confirmed up-regulation of four microRNAs in the adult hippocampus, and one of the microRNAs was shown to target the 3'UTR of *Slc17a6* in an *in vitro* reporter gene assay. Our results demonstrate that relatively moderate ethanol exposure during early pregnancy leads to significant changes in brain structure, function and gene expression in adulthood. . They also raise the possibility that some phenotypes observed in FASDs may be due to altered epigenetic and/or microRNA-based control of glutamate neurotransmission in the hippocampus.

O8

NOTCH4 DRIVES MESENCHYMAL-EPITHELIAL TRANSITION IN MELANOMA

Ehsan Bonyadi Rad[1,2], Heinz Hammerlindl[1,2], Dinoop R Menon[1,2,4,5], Peter Soyer[1,2], Helmut Bergler[3], Helmut Schaider[1,2,3,4]

1. Cancer Biology Unit, Department of Dermatology, Medical University Graz, Austria. 2. Center for Medical Research (ZMF), Medical University Graz, Austria. 3. Institute of Molecular Biology and Biochemistry, Center of Molecular Medicine, University of Graz, Graz, Austria. 4. Dermatology Research Centre, The University of Queensland, School of Medicine, Southern Clinical Division, Translational Research Institute, Queensland, Australia. 5. University of Queensland Diamantina Institute, Translational Research Institute, Brisbane, Queensland, Australia

Notch signaling is fundamental in regulating development and adult tissue homeostasis. It is involved in a variety of cellular mechanisms like cell fate specification, differentiation, proliferation, and epithelial-mesenchymal transition. In cancer, the effects of Notch signaling are highly context dependent and both, oncogenic and tumor suppressive functions of Notch have been described. Notch4 recently has been reported to regulate the embryonic morphogen Nodal, thus contributing indirectly to melanoma progression. Here we show that the overexpression of the constitutively active intracellular domain of N4 (N4ICD) caused a mesenchymal-epithelial switch in melanoma. The N4ICD overexpressing cell lines showed strongly reduced invasion, migration and proliferation. On the molecular level this switch was accomplished by down regulation of the epithelial-mesenchymal transition regulators Slug and Twist1. The N4ICD induced transcription factors Hey-1 and Hey-2 bind directly to the promoter regions of Slug and Twist1 and suppress gene transcription. Slug and Twist1 have been reported to activate Vimentin and MMP2, both of which are down regulated in N4ICD overexpressing cells. Therefore N4ICD overexpression indirectly leads to the formation of a less aggressive epithelial phenotype suggesting a role of Notch4 as a tumor suppressor in melanoma.

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ABSTRACTS

POSTER PRESENTATIONS

TRI MICROSCOPY CORE FACILITY

Sandrine Roy[1]

1. The University of Queensland Diamantina Institute, Translational Research Institute, Brisbane, Australia.

The aim of the Core Microscopy facility is to facilitate the imaging component of research for all TRI research partners and beyond and 2) to provide specialised and sample-specific training on appropriate instrumentation and assist with analysis and quantification of images from 2D to 4D. Several of the instruments in the core are unique in Queensland, including the OMX-Blaze 3D-SIM Super-resolution microscope, the LaVision Multiphoton, the 746nm laser available on the Olympus FV1200 Confocal microscope and the Nikon/spectral Spinning Disc Confocal microscope. There is a total of 17 instruments in the facility core, 5 are high end instruments. There is also a range of analysis softwares available and expert help to help you. This poster outlines the detailed specification of each instrument in the facility for your perusal.

TRI PROTEOMICS CORE FACILITY

Dorothy Loo[1] and Michelle Hill[1]

1. The University of Queensland Diamantina Institute, Translational Research Institute, Brisbane, Australia.

Proteomics is the study of proteins in a system. Traditional antibody base technologies only study one protein at a time. Mass spec based proteomics can study 100s to 1000s of protein in one assay. We have two types of mass spectrometers; QTOF discovery mass spectrometer and QQQ quantitative mass spectrometer. Mass spectrometers can be set up to identify proteins, study post translational modifications and quantify target molecules such as peptides, lipids, drugs and metabolites.

AN INDUCIBLE shRNA OVEREXPRESSION SCREEN FOR INHIBITORS OF p53 INDUCTION IN DIAMOND BLACKFAN ANEAMIA

Adam Stephenson[1], Dubravka Skalamera[2], Amee J George[3], Alex J Stevenson[2], Brian Gabrielli[2], Ross Hannan[3], Thomas J Gonda[1]

1. School of Pharmacy, The University of Queensland, Brisbane. 2. The University of Queensland Diamantina Institute, Translational Research Institute, Brisbane. 3. Oncogenic Signalling and Growth Control Program, Peter MacCallum Cancer Centre, Melbourne.

Diamond Blackfan Anaemia (DBA) is a rare congenital erythroid hypoplastic anaemia characterised by low red blood cell numbers and in many cases abnormalities of the face, head upper limbs and heart. The disease, which usually presents in infancy, is caused by deleterious mutations in a number of genes that encode ribosomal proteins, in particular ribosomal protein subunit 19 (RPS19) which accounts for 25% of all known cases. While a majority of patients respond well to current treatments including steroids and blood transfusions a significant proportion of DBA mortality and morbidity is due to complications arising from these treatments. It has recently been shown that disrupted ribosome biogenesis (as a consequence of increased nucleolar stress) results in the sequestration of HDM2 (MDM2) by certain ribosomal proteins leading to the accumulation of p53 and induced cell cycle arrest. We have developed retroviral tet-inducible construct containing a shRNA sequence targeting RPS19. This has been transduced into human alveolar adenocarcinoma cell line (A549). Upon treatment with doxycycline the induced RPS19 shRNA results in RPS19 knockdown, activation of p53 and cell cycle arrest. By using the automated high throughput genomic screening facilities at the ARVEC Functional Genomics Facility we have undertaken a gain of function screen against a 17,000-clone human ORF lentiviral expression library. This screen will identify genes that when overexpressed modulate the ability of RPS19 knockdown to activate p53 and cell cycle arrest. Using these techniques preliminary results have identified novel potential therapeutic targets for DBA.

COL1A1 CLEAVAGE SITE MUTATION CAUSES HIGH BONE MASS, BONE FRAGILITY AND JAW LESIONS: A NEW CAUSE OF GNATHODIAPHYSEAL DYSPLASIA?

Aideen McInerney-Leo[1], Paul J Leo[1], Brooke Gardiner[1], Linda A Bradbury[1], Jessica A Harris[1], Graeme R Clark[1,2], Matthew A Brown[1], Andreas Zankl[3.4], Emma L Duncan[1,5]

1. The University of Queensland Diamantina Institute, Translational Research Institute, Woolloongabba, QLD, Australia. 2. Department of Medical Genetics, Academic Laboratory of Medical Genetics, Addenbrooke's Hospital, Cambridge, England. 3. Discipline of Genetic Medicine, The University of Sydney, Sydney, Australia. 4. Academic Department of Medical Genetics, Sydney Children's Hospital Network (Westmead), Sydney, Australia. 5. Department of Endocrinology, Royal Brisbane and Women's Hospital, Butterfield Road, Herston, QLD, Australia.

Gnathodiaphyseal dysplasia (GDD) is a rare autosomal dominant condition characterized by bone fragility, irregular bone mineral density (BMD) and fibro-osseous lesions in the skull and jaw. Mutations in Anoctamin-5 (ANO5) have been identified in some cases. We aimed to identify the causative mutation in a family with features of GDD but no mutation in ANO5, using whole exome capture and massive parallel sequencing (WES). WES of two affected individuals (a mother and son) and the mother's unaffected parents identified a mutation in the C-propeptide cleavage site of COL1A1. Similar mutations have been reported in individuals with osteogenesis imperfecta (OI) and paradoxically increased BMD. C-propeptide cleavage site mutations in COL1A1 may not only cause 'high bone mass OI', but also the clinical features of GDD, specifically irregular sclerotic BMD and fibro-osseous lesions in the skull and jaw. GDD patients negative for ANO5 mutations should be assessed for mutations in type I collagen C-propeptide cleavage sites.

TARGETING EMT TO MODULATE PROSTATE CANCER CELL CHEMORESISTANCE

A Upadhyaya[1,2,3], N Stylianou[1,2,3], BG Hollier[1,2,3,4], ED Williams[1,2,3]

1. Chronic Disease and Ageing Theme, Institute of Health and Biomedical Innovation, Queensland University of Technology. 2. Australian Prostate Cancer Research Centre – Queensland. 3. Translational Research Institute. 4. Injury Prevention and Trauma Management Theme, Institute of Health and Biomedical Innovation, Queensland University of Technology.

Introduction: Metastatic prostate cancer (PC) is the second leading cause of cancer deaths in Australian males. Radical prostatectomy, followed by androgen deprivation therapy (ADT) upon recurrence of disease is the conventional form of treatment. Further intervention involves chemotherapy along with androgen targeted therapy (ATT). These treatments are not curative and the disease ultimately progresses. Epithelial to mesenchymal transition (EMT) is a reversible process that can facilitate tumour spread and has also been associated with chemoresistance. We therefore aim to determine how EMT and subsequent reversal of this state (MET) influences the chemoresistance profile of PC cells.

Method: Using a panel of inducible EMT PC cell line (LNCaP) models, the effect of each defined phenotypic state to a panel of clinically relevant drugs will be measured using various cellular and molecular assays. Further, cells will be treated with anti-androgens, such as bicalutamide and enzalutamide, to determine whether this interaction further alters chemoresistance profiles.

Results: The inducible EMT models have been validated using cell shape, qRT-PCR and western blotting analyses. The induction of EMT-transcription factors induced an increased expression of mesenchymal marker vimentin and decreased expression of epithelial markers E-cadherin and EpCam. The temporal pattern of these dynamic changes has now been established and time points following EMT and MET induction selected. Preliminary chemoresistance assays have been initiated using doxorubicin to define the workflow prior to initiating experiments using a panel of chemotherapy agents.

Conclusions: Future work will involve examination of the effect of EMT and MET state on the chemoresistance profile of PC cells in the presence or absence of ATT.

HIGH-THROUGHPUT SIRNA SCREENING FOR NOVEL CANCER THERAPEUTICS

AJ Stevenson[1], FF Bokhari[1], M Ranall[1], L Spoerri[1], D Skalamera[1], NA McMillan[2], TJ Gonda[3], B Gabrielli[1]

1. The University of Queensland Diamantina Institute, Translational Research Institute, Brisbane, Australia. 2. Griffith Health Institute, Griffith University, Parklands Drive Southport, Queensland, Australia. 3. School of Pharmacy, UQ, Australia

One of the common issues relating to anti-cancer drugs is the toxicity and side effects associated with their use. Traditionally cancer treatments are found through screening for effective tumour cell killers. Synthetic-lethal high-throughput siRNA screening presents an alternative, which allows for identifying targets whose depletion is lethal to cancer cells while non-lethal to non-tumourigenic cells. Using the ARVEC high-content screening platform we have performed multiple synthetic-lethal screens targeting a variety of cancers. The tumourigenic and non-tumourigenic lines were reverse-transfected in parallel with siRNA agents targeting select gene sets, eg. kinome. Plate reader and high-content imaging outputs were then used to select putative oncogenic targets. One kinome library screen (779 genes) performed for HPV positive cervical cancer identified Aurora A kinase (AURKA) as a potential treatment for HPV positive cancers. A commercial selective inhibitor for AURKA (Alisertib) selectively killed cells expressing the HPV-E7 protein both in vitro and in mice. We are currently analysing the data from several screens targeting other cancers including melanoma and mesothelioma. Together our results, demonstrate the utility and versatility of the model of synthetic-lethal siRNA screening for identifying novel anti-cancer therapeutics.

IMMUNITY TO NONTYPEABLE HAEMOPHILUS INFLUENZAE IN PROTRACTED BACTERIAL BRONCHITIS

Alice C-H Chen[1], Melanie Carroll[1], Helen Petsky[2], Susan Pizzutto[3], Stephanie Yerkovich[4], Katherine Baines[5], Jodie Simpson[5], Peter Gibson[5], Greg Hodge[6], Sandra Hodge[6], Anne Chang[2] and John Upham[1]

1. School of Medicine, The University of Queensland, Brisbane. 2. Royal Children's Hospital, Brisbane. 3. Charles Darwin Hospital, Darwin. 4. The Prince Charles Hospital, Brisbane. 5. The University of Newcastle, Newcastle. 6. Hanson Institute, Adelaide

Background: Protracted bacterial bronchitis (PBB) is characterised by chronic wet cough in young children and isolation of bacteria such as nontypeable Haemophilus influenza (NTHi). Some children with PBB are thought to develop bronchiectasis (BE) but there is a paucity of information available on the pathogenesis of PBB and the factors associated with persistent bacterial infection and progression to BE.

Aims: To compare immune responses against NTHi in patients with PBB (n=20), healthy control subjects (n=20) and BE (n=20), and to determine the importance of the IL-1β pathway.

Results: Unstimulated PBMC from children with PBB produced higher IL-10 and IFN-γ than PBMC from controls (n=20). NTHi stimulation induced high concentrations of innate and adaptive cytokine production in all three groups, including IL-1β, IL-6, IL-10 and IFN-γ. As expected, IL-1β production was caspase-1 dependent; the presence of the caspase-1 inhibitor (Z-YVAD-FMK) in cultures reduced IL-1β production by ~80%. Both caspase-1 inhibition and the IL-1 receptor antagonist anakinra inhibited IL-10 and IFN-γ production by PBMC. In PBB children (n=10), lung lavage macrophages produced high amounts of IL-1β, IL-6 and IL-8 in the absence of in vitro stimulation, and were relatively refractory to further stimulation with NTHi. Production of IL-1β was reduced in lung macrophage by caspase-1 inhibition, whereas in lung neutrophils, IL-1β production was reduced by anakinra but not by caspase-1 inhibition.

Conclusions: Our findings highlight activation of caspase-1 dependent, pro-inflammatory pathways in PBB. While blocking the IL-1β pathway has the potential to reduce inflammation, this may come at the cost of protective adaptive immunity against NTHi. Chronic lung infection in PBB may modify lung macrophage function, making these cells refractory to a secondary infection.

ONLINE QUANTITATIVE PROTEOMICS P-VALUE CALCULATOR FOR PERMUTATION-BASED STATISTICAL TESTING OF PEPTIDE RATIOS

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The utility of high-throughput quantitative proteomics to identify differentially abundant proteins en-masse relies on suitable and accessible statistical methodology, which remains mostly an unmet need. We present a free web-based tool, called Quantitative Proteomics p-value Calculator (QPPC), designed for accessibility and usability by proteomics scientists and biologists. Being an online tool, there is no requirement for software installation. Furthermore, QPPC accepts generic peptide ratio data generated by any mass spectrometer and database search engine. Importantly, QPPC utilizes the permutation test that we recently found to be superior to other methods for analysis of peptide ratios because it does not assume normal distributions. QPPC assists the user in selecting significantly altered proteins based on numerical fold change, or standard deviation from the mean or median, together with the permutation p-value. Output is in the form of comma separated values files, along with graphical visualization using volcano plots and histograms. We evaluate the optimal parameters for use of QPPC, including the permutation level and the effect of outlier and contaminant peptides on p-value variability. The optimal parameters defined are deployed as default for the web-tool at http://qppc.di.uq.edu.au/ .

DETECTING CIRCULATING TUMOUR CELLS IN METASTATIC HEAD AND NECK CANCER

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Head and neck squamous cell carcinoma (HNSCC) is the 6th most common cancer globally with less than 40% survival beyond 5 years. Locoregional and distant metastatic disease is responsible for 88% of patient deaths within 12 months of diagnosis. The ability to identify high risk patients with disseminated disease prior to presenting with clinically detectable metastases holds remarkable potential. Circulating tumour cells (CTCs) are a hallmark of invasive cancer cells expressing epithelial cell adhesion molecule (EpCAM) and key to metastasis. CTCs have been used as surrogate markers of overall survival and progression free survival for breast, prostate and colorectal cancers using the CellSearch® system. The aim of this study is to use CellSearch® (FDA-approved) and ScreenCell® to detect and compare CTCs in HNSCC. In a cohort of 25 HNSCC patients from the Princess Alexandra Hospital (PAH), CTCs were detected in 7 patients. To increase the number of CTCs, we are looking at a broader range of epithelial and mesenchymal markers which are not selected for by the CellSearch®. Preliminary data displays some degree of mesenchymal nature is present, suggesting of an epithelial to mesenchymal transition.

DEVELOPMENT OF TISSUE RESIDENT MEMORY T CELLS (TRM) IN PRECANCEROUS MODELS OF NON-MELANOMA SKIN CANCER

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Tissue resident memory T (TRM) cells of the skin are characterised by their infiltration and persistence within the epidermis and expression of surface markers such as CD103 and CD69. These cells have been characterised in the context of viral infections such as herpes simplex virus (HSV) where they provide protection against re-infection. In contrast, the function and persistence of TRM cells in precancerous or cancerous tissue is less well understood. Our aim was to determine the presence and functionality of TRM cells within two non-melanoma skin cancer mouse models expressing either transgenic human papilloma virus derived E7 (K14E7.C57) protein or an inducible mutant K-ras (LSLkRASG12D) protein. Both of these mice model precancerous, hyperplastic skin. Preliminary analysis showed an increased presence of TRM cells within the hyperplastic skin of both models compared to that of the control group. Despite the increased presence of TRM cells preliminary data suggests that their expression of interferon gamma, an important effector cytokine, was altered in K14E7 mice. Within the K14E7 model, TRM development was not altered by removal of NKT cells which have previously been shown to be immunosuppressive. The resulting data suggests that precancerous skin supports the initial development of cells with a TRM phenotype and that removing suppressive NKT cells does not alter the accumulation of TRM. Further studies looking at TRM function and persistence within precancers will be important in the future.

COMPUTATIONAL ANALYSIS OF DNA REPAIR PATHWAYS IN BREAST CANCER USING GENE EXPRESSION DATA

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Human DNA is constantly subject to threats posed by various endogenous and exogenous factors, such as ultraviolet radiation, cigarette smoke and oxidative byproducts from cellular respiration. At least five DNA repair pathways have been developed to counteract these threats. The difference in activity of these repair pathways amongst subgroups of various cancers has been associated with radio- and chemoresistance, and more recently with response to poly [ADP-ribose] polymerase 1 (PARP1) inhibitor-related targeted therapies for breast and ovarian cancer (1-3). It is thus important to systematically investigate the status of all these repair pathways in breast cancer, but to our knowledge no such studies have been done. As DNA repair research is a fast-advancing area, we have first manually curated these pathways by combining literature search and domain expertise to provide up-to-date knowledge of these pathways (4). We then applied the Pathifier algorithm (5) to expression data from 458 breast tumour samples and 22 normal breast tissue samples from The Cancer Genome Atlas (TCGA) to generate a pathway deregulation score for each of the curated repair pathway in individual tumour samples. The initial results showed that breast cancer subtypes exhibit substantial difference in terms of DNA repair capacities, and there seems to exist a regulatory mechanism that regulates four of the five repair pathways. These results thus provide new insights into the DNA repair mechanisms in breast cancer and have the potential to help predict tumour-specific response to DNA damaging-related chemoradiotherapy and PARP1 inhibitor-related targeted therapies.

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THE LNCRNA, EVX1AS IS NECESSARY AND SUFFICIENT FOR GASTRULATION

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Long non-coding RNAs (lncRNAs) are dynamically expressed during development and differentiation. There is increasing evidence to suggest they play important roles in epigenetic regulation of gene expression. We performed a screen for lncRNAs that are dynamically expressed during ES cell differentiation into embryoid bodies (EBs). We identified Evx1as lncRNA as a novel gene that is expressed in an antisense direction with respect to Evx1, a Hox gene at the 3' end of the HoxA cluster. Like Evx1, Mixl1, and T, expression of Evx1as is limited to the primitive streak from E7.5 to E9.5 in vivo. Knockdown of Evx1as results in complete failure of the primitive streak wave of differentiation from day 3 of EB differentiation from ES cells. We performed RNA-seq to determine the transcriptome alterations upon loss of Evx1as. Evx1, Mixl1, T, other primitive streak genes are poorly expressed in the absence of Evx1as. Surprisingly, there is also loss of expression of visceral endoderm (VE) genes such as BMPs, Wnts, Cer1 and Dkk1. In contrast, ES cell and epiblast (pre-streak) specific gene programs are up-regulated suggesting a specific role for Evx1as in primitive streak and VE derivation from epiblast. We also engineered a conditional Evx1as expression construct into the ROSA26 locus. Activation of Evx1as with taxmoxifen results in persistent expression of VE and primitive streak programs past D4 of EB differentiation and a failure to express genes expressed from mesoderm and endoderm tissue, as determined by RNA-seq. ChIP-seq in Evx1as-knockdown EBs shows loss of occupancy of H3K4me3 'marks' at certain gene promoters and a spread of occupancy at other promoters. Thus, we suggest Evx1as normally functions to limit the activity of MLL1 or similar histone methyl-transferases during development. In short, Evx1as is necessary and sufficient (in trans) for regulation of primitive streak and VE gene expression programs and differentiation of the second (VE) and third (mesoderm) germ layers from epiblast.

THE TOLL-LIKE RECEPTOR RP105 PROMOTES INNATE IMMUNITY VIA NON-CANONICAL PATHWAY

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Mycobacterial infections, in particular tuberculosis, remain a significant public health concern worldwide. Macrophages are the major host cells for pathogenic mycobacteria and play a central role in containing the pathogen and initiating inflammatory cytokine responses. Radioprotective 105 kDa (RP105) is a member of the Toll-like receptor (TLR) family that has been demonstrated to facilitate B cell proliferation but limit LPS-driven cytokine production by antigen-presenting cells. Our group recently identified a novel role for RP105 in promoting macrophage cytokine production during infection with pathogenic mycobacteria. RP105-deficient macrophages showed reduced cytokine secretion upon infection with Mycobacterium tuberculosis (Mtb) and Mycobacterium bovis, BCG. While we previously observed physical and functional interactions between RP105 and TLR2, we now show that canonical TLR signalling such as activation of MAP kinase and NF-κB signalling remained intact in RP105-deficient macrophages during mycobacterial infection. Furthermore, mycobacteria-induced mRNA and protein expression of cytokines such as TNF and IL-6 were comparable in WT and RP105-deficient macrophages. In contrast, RP105-deficient macrophages infected with mycobacteria displayed reduced cell surface-associated TNF suggesting diminished TNF trafficking in these cells. We further observed that PI3K signalling is significantly inhibited in RP105-/- macrophages and identified that PI3Kδ activity is involved in regulating mycobacteria-induced macrophage TNF secretion downstream of RP105. Furthermore, expression of components of the cytokine trafficking machinery were found to be significantly diminished in RP105-/- macrophages infected with mycobacteria. Taken together, our observations identify RP105 as an integral part of the innate immune receptor complex for pathogenic mycobacteria that contributes to macrophage responses by regulating trafficking of TNF and possibly other cytokines.

MECHANISM OF ACTION STUDIES IN PROSTATE CANCER CELLS OF A NEW COMPOUND ISOLATED FROM AN AUSTRALIAN ENDEMIC RAINFOREST TREE

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Objectives: Currently, there is no cure for advanced prostate cancer (PCa), highlighting the need for new and more efficient drugs to fight this disease. Nature is a rich source of potent antineoplastic agents, many of which have been shown to display new mechanisms of action.

Methods: We screened fractions derived from 36 Australian endemic plants for inhibiting viability of LNCaP prostate cancer cells. Cytotoxicity of the purified compounds was characterized by live cell imaging, and Alamar Blue assay in a panel of PCa cell lines. Cell death was investigated by Western blotting and immunofluorescence microscopy. Cell cycle analysis was performed by FACS and Western blotting. DNA damage and mechanism of action studies were investigated by immunofluorescence microscopy.

Results: Detailed chemical analysis of one plant led to the purification and characterization of a series of alkaloidal analogues which showed strong potency in inducing cell death in PCa cells, with IC50 values between 2.3 nM and 266 nM in LNCaP cells, providing critical structure-activity relationship information. Analogue 6-AA, a compound which has not been reported in the literature, was the most potent of the series. 6-AA caused a mitotic arrest with misaligned chromosome, ultimately leading to cell death via apoptosis. Furthermore, in vitro assay showed that 6-AA inhibited tubulin polymerization.

Conclusions: 6-AA is a new inhibitor of mitosis, causing cell death through apoptosis, without DNA damages. This new plant metabolite disrupts microtubule dynamics, leading to multipolar spindles and inhibits tubulin polymerization.

MITF CORRELATES TO ENHANCED PROLIFERATION AND EPITHELIAL TO MESENCHYMAL TRANSITION, AND DEFINES MELANOMA TUMOR SUB-POPULATIONS.

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Tumor heterogeneity, which is the occurrence of different sub-populations of cancer cells within a tumor, results in multiple cellular phenotypes within a single tumor site. As these cells display various behaviors, they could respond to therapies uniquely. Understanding the molecular signature influencing different sub-populations is therefore crucial to design the most effective therapeutic regimen. The fluorescence ubiquitination cell cycle indicator(FUCCI) system, which visually delineates phases of the cell cycle, was employed to better understand tumor heterogeneity. Using this model, it was found that tumor xenografts grown from melanoma cells produce two cohorts. One that contained distinct clusters of arrested or proliferating cells, and another that displayed more homogenous cell type dispersion. These cohorts were discovered to display either low or high levels of microphthalmia-associated transcription factor (MITF) expression, respectively. Additionally, knockdown of MITF by shRNA resulted in conversion of WM164 cells, which typically give rise to a homogenous xenograft, to produce a clustered tumor phenotype. Furthermore, in a 3D in vitro tumor spheroid model, MITF expression was predominantly found in the periphery of the spheroid, which corresponds with the region of highly proliferative cells. This area also has high Slug and Vimentin expression, indicative of an Epithelial to Mesenchymal Transition(EMT). Additionally, serum starvation resulted in cell cycle arrest and decreased MITF levels, while knockdown of MITF by shRNA gave rise to more cells arrested in G1. These data outline how MITF and tumor heterogeneity are tightly intertwined within tumor architecture, making it an important marker for therapy design.

STRESS INDUCED PHENOTYPIC PLASTICITY DRIVES MULTI-DRUG TOLERANCE ALONG WITH STEMNESS IN CANCER

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Drug tolerant cancer stem cell like subpopulations constitutes a major challenge for effective cancer therapies and is often related to relapse. However their mode of formation and the dynamics leading to resistance are poorly understood but are important to design better treatment strategies. Here we show that cancer cells in general exhibit an early innate response as a primary survival reaction towards unfavourable environmental conditions or drug exposure, inducing a transition into multiple drug tolerant stem like cells termed induced drug tolerant cells (IDTC). This response is led by global chromatin remodeling, activation of multiple signaling cascades, and transition into highly tumorigenic cell population. IDTCs exhibit persistent rewiring capabilities of signalling cascades making them a difficult target. Upon continuous drug exposure, they eventually transform into permanent drug resistant cells suggesting a dedifferentiation. Our results point to a need of alternative treatment strategies including drug holidays to prevent or delay the emergence of IDTCs and acquired drug resistance.

TARGETING MELANOMA-ASSOCIATED POINT MUTATIONS BY OVEREXPRESSION SCREENING USING LENTIVIRAL VECTORS

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Melanoma, the third most common cancer in Australia, has been extensively studied at the molecular level. As is the case with most other cancers, the genetic defects detected in melanoma tumours are so numerous that it is difficult to identify mutations driving the disease using conventional-throughput methods. To address this problem we have generated a pilot library of 80 recurrent melanoma mutations in a Gateway-compatible entry vector. Based on the available analysis of more than 11,000 documented melanoma mutations, from over 219 tumour samples, we selected the ones that were most likely to be dominant drivers of oncogenesis. Gateway entry clones were then transferred into a lentiviral expression vector which has been shown to deliver high levels of stable transgene expression in a variety of human cell types, including primary melanoblasts, immortalised keratinocytes and tumour-derived cell lines. We are using this library in cell-based assays that can detect mutations conferring cancer-associated phenotypes such as resistance to growth-arrest, apoptosis or chemotherapeutic agents as well as increased proliferation, migration and invasiveness. In the first instance, each mutation is tested individually on primary melanoblasts genotyped for the melanocortin receptor 1 (MC1R), as the MC1R locus is a known melanoma risk predictor. Subsequently mutations will be confirmed in other cell types. Preliminary data suggest that this is a robust expandable screening platform that can be used to identify novel therapy targets in melanoma and other cancers.

FUNCTIONAL ANALYSIS OF EPITHELIAL MESENCHYMAL PLASTICITY IN CARCINOMA SYSTEMS

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Epithelial mesenchymal plasticity (EMP) allows carcinoma cells to adopt phenotypes across the full spectrum from proliferative, organised epithelial collectives to individual migratory mesenchymal cells. Through EMP, carcinoma cells are empowered for migration, invasion, therapy resistance, dormancy and metastasis. Using breast and bladder cancer model systems, we have demonstrated both stable states and dynamic transitions across the EMP spectrum, in association with differential malignant potential. Stable variants manifesting through epigenomic and transcriptomic differences exist in the PMC42 human breast cancer model, where the mesenchymal state facilitates tumourigencity. Metastatically competent variants of the T24 TSU-Pr1 human bladder carcinoma model show an epithelial change via altered transcriptomic profiles. Both models have been subjected to a functional screen using siRNA smartpools, along with expression profiling. The mesenchymal state in each model was associated with an activated TGFβ signaling pathway, which was possibly held in check by IRS1 suppression in the epithelial state.

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DEVELOPMENT AND EVALUATION OF NANOEMULSION FORMULATIONS FOR TRANSDERMAL DELIVERY OF NAPROXEN

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Nanoemulsions are efficient vehicles for topical drug delivery. This study aimed to develop and evaluate superior nanoemulsions incorporating known skin penetration enhancers oleic acid (OA) or eucalyptol (EU) for enhanced skin penetration of the lipophilic drug naproxen. Two oil-in-water (O/W) nanoemulsions containing naproxen (2%) and either OA or EU as oil phases and surfactant, co-surfactant and aqueous phase proportions chosen with pseudo ternary phase diagrams were prepared and fully characterized. Nanoemulsions and control (2% naproxen in 60% ethanol/water) were applied to full thickness excised human abdominal skin in Franz diffusion cells (n=4) for 8 h and naproxen concentrations in receptor fluid and extracts of tape strips and skin were measured by HPLC. Skin treated with nanoemulsions was also imaged by multiphoton microscopy. For both nanoemulsions, the rate of penetration of naproxen through the skin (flux) and the amount extracted from residual skin (retention) were significantly greater than for controls (all $P<0.005$), whereas there was no significant difference in flux or skin retention between the nanoemulsions. Images of nanoemulsion treated skin showed altered stratum corneum barrier structure. In conclusion, both the nanoemulsion systems in this study gave significantly enhanced penetration of a model lipophilic drug, naproxen. The enhancement is likely due to alterations in the stratum corneum barrier structure promoted by eucalyptol and oleic acid, which are known penetration enhancers. This work is currently being extended to apply theoretical principles based on Hansen Solubility Parameters to design formulations for better targeting to specific skin layers.

DIFFERENTIAL MIGRATION OF METASTATIC PROSTATE CANCER CELLS TO BONE MARROW STROMAL CELLS

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Background and aim: Prostate cancer (PCa) is the second most common cancer worldwide, and the most common cancer in men in developed countries. In the advanced aggressive form of the disease, the bone marrow is the most common secondary tumour site. Bone metastasis is associated with morbidity and mortality. It is hypothesized that metastatic PCa cells are able to feed off niche signals and this enables them to achieve PCa quiescence, evade the immune system and chemotherapy. The bone marrow HSC niche is a complex microenvironment consisting of array of cells of different phenotypes, growth factors and extracellular matrix. To better understand how the HSC niche impacts PCa cells migration, growth and sensitivity to chemotherapy; we have cultured PCa cells in the presence of mesenchymal stem/stromal cells (MSCs) and their differentiated progeny.

Methods: Bone marrow-derived MSCs were used as a source of different bone marrow niche cells. MSCs were either cultured in maintenance medium, or differentiated into osteoblasts or adipocytes over 14 days. The proliferation rate of PCa cells was assayed following direct co-culturing with either MSCs, adipocytes or osteoblasts. In-direct co-cultures were performed using transwell assays to estimate the migratory capacity of the PCa cells towards all three linages.

Results: Different prostate cancer cell lines, representing different stages of the disease, respond differently to the different cells types of the bone marrow niche.

Conclusion: A varied response of PCa cells was observed in response to different MSCs-derived populations. We are now using gene expression and ELISA to characterize the different signalling profiles.

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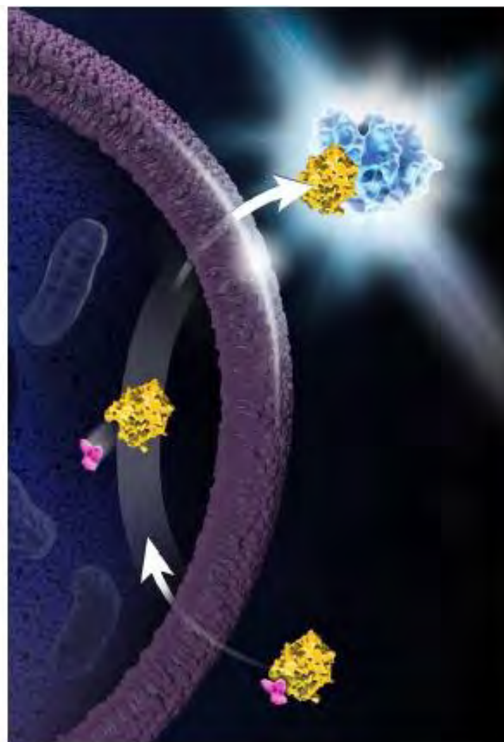

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REPOSITIONING "OLD" DRUGS FOR NEW CAUSES: IDENTIFYING NEW TREATMENTS FOR PROSTATE CANCER

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The majority of prostate cancer (PCa) deaths occur due to the metastatic spread of tumour cells to distant organs and the establishment of secondary tumours, accounting for more than 3000 deaths per year in Australia alone. Currently, there is a lack of effective therapies once tumour cells have spread outside the prostate. It is therefore imperative to rapidly develop therapeutics to inhibit the metastatic spread of tumour cells in order to promote the survival of men suffering from aggressive forms of PCa. The proposed research will develop a cell-based primary screening assay that will help identify existing drugs that are capable of inhibiting the migratory and invasive properties of the highly metastatic PCa cells. These drugs have been previously approved by the Food and Drug Administration (FDA) and are a part of a drug library that comprises 420 candidates. At the end of the primary screen, a number of drugs have been identified that can either inhibit migration or are cytotoxic to the PCa cells. Dose response studies were performed to identify the optimal drug concentration that can inhibit migration. Following which, a series of validation studies will be performed to identify the most potent drug with minimal side effects which can be used for further clinical studies. By employing the drug repositioning strategy instead of the traditional de-novo drug discovery and development strategy, the potential drug candidate(s) could be rapidly translated into the clinic for the management of men with aggressive forms of PCa.

GENETIC CONTROL OF REGULATORY T CELL FUNCTION IN TYPE 1 DIABETES

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Defects in regulatory T cell (Treg cell) development and function are commonly believed to contribute to the loss of immune cell tolerance to islet antigens leading to type 1 diabetes (T1D). Multiple genetic polymorphisms are associated with T1D and variants in key immune pathways such as IL-2 signaling are believed to cause impairments in Treg cell suppressive function. In this study we examine defects in Treg cell development and function in T1D prone NOD mice compared with T1D protected strains including C57BL/6 and NOD congenic mice carrying protective alleles at Idd3 (Il2) or Idd5 (Ctla4, Scl11a1 and Acadl). When compared with C57BL/6 mice, NOD mice had significantly reduced frequencies of Treg cells in peripheral lymphoid organs other than the spleen. NOD Treg cells in the spleen and Peyer's patches were skewed towards IL-2 independent effector Treg cells. NOD mice had impaired expression of CD25, the immunosuppressive molecules CD39 and CD73 and produced less IL-10. Expression of protective Idd3/5 alleles did not significantly increase Treg cell frequency. Nonetheless, the protective alleles of both Idd3 and Idd3 interacted to increase the expression of CD25 on Treg cells and enhance IL-10 production. These studies uncovered novel defects in Treg cell phenotype in T1D susceptible mice and determined which phenotypes were dependent on IL-2 signaling. The T1D related Treg cell phenotypes we have identified would be of interest to investigate in T1D patients, who share common gene pathways with NOD mice.

DEVELOPMENT OF A NEW BIOPSY DEVICE FOR THE "ASEPTIC" COLLECTION OF INTESTINAL MICROBIOTA SAMPLES

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The microbiota inhabiting the gastrointestinal tract plays an essential role in maintaining gut health. Understanding the role and importance of specific components within the microbiota is essential to establishing the underlying causes of a variety of inflammatory and metabolic disorders. Although mucosal tissue samples are being increasingly used for microbiota studies, their cross-contamination with luminal contents constrains our ability to precisely define the microbes associated closely with the mucosal layer, and in particular the host-microbe interactions relevant to health and disease. To overcome this limitation we have developed and patented a new "aseptic" biopsy device that is now commercially produced by MTW (Germany), which can be used during endoscopic procedures to obtain biopsies from the gastrointestinal tract that are not contaminated by luminal content or material deposited in the endoscope biopsy channel. The device has a biopsy forceps that is housed within a sterile sheath and the distal opening is sealed by a membrane, protecting the forceps and working channel from exposure to luminal contents. After appropriate positioning of the biopsy device during an endoscopy, the forceps are advanced through the membrane, and biopsies are obtained via a "clean" channel, avoiding cross-contamination. In preliminary studies, microbiota profiles from matched samples of duodenal tissue collected by standard and "aseptic" devices are different, suggesting the "aseptic" device does minimize cross-contamination, and provides superior sampling of the microbes closely associated with the mucosa. Accurately characterising the mucosa associated microbiota will facilitate development of diagnostic tests, novel treatments and improved clinical outcomes for patients.

ENDOCHONDRAL BONE FORMATION AND ADVANCED ENTHESITIS ARE KEY FEATURES OF PGISP MOUSE MODEL OF ANKYLOSING SPONDYLITIS

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Ankylosing spondylitis (AS) is an arthritis predominantly targeting the spine and pelvis characterized by an initial inflammatory phase followed by an osteoproliferative phase that can result in joint fusion (ankylosis). The mechanisms controlling the inflammation-bone formation transition are poorly understood due to the lack of human bone samples. Therefore, we used a proteoglycan (PG)-induced spondylitis (PGISp) mouse model to delineate the morphology and molecular change during axial disease progression in a 6-month time course study. The histology depicted morphological features including inflammation, destruction and excessive tissue formation. Mild inflammation initiated at the peripheral disc and then invaded the disc and longitudinal ligament, the enthesis in the spine. The up-regulation of TNF-a, matrix metalloproteinase (MMP)-3, MMP-13 mRNA expression accompanied inflammation and erosion. Mesenchymal cell expansion and chondrocyte formation appeared around disc remnants when inflammation started to decline. The advanced disease was characterised by excess tissue formation around the disc and ectopic chondrocyte expansion along the vertebrae in the absence of inflammatory infiltrate. These excess tissues were composed of cartilage matrixes, including proteoglycan, type II and X collagen but not bone matrix type I collagen. Expression of cartilage markers also increased. The hypertrophic phenotype suggested endochondral ossification is involved in the excessive tissue formation. Moreover, the massive cartilage and hypertrophic chondrocyte formation suggest endochondral ossification mediates the excessive tissue formation in AS. Therefore, the PGISp mouse model is an ideal model for studying inflammation-driven excess tissue formation.

UNDERSTANDING NUTRIENT SULPHATE DEFICIENCY IN PRETERM BABIES

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Background & Aims: Sulphate is an obligate nutrient for fetal growth and development. The fetus is completely reliant on sulphate derived from the maternal circulation via the placenta. Sulphate generation is mediated by the enzyme cysteine dioxygenase 1 (CDO1), which is absent in early gestational fetal tissues. This is relevant to preterm babies that are born at a gestational age when they may lack CDO1 expression and are thereby likely to become sulfate deficient. In this study, we aimed to understand the molecular regulation of CDO1 in a mouse model, as well as investigate sulphate levels in preterm babies.

Method: We quantitated the expression of placental CDO1 in mouse gestation using qRT-PCR and Western blotting. Promoter analysis of human CDO1 was performed using a luciferase reporter assay in cultured placental cells. Plasma and urinary sulphate levels in preterm babies (<30 weeks gestation) were quantitated using ion chromatography.

Results: In mice, CDO1 expression increased from mid-gestation and was localised the decidual region of the placenta. The human CDO1 promoter contains several putative regulatory motifs that are conserved across numerous species. Preterm babies (n=50) rapidly become sulphate deficient in the neonatal period.

Conclusion: Since preterm babies rapidly become sulphate deficient, then it is highly likely that their capacity to generate sulphate is not fully functional. This is relevant to our findings of increasing CDO1 expression from mid-gestation in mouse fetal tissues. Collectively, these findings warrant further investigation of the consequences of sulphate deficiency in preterm babies.

INTESTINAL CHANGES MAY PRECEDE THE DEVELOPMENT OF TYPE 1 DIABETES IN NOD MICE

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The gut microbiota has a critical role in metabolism and normal immune system development. The microbiota are thought to contribute to a variety of disease states when inappropriately skewed or when less diverse microbial populations are present. While type I diabetes (T1D) results from complex interactions between both genetic and non-genetic factors, microbial agents are likely to be one of the most important environmental factors influencing this disease in both humans and animal models. To understand more fully the role of the microbiota in shaping host immunity and T1D, diabetes susceptible NOD mice were compared with diabetes-protected strains to observe differences that might be attributed to microbiota in a number of preliminary studies. NOD mice had a functionally different microbiota to C57BL/6 mice as assessed by short-chain fatty acid analysis. A detailed histological examination of the intestine revealed that prediabetic NOD mice harbor a number of subclinical abnormalities and signs of inflammation compared with the protected strains examined. These included neutrophilic infiltration and paneth cell abnormalities. QRT-PCR results suggest that NOD mice have alterations in microbial defense genes. Our studies demonstrate that changes in the intestinal immune response may precede the development of diabetes.

THERAPEUTIC INDUCTION OF TOLERANCE TO REVERSE ALLERGEN-INDUCED AIRWAY INFLAMMATION

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Allergic asthma is a lower airways inflammatory disease resulting from dysregulated T-cell responses to inhaled allergens. Chronic use of pharmacologic disease modifiers employed to control symptoms has detrimental complications and current allergen-specific immunotherapies are poorly efficacious or have other disadvantages. Previously, we have shown that antigen genetically targeted to dendritic cells (DC) inactivates naive, memory and effector CD4+ and CD8+ T cells. We propose this as the basis for a new approach to 'turn off' dysregulated T cells in asthma. We first tested whether DC expression of the model allergen ovalbumin (OVA) prevented sensitisation and airways inflammation that normally results from OVA323-339/alum immunisation and intranasal (i.n.) OVA challenge. Production of IL-4, IL-5 and IL-13 in recall assays was significantly decreased to baseline levels in OVA-expressing mice relative to non-Tg control and little or no inflammatory cell infiltrate was present in bronchoalveolar lavage fluid (BALF) in OVA-expressing mice after sensitisation and i.n. challenge respectively. Next, we investigated whether pre-existing dysregulated (memory) T-cell responses could be inactivated in mice with established airways inflammation. Using non-myeloablative conditions, OVA-encoding or non-Tg BM was transferred to OVA323-339/alum-immunised and OVA-intranasally challenged BALB/c mice. Production of IL-4, 5, 13 by OVA323-339 restimulated spleen cells and MLNC was dramatically reduced in recipients of OVA-encoding, but not non-Tg, BM. Eosinophil content in BALF and histological evidence of mucus hypersecretion were also reduced indicating reversal of pathogenic processes associated with dysregulated T cell responses. Therefore, expression of an allergen in DC prevents allergic sensitisation and subsequent respiratory immune responses to allergen challenge. Allergen-encoding BM transfer under non-myeloablative conditions terminates established allergic T-cell responses and reduces allergen-induced airway inflammation.

REGIONAL AND SEASONAL VARIATION IN AIRBORNE GRASS POLLEN LEVELS BETWEEN CITIES OF AUSTRALIA AND NEW ZEALAND

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Background: Grass pollen is widely regarded as the major outdoor aeroallergen source in Australia and New Zealand (NZ). However, no assemblage of airborne pollen data for the region has been previously compiled.

Methods: Grass pollen count data collected at 14 urban sites in Australia and NZ over periods ranging from one to 17 years were acquired, assembled and compared, revealing considerable spatiotemporal variability.

Results: Grass pollen seasons tended to have more than one peak from tropics to latitudes of 37°S and single peaks at sites south of this latitude. A longer grass pollen season was therefore found at sites below 37°S, driven by later seasonal end dates for grass growth and flowering. Daily pollen counts increased with latitude; subtropical regions had seasons of both high intensity and long duration. At higher latitude sites the single springtime grass pollen peak is potentially due to a cooler growing season and a predominance of pollen from C3 grasses. The multiple peaks at lower latitude sites may be due to a warmer season and the predominance of pollen from C4 grasses.

Conclusions: Prevalence and duration of seasonal allergies may reflect the differing pollen seasons across Australia and NZ. Spatiotemporal differences in grass pollen counts indicate that local, current, standardized pollen monitoring would assist with the management of pollen allergen exposure for patients at risk of allergic rhinitis and asthma.

A LINEAR MIXED MODEL SPLINE FRAMEWORK FOR ANALYZING TIME COURSE 'OMICS' DATA

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With the advent of high-throughput technologies it is feasible and practical now to study a biological system at all levels of metabolism. Studying time-resolved 'omics' is a powerful method to study an organisms molecular response to perturbation, in developmental process or during reoccurring events. The analysis of these data remains challenging due to its high-dimensionality and different noise components. Therefore, we developed a unified approach, integrating quality control and filtering, linear mixed effect models, supervised and unsupervised methods to explore and analyse time-resolved 'omics'.

UNCOVERING NEW PATHWAYS OF CD8+ T-CELL REGULATION IN THE SKIN

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Tumor specific CD8+ T-cells are important in eliciting tumor rejection in the skin, however, in some cases the tumor microenvironment is able to render them dysfunctional. Regulation of CD8+ T-cell responses in the tumor microenvironment is poorly understood. Using a skin grafting model, we aimed to explore the mechanisms involved in the regulation of CD8+ T-cells in the skin microenvironment. Highly purified adjuvant-activated CD8β+ T-cells were introduced into RAG1KO mice to assess CD8+ T-cell deregulation in the absence of conventional T-cells. Autoimmune mediated deregulation of CD8+ T-cell in the ear skin occurred approximately 37 days post CD8β+ T-cells transfer when co-administrated with CD4-depleting antibody. Transfer of activated CD8β+ T-cells alone did not lead to the development of CD8+ T-cell deregulation. The timing of the destruction suggests that the CD4-expressing cell exerts regulation over a CD8+ T-cell memory response. Preliminary assessment revealed immune infiltrates were confined to the skin and cervix, suggesting that the target antigen(s) may be restricted to stratified squamous epithelium. CD4-depleting antibody treatment led to increased numbers of CD8+ T-cells in the skin. Flow-cytometry of lymph nodes 30 days post CD8β+ T-cell transfer showed no evidence of classical CD4+FoxP3+ regulatory T-cells (Treg) indicating regulation is mediated by a separate, distinct cell type. As yet unidentified CD4+expressing cells were observed in RAG1KO skin by immunofluorescent staining. Using gene-knockout mice as a source of donor CD8+ T-cells, it was established that interferon-gamma was a key mediator of destruction, but not interleukin-17 or perforin. The data suggests that the removal of a CD4+ expressing regulatory cell leads to the breakdown in peripheral tolerance resulting in CD8+ T-cell mediated skin destruction. We plan to use our established model to identify the CD4+ expressing cells, which are distinct from classical Treg, and mediate a regulatory influence on CD8+ T-cell function in the skin.

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PROTEOLYSIS OF THE EPHB4 RECEPTOR IN PROSTATE CANCER; DOES IT PRODUCE BIOACTIVE CLEAVAGE FRAGMENTS?

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EphB4, a receptor tyrosine kinase, is over-expressed in 66% of prostate cancers (PCa) where it promotes tumour angiogenesis, increases cancer cell survival and facilitates cell invasion and migration. Recently, we have identified the presence of novel EphB4 fragments in PCa cell lines that we predicted to be the result of sequential proteolytic cleavage events releasing both extracellular (70kDa) and intracellular (50 kDa and 47 kDa) fragments. The PCa-associated protease KLK4 was identified as the mediator of the first cleavage event using recombinant KLK4 on PCa cells and the cleavage site was determined by N-terminal sequencing (arginine 508), consistent with the identified fragments (70 and 50 kDa). The second fragment of 47 kDa was lost upon γ-secretase inhibition suggesting that the production of this fragment was due to the action of this intramembrane protease. Subcellular fractionation demonstrated that the 47 kDa fragment was present in the nuclear fraction suggesting nuclear translocation of this fragment. DU145 and PC-3 PCa cells were engineered to over-express the 47 kDa, with the DU145 cells acquiring a more mesenchymal-like morphology compared to control cells, suggesting an EMT had occurred, which was further validated by qRT-PCR with EMT markers. This study provides the first evidence of proteolytic regulation of EphB4 and has possibly identified a mechanism for the regulation of EphB4 signalling through the production of bioactive EphB4 protein fragments. Further understanding the function of EphB4 fragments and the proteases that regulate it may identify novel avenues for anti-prostate cancer therapies.

CONTRIBUTION OF IMMUNOSUPPRESSIVE DRUGS TO NON-MELANOMA SKIN CANCER DEVELOPMENT: A ROLE FOR MEMORY T-CELLS?

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Organ transplant recipients have an increased risk of non-melanoma skin cancer (NMSC) development. Immunosuppressive agents, given to these patients to prevent organ rejection, are widely recognised to play a key role in the increased incidence of malignancy. However, different immunosuppressive agents, such as rapamycin and tacrolimus, which both prevent organ rejection, convey different risks of tumour formation. Using a UV-induced murine model of squamous cell carcinoma (SCC); HPV38 E6E7, we aim to determine whether these discrepancies are due to differences in the mechanism of immune suppression, in contrast to the much discussed direct effects of these drugs on tumour cells (i.e. immune-independent). Using customised rapamycin- and tacrolimus-incorporated feed to allow long-term administration, clinically relevant whole blood drug concentrations were obtained and validated using LC-MS/MS. Both systemic and local immunosuppression was functionally determined via adoptively transferred transgenic T-cell assay and skin allograft rejection profile, respectively. Notably however, preliminary data suggests phenotypic and functional differences of skin-residing T-cell subpopulations may exist when mice are treated with either rapamycin or tacrolimus, alluding to their potential role in the pathogenesis of NMSC. Using established UV dosing schedules for SCC development, the kinetics of tumour formation will be assessed under the influence of these drugs. In addition, immunosuppressant treated HPV38 E6E7 mice backcrossed onto Rag1-/- mice (lacking T and B cells) will further elucidate the contribution of these drugs in SSC development. Through this project, we will expand our knowledge base regarding mechanisms of SCC formation in immunosuppressed patients.

SOCIAL INTERACTION REDUCES ETHANOL BUT NOT SUCROSE CONSUMPTION IN ADOLESCENT MICE

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The environment in which alcohol is consumed plays a critical role in the development of and relapse to alcohol consumption. Social interactions influence both positive and negative aspects of alcoholism and are especially important during adolescence when peer group behaviour can either increase or decrease the risk to binge-drinking behaviours. This project aimed to determine whether the Intellicage (NewBehaviour AG) is suitable for examining the influence of social environment on the acquisition of alcohol consumption behaviours in adolescent mice. To achieve this aim we compared two-bottle choice ethanol and sucrose consumption in the Intellicage, an automated device capable of simultaneously measuring behaviours of up to 16 mice living in an enriched social environment, with traditional social and isolated two-bottle choice drinking models. Mice drinking in the Intellicage (social and enriched environment) had significantly lower preference for 20% (v/v) ethanol than those drinking in an unenriched socially-isolated environment. Ethanol preference in the Intellicage was similar to mice drinking in an unenriched social environment. To determine if this effect was specific to ethanol we compared consumption of 5% (w/v) sucrose in the Intellicage with the unenriched socially-isolated environment. There was no effect of drinking environment on sucrose preference. These results suggest that social interaction is more important than other forms of environmental enrichment in ethanol but not sucrose consumption and are consistent with current epidemiologic evidence indicating that specific forms of social interaction can influence binge drinking during adolescence.

IMPROVING CELL MEDIATED IMMUNITY TO NON-TYPEABLE HAEMOPHILUS INFLUENZAE IN CHILDREN WITH CHRONIC SUPPURATIVE LUNG DISEASE.

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Finding an effective vaccine to protect against non-typeable Haemophilus influenzae (NTHi) is an important goal in bronchiectasis & chronic suppurative lung disease (CSLD). NTHi infection is common in both conditions and impaired cell-mediated immunity against NTHi has been documented. Aim: Determine if a H. influenzae protein D containing conjugate vaccine improves cytokine responses to NTHi in children with CSLD. Methods: 108 children with CSLD and 32 healthy children (median age 2 yrs) were included. Those who received the 10-valent pneumococcal H. influenza protein D conjugate vaccine (PHiD-CV) were compared with those who received multi-valent pneumococcal vaccine without protein D. Blood mononuclear cells were cultured with live NTHi. Results: Cells from CSLD children who had received ≥ 3 doses of PHiD-CV produced significantly more IFN-γ (median 939; IQ range 247-2142 pg/ml) than cells from children who had received the alternative vaccines (median 338; IQ range 108-938 pg/ml; p=0.007). Importantly, the amount of IFNγ produced by cells from CSLD children receiving ≥ 3 doses of PHiD-CV approached that seen with healthy children. Having received ≥ 3 doses of PHiD-CV was also associated with small but significant increases in IL-13 and IL-5 (p<0.001 and p=0.007 respectively). Plasma protein D-specific IgG1 levels correlated with the number of PHiD-CV doses (p=0.02). Conclusions: Vaccination with PHiD-CV augments NTHi-specific cell mediated immunity in children with CSLD. Prospective studies are needed to determine whether these effects translate into improved protection against NTHi infections in children with CSLD.

TIE-2 REGULATES THE STEMNESS OF PROSTATE CANCER CELLS

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Prostate cancer (PCa) is the most commonly diagnosed male cancer in Western countries. Currently, the major treatment challenge for PCa patients is the development of tumor metastasis. Ample evidence supports the idea that tumor metastasis originates from a rare population of cancer cells known as cancer stem cells (CSCs). Unfortunately, little is known about the identity of these cells, making it difficult to target prostate tumor metastasis. Here we reported the identification of a rare population of PCa cells which express the Tie-2 protein, a tyrosine kinase receptor required for the bone marrow homing and colonization of hematopoietic stem cells. Notably, this Tie-2+ population exists exclusively in highly metastatic and aggressive PCa cell lines. Data from our study revealed that prostate CSC markers were upregulated in Tie-2+ cells when compared to the Tie-2- population. Meanwhile, Tie-2+ cells are highly adhesive to both osteoblast and endothelial cells, a characteristic necessary for tumor metastasis. We also found that Tie-2+ cells are more quiescent and resistant to the chemotherapeutic drug cabazitaxel, further support that these cells possess CSC-like characteristics. More importantly, we found that Tie-2+ cells, but not the Tie-2- population, developed metastatic tumor in vivo. Our data suggested that Tie-2 plays an important role in the development of drug resistance and prostate tumor metastasis. Thus, Tie-2 might be a novel therapeutic target for the treatment of advanced PCa patients.

MIRAGE IN A GENE DESERT

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Background: Ankylosing spondylitis (AS) is a severely debilitating inflammatory arthritis of unknown aetiology which primarily affects the joints of the spine and pelvis. Large-scale international genome-wide association studies lead by our group have identified a number of loci strongly associated with AS including the “gene desert” at 21q22.

Methods: In order to fully characterise transcriptional activity from this locus, we have designed an RNA-Seq capture array (CaptureSeq) which enables ultra-deep targeted sequencing to identify very rare transcripts around 21q22. We have investigated expression from this area in peripheral blood mononuclear cells from five AS patients and five healthy controls.

Results: There is evidence of bi-directional transcription from the 21q22 locus, indicative of enhancer RNAs (eRNAs). The presence of these eRNAs was confirmed in a large RNA-Seq study on AS patients, and in a third cohort using qPCR. Quantitation of data from CaptureSeq, RNA-Seq and qPCR shows higher expression of these transcripts in AS patients compared to healthy controls, implying higher enhancer activity in AS patients. 21q22 eRNAs are exclusively expressed in CD14+ monocytes, and their expression was further enhanced on microbial stimulus.

Conclusions: This is the first example of a role for a non-coding RNA in AS, and one of the first examples where a polymorphism influences disease by effects on an ncRNA. Our findings strongly support a role for monocytes in AS aetiology, possibly through responses to microbes. Aberrant expression of the microbial-induced 21q22 transcript in CD14+ monocytes presents a novel potential mechanism through which AS may be modulated by microbes.

MUTATIONS IN THE ZINC FINGER DOMAIN OF HUMAN AND MOUSE KLF1 CAUSE CONGENITAL DYSERYTHROPOIETIC ANEMIA (CDA) VIA PROMISCUOUS DNA

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Krüppel-like factor-1 (KLF1) is an essential erythroid-specific transcription factor. A number of studies have shown up to ~700 genes are poorly expressed when KLF1 is absent. This global loss of expression is responsible for failure of effective red blood cell production in KLF1 knockout mice, and partly responsible for congenital dyserythropoietic anemia type IV (CDA-IV) observed in humans with dominant mutations in the DNA-binding domain of KLF1. Recently an ENU-generated mouse model of neonatal anemia, 'nan', was also reported to harbour a mutation in the second zinc-finger of KLF1. Remarkably, the 'nan' mutation (E339D) resides at the equivalent residue in human CDA IV (= E325 in humans). Unlike loss of function point mutations in KLF1, this mutation leads to a more severe phenotype than the KLF1 null allele, suggesting it is an unusual dominant mutation. To investigate how this mutation might cause disease, we performed ChIP-seq to determine differences in genome occupancy in vivo, and identified novel sites occupied by EKLF-E339D but not by wild type KLF1. Ectopic binding to non-erythroid gene promoters is accompanied by aberrant gene expression as determined by 4sU labelling and deep sequencing of nascent RNAs. Together RNA-seq and ChIP-seq studies have provided a novel explanation for how mutations in KLF1 result in dominant anaemia in mice and man. To our knowledge this mechanism, whereby a transcription factor DNA-binding domain mutation leads to promiscuous binding, activation of an aberrant transcriptional program and subsequent derailing of co-ordinated differentiation, is novel.

TARGETING ANTIGEN TO HUMAN CD141+DC VIA CLEC9A PRODUCES SUPERIOR CROSS-PRESENTATION TO CD8+T CELLS COMPARED TO TARGETING DEC-205

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Cytotoxic T lymphocyte (CTL) responses are initiated by dendritic cells (DC) and are required for immune-mediated clearance of tumours and many pathogens for which there are currently no effective vaccines. Targeting antigen to DC in vivo is an attractive strategy for vaccine development and clinical trials are currently underway using antibodies specific for the DEC-205 receptor to deliver tumour antigen to DC in patients with solid malignancies. Human CD141+ DC have been identified as the main subtype involved in antigen cross-presentation and are considered the most effective subset to target for CTL induction. The C-type lectin-like receptor CLEC9A is specifically expressed on CD141+DC and facilitates cross-presentation of dead cell antigen, making it an attractive candidate for specifically targeting this DC subset. We have produced and validated recombinant human chimearic IgG4 antibody specific for human CLEC9A and DEC-205. We fused a fragment of human CMVpp65, including the HLA-A2-restricted NLVPMVATV epitope, to the Ab heavy chains to create antibody-antigen fusion proteins. These fusion proteins retain binding specificity for their target receptor, are internalised by, and accumulate within DCs. We investigated the ability of CD141+ and CD1c+DCs to cross-present the NLVPMVATV epitope to NLV-specific CTL lines following uptake of fusion proteins. Our data demonstrate that anti-CLEC9A antibodies are more effective at delivering antigen for cross-presentation by CD141+DCs compared to anti-DEC-205 antibodies, while CD1c+DCs did not cross-present antigen from either antibody. Thus, targeting of human CD141+DC via CLEC9A is a strategy that warrants development as an immunotherapeutic for cancer or infectious disease.

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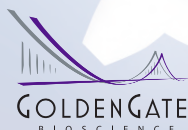

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CD83 ANTIBODY: A POTENTIAL NEW THERAPY FOR AUTOIMMUNE DISEASE AND TRANSPLANT REJECTION

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CD83 is expressed on the plasma membrane during maturation of dendritic cells (DC). It is also expressed on activated CD4 T cells and activated B-cells in mouse and man. Anti-CD83 antibody depletes activated DC and CD4 T cells by NK cell mediated antibody dependent cellular cytoxicity (ADCC) and has potential for the control of graft-versus-host disease. We hypothesized that anti-CD83 antibody would also deplete CD83+ activated B-cells and inhibit B-cell responses, without depleting resting B-cells and therefore may have therapeutic advantages in antibody mediated autoimmunity and organ transplant rejection. In a 51-Cr release assay we showed that CD83 antibody can induce ADCC lysis by NK cells of activated B-cells but not of resting B-cells isolated from PBMC. Also, ELISA analysis showed that anti-CD83 inhibited human total IgM and IgG expression in vitro by allo-stimulated PBMC. To determine if CD83 antibody can modulate antigen specific B cell responses, CFSE-labelled human PBMC were cultured with the recall antigen tetanus toxoid (TT) in the presence of anti-CD83 or control antibody. Flow cytometric analysis showed that anti-CD83 significantly suppressed TT-responsive B-cell and CD4 T-helper cell proliferation ($p<0.001$, $n=9$) without affecting non-proliferating cells. Bioplex analysis showed that expression of the autoimmune disease associated cytokines IFN-γ and IL-17A were significantly reduced. In conclusion, CD83 antibody depletes activated CD83+ B-cells, as well as activated CD4 T cells and DC, all of which play key roles in autoimmunity and transplant rejection. Accordingly, CD83 antibody may be a novel and useful approach to treating or preventing these conditions.

TRANSCRIPTIONAL PROFILING OF NAIVE AND MEMORY CD8+ T CELLS UNDERGOING TOLERANCE INDUCTION

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Dendritic cells (DC) expressing cognate antigen can induce peripheral tolerance in naive and memory CD4+ and CD8+ T cells. To understand and compare the mechanisms that contribute to peripheral tolerance induction in naive and memory CD8+ T cells, we performed transcriptional profiling. Naive or memory TCR transgenic CD8+ T cells (OT-I) were transferred to 11c.OVA mice that express OVA in DC. mRNA was isolated (Quiagen RNeasy) from OT-I T cells recovered at day 2 (expansion phase), 10 (deletion phase) and 28 (tolerant phase) after transfer and control cells processed and hybridised to the Illumina 48K Mouse Ref-6 V2.0 Expression Chip. We compared gene expression longitudinally across time points and between naive and memory T cells at each time point. After transfer of naïve OT-I to 11c.OVA, differentially expressed genes (DEGs; ≥2-fold, $p<0.05$) were 1213 at d2. By d10, the number of DEGs was reduced 866 and by d28, only 566 were differentially expressed relative to naive OT-I. This indicates that after the initial activation occurring between d0 and d2, and as tolerance proceeds, naive OT-I T cells revert to a state more resembling naive cell (d0). At transfer, memory OT-I were transcriptionally distinguishable from naive OT-I (1884 genes ≥2-fold, $p<0.05$), but as tolerance induction proceeded they developed a profile that resembled 'tolerant' naive OT-I. K-means clustering and functional annotation of clusters revealed remarkable similarity in the genes regulated in naive and memory OT-I during tolerance induction. This study provides a transcriptional framework for understanding peripheral T cell tolerance.

DETERMINATION OF NOVEL MOLECULAR PATHWAYS REGULATED BY KALLIKREIN-RELATED PEPTIDASE 7 IN OVARIAN CANCER

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Kallikrein-related peptidase 7 is a serine protease that is highly expressed in ovarian tumour, compared to normal ovaries, and is associated with the progression of this cancer. Thus far, only a few KLK7 substrates have been identified in a biochemical context, which has linked KLK7 action to tumour progression. In addition, a proteome-wide study to define the KLK7 biological function in this cancer is long overdue. Hence, we sought to define the substrate mediators of KLK7; employing a novel N-terminomics based technique, namely Terminal Amine Isotopic Labelling of Substrates (TAILS). TAILS employs digestion of native proteins secreted by ovarian cancer cells such as SKOV-3 and OV-MZ-6 with recombinant active KLK7 and an inactive double mutant (dm)KLK7 (D112N and S205A in the catalytic triad) as control, followed by labelling of native and KLK7-derived N-termini by dimethylation with light and heavy formaldehyde. N-terminally labelled peptides are digested with Trypsin/GluC and enriched by negative selection followed by liquid chromatography (LC)-MS/MS. Thirty two potential KLK7 substrates were identified along with two known substrates, which validated our approach. The majority were novel substrates, including extra cellular matrix components, growth factors, cytokines and cell surface receptors, degradation of which is associated with increased tumour progression. For an instance, mediators of the TGFβ1 signalling pathway, such as Thrombospondin 1 and Integrin β6 are identified as two novel substrates of KLK7, degradation of which may facilitate the progression of ovarian cancer. Cell-based validation of the effects of KLK7 action on ovarian cancer epithelial cells and validation of the novel substrates in vitro and in vivo is currently being undertaken.

MATERNAL NICOTINE CONSUMPTION IS ASSOCIATED WITH PHENOTYPIC AND TRANSCRIPTIONAL CHANGES IN THE EMBRYO

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Maternal tobacco usage in pregnancy is associated with increased risk of both adverse gestational outcomes (such as fetal growth restriction) and long-term outcomes including behavioural disorders and obesity in the resultant children. Australian data from 2010 suggest that 11.7% of pregnant women admit to smoking prior to confirming their pregnancies. For almost half of Australian women, confirmation of their pregnancies occurs six or more weeks after conception. What effect, if any, that this early exposure to nicotine products has on the developing embryo has yet to be elucidated.

We have developed a mouse model for chronic moderate nicotine exposure during early gestation. Pregnant C57BL/6 mice were provided with ad libitum access to 100 μg/ml nicotine from 0.5 to 8.5 dpc (equivalent to the first 3-4 weeks of human gestation). Resultant embryos were then assayed for morphological and transcriptional changes at 9.5 dpc. A reduction in crown-rump length, but not in somite number following this nicotine exposure was observed. 105 genes from a genome-wide mRNA microarray were identified to be differentially expressed (fold change ≥ 1.5, uncorrected p-value ≤ 0.05), of which 21 were correlated (absolute Pearson's $r \geq 0.5$) to a measure of embryonic size (crown-rump length normalised to somite count). Many of these genes had annotated roles in growth and metabolism. The regulation and functional significance of these transcriptional changes is currently being investigated.

These data suggest that maternal nicotine consumption, even if not for the entirety of gestation, has both phenotypic and transcriptional consequences in the mouse.

INVESTIGATION OF AN ATYPICAL CELL CYCLE CHECKPOINT DEFECT IN MELANOMA: A NEW THERAPEUTIC TARGET?

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Recently discovered targeted therapies for melanoma have proven good response in patients. However such treatments are limited by rapid development of drug resistance, instigating research towards the identification of novel therapeutic targets. Cell cycle checkpoints are control mechanisms that preserve genomic integrity and control cell proliferation. Defective cell cycle checkpoints provide obvious growth advantages to tumours, however they are also thought to increase cell reliance on survival mechanisms in order to cope with accumulation of DNA damage. Ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related (ATR) together with their respective downstream effectors checkpoint kinase 1 and checkpoint kinase 2 protein (Chk1 and Chk2) are crucial kinase proteins for checkpoint activation. We have found that more than 60% of melanoma cell lines have a defective ATM-dependent checkpoint arrest. The novelty of this defect is that ATM-Chk2 activation is normal but it is unable to establish a stable cell cycle arrest. This differentiates the ATM-checkpoint defect from checkpoint defects caused by mutations that inactivate ATM signal. The cause of impaired cell cycle arrest appears to be upregulated Plk1 activity, a cell cycle promoting kinase whose activation is blocked by ATM-Chk2 and ATR-Chk1 signalling. Interestingly, the ATM-checkpoint defect enhances cellular survival in response to genotoxic stress (DNA double strand breaks) but make the cells reliant on ATR-Chk1 activity. Elucidation of the molecular mechanism of the ATM-checkpoint defect and its understanding in the context of melanoma treatment might be beneficial for the advancement of targeted melanoma therapy.

CHARACTERISATION OF A CHECKPOINT AND REPAIR RESPONSE TO UVB-EXPOSURE THAT IS COMMONLY DEFECTIVE IN MELANOMA

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Background: The high load of UV signature DNA mutations found in melanomas is evidence of not only the role of UV in melanomagenesis but also of a defect in repair of UV-induced lesions. We have previously identified a G2 phase cell cycle checkpoint and repair response to low-dose UVB that is commonly defective in melanoma cell lines. Here we aim to identify the components of the checkpoint and repair pathways involved in this UV-induced response.

Methods: We have investigated the global alterations in: transcription; translation; protein abundance; and microRNAs associated with the UV response. We have used two model systems previously characterised for the UV-induced G2 delay, an A2058 melanoma cell line model and a whole skin organ culture model. Total mRNA and polysome mRNA were analysed using Illumina whole genome microarrays, microRNAs were analysed by RNA-seq, and protein changes were analysed by
SILAC proteomics.

Results: Data was combined from these analyses to identify a set of 45 high probability candidates, and 11 UV-responsive mircroRNAs potentially contributing to this response. Candidates are being further examined using high-throughput functional analyses, to determine their involvement in the normal UV response. A summary of ongoing work characterising the UV response will be presented.

Conclusions: Once the mechanisms of the normal UV response have been characterised, we can identify defects in the normal surveillance, checkpoint and/or repair mechanisms which allow escape of UV-induced mutations. This may offer opportunities to expand diagnostic (and potentially therapeutic) options for melanoma patients.

GENETIC CONTROL OF THYMUS SELECTION IN TYPE 1 DIABETES

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Type 1 Diabetes (T1D) is a chronic disease mediated by autoreactive T cells specific for islet autoantigens destructing the insulin-producing β cells of the pancreatic islets of Langerhans. Several studies have suggested that the thymic negative selection of Non-Obese Diabetic (NOD) mice is defective. NOD mice spontaneously develop T1D, serving as a model of understanding the genetic and immunological basis of T1D. Although it is unclear which genetic loci control defective negative selection in NOD mice, crude mapping studies have implicated several diabetes susceptibility regions including Idd3 and Idd5 that have not been confirmed. Peripheral tolerance was found to be restored when both T1D protective-Idd3 and –Idd5 alleles derived from diabetes-resistant mice were expressed within diabetes-susceptible NOD mice. There is, however, a critical knowledge gap existing between the association of negative selection defect with Idd alleles and the actual functionality that these genetic regions promote. Therefore, by using congenic-NOD mice expressing Idd3 and Idd5 alleles, this project aims to further investigate the role of these genetic regions in central tolerance. By using MHC (Kd) tetramers loaded with specific islet autoantigens, islet-specific glucose-6-phosphate catalytic subunit-related protein (IGRP) and insulin, the amount and the functionality of CD8+ T cells specific for islet autoantigens were investigated in NOD mice and Idd3/5-congenic NOD mice. It is believed that protective-Idd3 and –Idd5 alleles would function together to promote the reduction of overall frequency of autoreactive T cells by increasing the efficiency of thymic deletion or purging of autoreactive T cells within NOD mice.

T-BET: AN IMPORTANT REGULATOR OF INFLAMMATION IN ANKYLOSING SPONDYLITIS

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Ankylosing spondylitis (AS) is an inflammatory arthritis of the spine and pelvis, driven by aberrant immune responses. Genetic variants in TBX21 are associated with increased likelihood of developing AS. TBX21 encodes the transcription factor T-bet, a key regulator of innate and adaptive immunity. However, the importance of T-bet in AS pathogenesis remains unclear. Our study is the first to address this. To determine if T-bet expression is altered in AS, we compared gene and protein expression in peripheral blood mononuclear cells between AS patients (n=172) and healthy individuals (n=83) by quantitative real-time PCR and multicolour flow cytometry respectively. Using flow cytometric analyses of peripheral lymph nodes, the draining sites of inflammation, we examined the number and function of T-bet-dependent cells across the timecourse of disease in the SKG mouse model of AS. Our data show higher TBX21 expression in AS patients than healthy individuals ($P<0.0001$). Enhanced T-bet expression is predominantly driven by NK cells ($P=0.0200$) and CD8 T cells ($P<0.0001$). In SKG mice, T-bet expression increases early after disease initiation and persists throughout the course of disease with activated NK cells and CD8 T cells being the dominant sources of T-bet. These cells secreted more pro-inflammatory cytokines IFN-gamma and IL-17. To formally test the role of T-bet in AS pathogenesis, we are now examining the rate and fate of disease progression in Tbx21-/- SKG mice. Further understanding of the immune landscape of disease is critical for identifying targetable cells, particularly as T-bet may be a useful biomarker of disease activity.

SALIVARY GLYCOPROTEIN CHARACTERIZATION USING LECTIN MAGNETIC BEAD ARRAYS

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Protein glycosylation is an attractive source for discovering biomarkers of systemic diseases. Many cancers have profound effects on cells' glycosylation machinery, thus glycoproteins can carry structures which are markedly different to those from normal cells. Human saliva is an emerging diagnostic fluid, as not only is it easy to handle and ideal for remote site testing but more than 80% of the salivary proteome is glycosylated. The aim of this study is therefore to develop a high-throughput methodology for glycoprotein enrichment, optimized for human saliva, using lectin magnetic bead arrays (LeMBA). We have titrated the amount of protein binding to beads and optimized the buffers for denaturing to correspond to the overall low protein concentration in human saliva compared to serum. We have shown the methodology is reproducible in different human saliva fractions from the same biological source using SDS-PAGE separation and high-performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), suggesting that our methodology is robust and suitable for future clinical validations. In summary we suggest that saliva optimized with LeMBA (saLeMBA) is a powerful tool for glycoprotein enrichment which could be used for biomarker discovery using human saliva in systemic diseases such as cancer.

BDX1: A NATURAL SMALL MOLECULE ACTIVATOR OF THE AMINO ACID STRESS RESPONSE PATHWAY IN PROSTATE CANCER CELLS

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Objectives: Ascidians are a known source of anticancer compounds with potentially new mechanisms of action. Yet, most of previously isolated compounds are unknown for their mechanism of action. During a screening campaign of ascidian natural products from the Great Barrier Reef, we isolated the cytotoxic compound BDX1.

Methods: BDX1 was investigated in a panel of prostate cancer cell lines, and mechanism of action studies were carried out in LNCaP cells. IC50 values were determined by IncuCyte and Alamar Blue assays. Cell cycle distribution was determined by FACS analysis. Target identification was initiated by DNA microarray and validated by qRT- PCR and Western blotting.

Results: BDX1 was more active in LNCaP cells compared to other PCa cell lines, with low cytotoxicity in MDA-MB-231 breast cancer cells and non-malignant NFF cells. BDX1 caused a temporary G0/G1 arrest in LNCaP cells and elevated levels of the autophagy marker LC3B-II. Transcript profiling and Ingenuity pathway analysis highlighted genes involved in amino acid (AA) synthesis and transport and tRNA charging. qRT-PCR of markers of AA and endoplasmic reticulum stress response confirmed that BDX1 induced an AA stress response in LNCaP cells. Consistent with this, BDX1 activated the phosphorylation of the AA deficiency sensor kinase GCN2, leading to the inhibitory phosphorylation of eIF2a and translation of ATF4, ultimately causing a reduction in protein synthesis.

Conclusions: BDX1 is a promising lead molecule for therapeutic development, targeting the amino acid metabolism in prostate cancer cells. Ongoing experiments investigate the molecular target of BDX1 and its chemical synthesis.

TARGETING LIVER MACROPHAGES WITH CURCUMIN AND REGULATORY T CELLS PROTECTS MICE FROM DIET INDUCED STEATOHEPATITIS

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Background: Nonalcoholic steatohepatitis (NASH) is characterized by an increase in the number of hepatic macrophages with a pro-inflammatory phenotype. To preserve macrophage integrity but reduce inflammatory activation in NASH we have developed liposomes with anti-inflammatory drug curcumin and ovalbumin antigen entrapped and injected these nanoparticles together with ovalbumin-specific regulatory Tcells (Tregs) into mice fed methionine and choline deficient (MCD)-diet and tested their capacity to target inflammatory macrophages, reduce inflammation and treat disease.

Methods: C57BL/6 mice were fed MCD-diet for 2 weeks and then intravenously injected with 100 µL of DiI-labelled curcumin-ovalbumin liposomes and 24 hours later adoptively transferred with 8x105 ovalbumin-specific Tregs. Serum and livers were harvested 1-week post treatment, immune cells were isolated and analysed using flow cytometry. Stage of disease and severity was assessed by measuring serum ALT levels, and by H&E staining.

Results: Mice fed MCD-diet for 3 weeks developed severe steatohepatitis with cellular recruitment of pro-inflammatory cells including CD11b+F4/80+ macrophages and dendritic cells compared to mice on control diet. Curcumin-ovalbumin liposomes targeted hepatic macrophages and DCs. The outcome on targeting hepatic APCs with curcumin-ovalbumin liposomes was reduced macrophage activation and reduced proportion of inflammatory DCs which prevented MCD-diet induced liver damage as measured by serum ALT and severity of disease as measured by NAFLD assessing score. Addition of ovalbumin-specific Tregs with curcumin liposomes containing ovalbumin further reduced hepatocyte damage and reduced neutrophil proportions in the liver.

Conclusion: Targeting curcumin and regulatory T cells to hepatic inflammatory APC is a novel approach to prevent NASH progression.

INVESTIGATING THE ROLE OF THE EPITHELIAL-TO-MESENCHYMAL PLASTICITY IN PROSTATE CANCER METASTASIS

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The majority of prostate cancer (PC) complications and mortality occur due to the outgrowth of metastatic deposits rather than from the primary tumour itself. Cancer metastasis is hypothesised to be linked with the reactivation of the latent embryonic programs known as the Epithelial to Mesenchymal Transition (EMT) and the reverse, Mesenchymal to Epithelial Transition (MET). The activation of EMT imparts epithelial cancer cells with invasive characteristics, aiding their dissemination to secondary tissue locations. However, recent research suggests that a reversion back to their proliferative epithelial phenotype via an MET is imperative for the growth of the secondary tumours. With increasing evidence that cell plasticity plays an important role in cancer progression, more accurate models are required to study these events more thoroughly. We engineered LNCaP PC cells with doxycycline (dox) inducible expression of the potent EMT-inducing transcription factors Snail and Slug. Exposure of cells to dox induced a rapid transition of cells to a mesenchymal-like phenotype, which we confirmed via the reduction in epithelial specific markers and an increase in mesenchymal markers at the mRNA and protein level. Upon removal of dox from cells having undergone an EMT, we observed the converse change in these EMT markers, whereby they returned to prior baseline levels following MET. Cells induced into the EMT-state became highly invasive in 3D-on-top Matrigel™ assays, and this was paralleled by a loss of proliferative ability. These features were reversed following MET. Microarray profiling between EMT and MET states, revealed a significant association of MET enriched gene expression in metastatic PC samples, which also correlated with patient relapse and poorer overall survival. By increasing our understanding of the EMT and MET programs and their role in PC metastasis we hope to identify new biomarkers of disease progression and therapeutic targets to improve patient treatment and outcomes.

THE ROLE OF FKBP52 IN DNA DAMAGE REPAIR PATHWAYS

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Damage to genetic material represents a constant threat to genomic stability, with tens of thousands of DNA lesions occurring every day in every cell. If these lesions are not repaired correctly it can lead to disease states such as cancer and neurodegenerative disorders. To protect the integrity of the DNA, cells have evolved a global signalling network known as the DNA damage response. Recently FKBP52 has been implicated in DNA damage and repair pathways. FKBP52 is a peptidyl-prolyl isomerase, that has already been implicated in cancer biology with an up regulation observed in drug resistant breast carcinoma and can interact with DNA damage signalling proteins e.g. p53. The aim of this project is to determine if FKBP52 is a central mediator in the DNA damage response pathway. DNA damage was induced in the U20S cells, by Ionising Radiation (IR) and H2O2. FKBP52 was depleted using siRNAs and the cellular response was examined using western blots, immunoprecipitation and clonogenic assays. We have determined that depletion of FKBP52 increases genomic instability and cells display increased sensitivity to ionising radiation. Immunofluorescence studies demonstrate an up-regulation of FKBP52 levels in the nucleus in response to DNA damage. Thus suggesting a role for FKBP52 in either signalling or repair of DNA damage. Early studies indicate that FKBP52 may be involved in the DNA damage response pathway and may interact with other known DNA Damage repair proteins, it may also be needed for the repair of DNA breaks.

HUMAN SINGLE-STRANDED DNA-BINDING PROTEIN 1 (HSSB1) FUNCTIONS IN A POSITIVE FEEDBACK LOOP WITH THE ATR KINASE

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Introduction and aims: DNA damage can occur through a variety of means, including as a result of normal cellular metabolism and chemical or radiation exposure. The cell must repair these damages in order to maintain viability and prevent malignant transformation, conditions which may manifest in neurological degeneration, premature ageing, development defects and cancer. Human single-stranded DNA-binding protein 1 (hSSB1) is a central DNA repair protein required for the efficient resolution of double-strand DNA breaks and stabilisation of stalled replicative forks. These pathways are essential for the maintenance of genomic stability and prevention of malignant transformation. In this work we aim to further characterise the hSSB1 signalling events which govern these processes.

Methods: Replicative stress was induced in cells using the ribonucleotide reductase inhibitor hydroxyurea and phosphorylation of hSSB1 monitored using a poly-clonal antibody raised against a phosphorylated hSSB1 peptide. In order to assess the function of this modification we have generated a series of hSSB1 mutants using site directed mutagenesis. The activity of these mutant proteins was assessed both in vitro and in cells.

Results: In the current work we build on earlier data to suggest a novel signalling mechanism through which hSSB1 is activated following replication fork stalling. This we propose occurs through association with and subsequent phosphorylation by the ATR kinase. Phosphorylation then seems to alter the substrate-binding efficiency of hSSB1, stimulating DNA repair signalling.

Conclusions: Our data illustrates a novel feedback system through which the cell is able to detected, signal and restart stalled replication forks.

MITOCHONDRIAL TARGETING OF SUPEROXIDE BY MITOQ IMPROVES METABOLIC RENAL DYSFUNCTION

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Background: Pathological progression of diabetic renal disease is associated with mitochondrial dysfunction attributed in part to excess reactive oxygen species (ROS) generation.

Aim: Our objective was to investigate if targeting mitochondrial ROS using a unique anti-oxidant MitoQ could improve renal function in experimental diabetes.

Methods: Groups (n=10/group; 8W of age) of randomised male db/db and db/m mice to (i) Vehicle, (ii) MitoQ (MQ), (iii) Ramipril or (iv) Co-therapy were followed for 12 weeks. Human immortalized podocytes and proximal tubular cells (PTC) were also studied using a Seahorse XF24 analyser to define mitochondrial respiratory function after normal (NORM; 5.5mM glucose & 100pM insulin), diabetic-like (DIA; 15mM & 500pM) and modulating (MOD; 2 hour alternating treatments) conditions in the presence and absence of MQ.

Results: In Vivo, pharmacological interventions improved renal function (Sinistrin GFR and UAER) in db/db mice. Oral glucose tolerance testing suggested improvements in renal function with MQ were independent of improved glycaemic control. Analysis of isolated renal mitochondria showed improved function with MQ. In vitro, PTCs had greater maximal respiratory capacity (MRC) and ATP content than podocytes in NORM. Under DIA, mitochondrial respiratory function was not significantly altered. However, mimicking postprandial glucose excursions, via MOD, podocytes showed significantly elevated MRC and ATP content compared with NORM. MQ treatment facilitated increased podocyte mitochondrial function through improved basal respiration and ATP content.

Conclusions: Targeting of mitochondrial ROS by MQ has efficacy in experimental models of type 2 diabetes and mechanistically improves mitochondrial function both in vivo in renal cortical mitochondria and in cultured human cells.

REGULATION OF IRX4 AND ITS ANTI-SENSE LNCRNA NONHSAG039687 BY ANDROGENS IN PROSTATE CANCER

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Genome-wide association studies (GWAS) have identified more than 100 single nucleotide polymorphisms (SNPs) associated with men having increased risk of developing prostate cancer, with most of these SNPs occurring in non-protein coding DNA. A recent GWAS found the rs12653946 SNP at 5p15 to be significantly associated with prostate cancer risk in multi ethnic populations. This SNP is proximal to the IRX4 gene and the rs12653946 SNP genotype has been correlated with lower expression of the IRX4 transcript in prostate cancer cells. IRX4 has also recently been shown to suppress prostate cancer proliferation. We have found a novel long non-coding RNA (lncRNA), NONHSAG039687, in the antisense strand of IRX4, and interestingly, we observed an overexpression of this locus from RNA-seq data following androgen treatment. Therefore we hypothesis the expression of IRX4 and its anti-sense lncRNA NONHSAG039687 is regulated by androgens.

An androgen deprivation assay was performed in LNCaP and DuCaP prostate cancer cells followed by qRT-PCR of NONHSAG039687 and IRX4. We found an increased expression of NONHSAG039687 and IRX4 in DuCaP cells with DHT treatment, but not in LNCaP. We have found an ERG/AR binding region in the upstream of IRX4 from VCaP ERG/AR chip sequencing data. Therefore, we assume ERG over-expression makes NONHSAG039687 and IRX4 responsive to androgens in DuCaPs. In addition, the literature provides evidence for increased expression of IRX4 in TMPRESS2:ERG positive prostate cancer. Further validation is currently being undertaken.

This study will determine whether IRX4 and NONHSAG039687 are critical downstream genes of ERG in androgen mediated TMPRESS2:ERG fusion-positive prostate cancer.

IDENTIFICATION OF DE NOVO L1 INSERTIONS IN GLIOBLASTOMA MULTIFORME

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LINE 1 (L1) is the only autonomously active retrotransposon present in the human genome. Its mobilisation has been associated with >100 diseases, including several cancer types. However, to date, de novo L1 insertions have not been identified in glioblastoma (GBM) tumour samples. GBM is the most common and deadliest malignant primary brain tumour in humans, with a median survival of 12 months and relatively unclear disease aetiology. In this study, we examined tumour and non-tumour tissue samples from 14 GBM patients using RetrotransposonCapture-Sequencing. This approach allowed us to identify L1 atypical events for the first time in GBM. In 4 individuals we found de novo, tumour-specific L1 insertions, one of which was located within the MeCp2 gene. The MeCp2 protein binds methylated CpG regions inducing chromatin structure changes and altering transcription factors recruitment. Furthermore, MeCp2 has been previously associated with the regulation of L1mobilisation in the brain. Intriguingly, the de novo L1 insertion identified within MeCp2 significantly reduces MeCp2 expression in the relevant tumour sample compared to non-tumour sample from the same donor, as well as compared to brain tissue samples from unrelated donors. These results demonstrate L1 mobilisation is not restricted to epithelial tumours and indicate functional consequences of L1 mobilisation within GBM tumours.

THE GHRELIN RECEPTOR ANTISENSE GENE, GHSROS, IS A NEWLY IDENTIFIED ONCOGENIC LONG NON-CODING RNA IN PROSTATE CANCER

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Prostate cancer is the most common cancer in men in Australia and will affect one in eight men during their lifetime. Treatments for advanced prostate cancer have improved considerably in recent years, however, metastatic prostate cancer remains incurable. The roles of long non-coding RNA (lncRNA) in disease are becoming increasingly appreciated, particularly in cancer. Non-coding RNAs have emerged as important orchestrators of gene expression and have significant potential as biomarkers for cancer. We have discovered a natural antisense long non-coding RNA (lncRNA), encoded by the opposite strand of the growth hormone secretagogue receptor (GHSR) gene, which we have termed GHSROS (GHSR opposite strand). GHSROS expression was investigated using strand-specific, quantitative real-time RT-PCR (qRT-PCR) in prostate cancer cell lines and clinical prostate cancer specimens. Using the xCELLigence real-time cell analysis system the effects of forced overexpression of GHSROS on proliferation, cell migration and invasion were measured (and compared to vector-only controls) in the PC3 prostate cancer cell line. GHSROS is expressed in prostate cancer cell lines and in clinical prostate cancer specimens and forced overexpression of GHSROS significantly increased cell proliferation and migration in PC3 and DU145 prostate cancer cells, compared to vector controls. Our study indicates that GHSROS may have clinical significance in prostate cancer progression as it is expressed in clinical cases of prostate cancer and functional studies indicate that it plays a role in cell proliferation and migration. GHSROS may provide a useful target for the development of novel antisense therapies for prostate cancer treatment.

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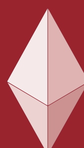

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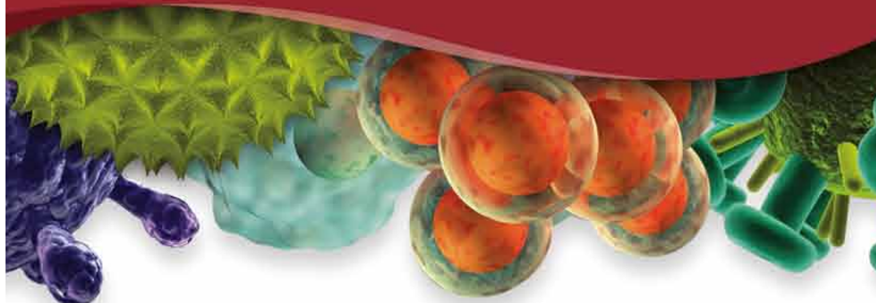

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TUMOUR VERSUS HOST uPAR: ROLE IN BREAST CANCER METASTASIS

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Elevated level of urokinase receptor (uPAR) is detected in various aggressive cancer types including breast cancer and is closely associated with poor prognosis. Both, by initiating a proteolytic cascade acting on extracellular matrix components, and by regulating the activity of important signal transducers, uPAR controls several signaling pathways modulating cell invasion and tumour progression. Within the tumour microenvironment several cell types express uPAR including: tumour cells, endothelial cells, fibroblasts and infiltrating immune cells. In human breast carcinoma uPAR as focally upregulated in both macrophages and myofibroblasts in microinvasive areas, but not in tumour cells. For this reason we decided to investigate the pro-metastatic activity of uPAR in the different tumoural compartments: host versus tumour cells. By performing a series of genetic crosses and bone marrow transplantation experiments involving the PyMT mouse model of breast carcinogenesis and uPAR KO mice, we were able to show that uPAR in the host compartment has minor effects on the growth of primary tumours, while it has a strong promoting effect on both spontaneous and experimental lung metastasis. More importantly, transplantation of uPAR WT bone marrow into uPAR KO mice completely rescued inhibition of experimental lung metastasis. Interestingly, lack of host uPAR reduced the amount of tumour infiltrating myeloid cells and the percentage of circulating Tie2 expressing monocytes, a pro-tumoural subpopulation of monocyte endowed with pro-angiogenic and immunosuppressive activities. These data provide the first in vivo functional evidence for a pro-metastatic role of uPAR in the tumour microenvironment.

TARGETING MULTIPLE ISLET AUTOANTIGENS FOR TOLERANCE INDUCTION

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Type 1 diabetes (T1D) is an autoimmune disease characterized by CD4+ and CD8+ T-cell mediated destruction of insulin-secreting pancreatic beta cells. Insulin-specific T cells appear early in the disease process and transferring hematopoietic progenitor cells (HPCs) encoding proinsulin prevents diabetes in young mice by inducing immunological tolerance to proinsulin. However, T cells specific to the other islet antigens appear later in the disease process and tolerization of these T cells may be required to prevent progression of disease in symptomatic individuals. The aim of this study is to evaluate whether targeting the presentation of multiple islet autoantigens to resting antigen presenting cells (APCs) can effectively tolerize autoreactive T cells with multiple specificities. To facilitate this, lentivirus vectors were generated containing tandem sequences encoding islet antigenic determinants from proinsulin, IGRP, AI4 (mimotope) and Chromogranin A. Here, we demonstrate that these lentivirus vectors transduce murine HPCs efficiently leading to expression of the polytope and the reporter GFP. The HPC enriched fraction contained the cKIt+Sca1+ (hematopoietic stem/Multipotent progenitors) cells that have the potential to mediate long-term engraftment and persistent expression of the polytope. HPCs transduced with the polytope encoding lentivirus vectors will be transferred into NOD mice to drive polytope expression to resting APCs. Subsequently, the capacity for simultaneous tolerization of autoreactive T cells with multiple specificities will be evaluated by transferring mixed populations of TCR transgenic T cells. These studies could lead to the development of effective cell-based therapeutic strategies even in symptomatic T1D individuals and other T cell mediated autoimmune disorders.

A PATIENT WITH PERINEURAL SQUAMOUS CELL CARCINOMA POSSESSES INTRATUMORAL T CELLS WHICH EXPRESS TIM-3 AND/OR PD-1

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The perineural spread of tumours cells along the cranial nerves is an often lethal complication associated with primary squamous cell carcinoma of the head and neck region. Very little is known about the immune response to perineural deposits of tumour. Our aim was to examine perineural SCC samples for the presence of T cell infiltrates and analyse their expression of inhibitory surface markers and functional capability. Although at a preliminary stage, our study has found a patient with substantial expression of PD-1 and/or TIM-3 on both CD4 and CD8 T cells isolated from the tumour microenvironment and analysed using flow cytometry. These markers were not expressed on blood-derived CD4 and CD8 T cells from the same patient. A second patient with neuronitis but no evidence of tumour cells had T cells which did not substantially express PD-1 or TIM-3. This preliminary data suggests that perineural SCC can be associated with T cells of an "exhausted" or inhibited phenotype. In future studies it will be important to determine the proportion of patients with T cells expressing these markers and determine the functional consequences of PD-1/TIM-3 expression.

STERILE INFLAMATION IS THE DETERMINANT OF ADAPTIVE IMMUNITY

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Our aim is to examine how signals delivered from distressed, dying and dead cells, in the absence of infection, impact on the instructions given by antigen presenting cells to immune effector cells, and on the type of immune response induced. We want to compare the input inflammatory stimuli with the output adaptive immune responses generated to non-self and self-antigens. The information from this comparison will inform direct manipulation of the class of immune response generated to an antigen, through the use of controlled tissue injury. It will also generate a data set that should clarify how the environment around APCs instructs APC and hence determines the programming of effector lymphocytes to a particular class of response.

A NOVEL APPROACH TO DETECT AND STRATIFY HEART FAILURE

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Background and aims: Approximately 30,000 Australians are diagnosed with HF and HF is estimated to be an AU$ 1 billion burden on our health system. Plasma NT-proBNP is currently used in clinical setting for HF diagnosis. However, the current assay for NT-proBNP which targets the full length molecule has its drawbacks due to it being truncated both at N and C termini as well as glycosylation. Our aim is to develop a novel immunoassay that can overcome above mentioned problems when measuring NT-proBNP in circulation.

Methods: Plasma samples were collected from healthy controls (n=54) and HF patients (n=63). Customized AlphaLISA® immunoassays were developed and validated to measure the concentrations and different NT-proBNP fragments (13-71, 28-71, 13-76) including the full length NT-proBNP. The diagnostic performance and predictive value of NT-proBNP fragments and their combinations were evaluated.

Results: Our novel NT-proBNP13-71 assay is useful in diagnosing HF patients. The diagnostic performance of NT-proBNP13-71 (sensitivity of 93.7% and specificity of 98.2%) showed improvement over the full length NT-proBNP (sensitivity of 76.2% and specificity of 98.2%). The median level of controls and HF patients classified with NYHA I, II and III was 225, 3800, 29824, and 223560 pg/mL respectively.

Conclusion: Our results demonstrate that novel NT-proBNP13-71 assay is better at diagnosing and stratifying HF patients. Thus this novel fragment may be of importance in monitoring HF.

A SALIVA-BASED TEST FOR THE DETECTION OF HPV-ASSOCIATED HEAD AND NECK CANCERS

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Background: Human papilloma virus (HPV) infection is a major risk factor for a distinct subset of head and neck squamous cell carcinoma (HNSCC). The incidence of HPV-associated HNSCC is increasing and there are no early detection methods with most cases at an advanced stage upon diagnosis. The current study aims to develop a saliva-based assay for the detection of oncogenic HPVs in patients with HNSCC.

Methods: Salivary rinse was collected from HNSCC patients recruited from the Head and Neck clinic at Princess Alexandra Hospital (PAH). Briefly, genomic DNA and RNA were extracted from rinse samples using a commercial kit and phenol-chloroform method respectively. PCR amplification was performed using MY11/MY09 primers that target >25 HPV strains as well as primers specific to oncogenic HPV16 and HPV18. HPV16-related transcripts (p16, E6 and E7) were detected using reverse transcription PCR (RT-PCR).

Results: Oncogenic HPV-16 DNA was detected in the salivary rinse of 31/35 (88.6%) patients diagnosed with HPV-positive HNSCC and none in the salivary rinse of patients with HPV-negative tumour (0/25). In addition, the presence of HPV-related mRNA was correlated with high viral load in patient rinse samples.

Conclusions: Salivary rinse is a promising diagnostic medium for the detection of oncogenic HPV in the oral cavity. The current study will aid in the detection of HPV infection in people at a high risk of developing HPV-associated HNSCC in a non-invasive and cost effective way. Early detection and intervention will significantly reduce the mortality and morbidity associated with HNSCC.

SEGMENTATION OF SKIN STRATA IN REFLECTANCE CONFOCAL MICROSCOPY DEPTH STACKS

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Background: Reflectance confocal microscopy (RCM) is an emerging tool for imaging human skin, but currently requires expert human assessment. To overcome the need for human experts it is necessary to develop automated tools for assessing reflectance confocal microscopy imagery.

Aim: To automatically classify the en-face optical sections from RCM depth stacks and hence segment the physiological strata of the skin.

Methodology: A bag of visual words framework was used to represent and classify the appearance of each en-face optical section. A dictionary of representative features was learned from whitened and normalised patches using spherical k-means. Each image was then represented by extracting a dense array of patches and encoding each with the most similar element in the dictionary. Logistic regression was used to for classification based on the histograms of visual words. Testing was performed on 311 depth stacks from 54 volunteers with varying degrees of photo-ageing. Parameters were tuned using leave one patient out cross validation on a training sub-set of the data, and final evaluation was performed on a held out test set.

Results: Cross validated classification accuracy was 80.5% across all four classes. Classified strata locations were physically plausible and consistent with the structure of the skin.

Conclusions: The proposed method is capable of accurate segmentation of the physiological strata of human skin.

IMPROVING THE EFFICACY OF MONOCLONAL ANTIBODY THERAPIES FOR THE TREATMENT OF BREAST CANCER

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Monoclonal antibodies (mAbs) are emerging as an important component of cancer therapy, for example, cetuximab for squamous cell carcinoma (SCC) and trastuzumab for breast cancer treatment. These antibodies target receptors expressed on the cell plasma membrane but outcomes are unpredictable and the biological determinants of antibody therapy sensitivity remain unknown. We hypothesised that the trafficking status of the epidermal growth factor receptor (EGFR) may impact on the efficacy of the mAb treatments directed at this receptor. Our current work is analyzing whether these pathways also influence the Human Epidermal Growth Factor Receptor 2 (HER2) activity and trastuzumab efficacy in breast cancer. Analysis of pre-treatment patient SCC tumours indeed showed EGFR trafficking defects which correlated to positive patient outcome after anti-EGFR mAb therapy. By modulating EGFR trafficking in vitro using endocytosis inhibitors which blocked the EGFR on the plasma membrane we were able to enhance anti-EGFR mAb (cetuximab)-induced SCC tumour cell death by antibody dependent cellular cytotoxicity (ADCC) in both cetuximab-sensitive and insensitive SCC cells. Using different classes of endocytosis inhibitors we find that this ADCC increase only occurs when the surface EGFR is clustered and this presents a new model for targeted therapy. These studies are now being extended to analyse trastuzumab action in breast cancer cells. We aim to increase our molecular understanding of HER2 trafficking and the internalisation of trastuzumab in different breast cancer sub-types. Our initial results suggest that by modifying receptor trafficking we can similarly enhance anti-HER2 mAb (trastuzumab)-induced cell death by ADCC in both trastuzumab-sensitive and insensitive breast cancer cell lines. Furthermore, HER2-negative and triple negative breast cancer cell lines could be made responsive to trastuzumab- and/or cetuximab-induced ADCC by modulation of receptor trafficking. Findings from this work have potential to impact the clinical management of tumours in which target receptors are spatially regulated.

G1 PHASE MELANOMA CELLS ESCAPE PROTEASOME INHIBITOR CYTOTOXICITY

Sheena M Daignault[1], David S Hill[2,3], Kimberley A Beaumont[2], Andrea Anfosso[2], Penny E Lovat[3], Wolfgang Weninger[2,4,5], Nikolas K Haass[1,2,4,5]

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Utilising the fluorescent ubiquitination-based cell cycle indicator (FUCCI), facilitating real-time cell cycle tracking, we demonstrate that melanomas are composed of differentially cycling tumour cells in a subcompartment-specific distribution. This study investigates the effect of ER stress-inducing agents, fenretinide (synthetic retinoid) and bortezomib (26S proteasome inhibitor), on the dynamics of cell division and cell death of individual melanoma cells within tumor microenvironments to increase therapy efficacy. FUCCI-melanoma cells were grown as 3D spheroids and embedded into a collagen matrix mimicing tumor microhabitats, or as xenografts in NOD/SCID mice. Utilising the F-XBP1ΔDBD-venus reporter construct that tags the cytoplasm in response to ER stress, we found that bortezomib induced ER stress and delayed cell cycle progression. Flow cytometry and confocal microscopy indicated that treatment of FUCCI-melanoma cells with bortezomib induced G2 accumulation in both cultures over 24 hours, and by 72 h were predominately in G1 phase. Additionally, heightened cell death in 2D and 3D culture was achieved with bortezomib in combination with fenretinide. Interestingly, bortezomib generated G1- and G2 arrest, but preferentially killed G2-phase cells. While temozolomide enhanced cytotoxic effects of bortezomib, MEK inhibitors blocked this effect in all melanoma cells, as did selective BRAF inhibitors in BRAF mutant cells. Our findings suggest that bortezomib combined with fenretinide or temozolamide is promising for treatment of BRAF-inhibitor insensitive or resistant melanoma. Importantly, melanoma cells arrested in G1 are protected from bortezomib cytotoxicity, excluding MAPK pathway inhibitors as combination options.

THE EFFECTS OF SOLUBLE RECEPTOR FOR ADVANCED GLYCATION END PRODUCTS (SRAGE) ON THE NON-OBESE DIABETIC MOUSE

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Type 1 diabetes mellitus involves the autoimmune destruction of pancreatic islets. Advanced glycation end products (AGEs) are compounds that may be crucial in mediating disease onset and they are formed by non-enzymatic reactions between sugars and proteins, lipids or nucleic acids. AGEs bind to numerous receptors including receptor for AGEs (RAGE), which generates inflammation possibly important in directing islet damage. There is discussion about the role of soluble receptor for AGEs (sRAGE) as a decoy receptor that mops up AGEs to decrease inflammatory AGE-RAGE interactions. We have tested the effect of prediabetic sRAGE therapy on the non-obese diabetic mouse model of human type 1 diabetes mellitus. The potentially prophylactic protein was either delivered chronically by adeno-associated virus (AAV) or twice daily for 2 weeks by intraperitoneal injections. Interestingly, AAV encoding for a sham (enhanced green fluorescent protein) resulted in improvements in fasting plasma glucose and insulin, and area under the glucose and insulin curves during an oral glucose tolerance test. When AAV encoded for sRAGE, the majority of improvements were abolished. Non-viral delivery of sRAGE resulted in an undesired increase in plasma glucose at 120 minutes. Further beneficial evidence of AAV includes decreased insulitis independent of the encoded protein. Non-virally delivered sRAGE resulted in increased insulitis. This data suggests that AAV may be beneficial in preventing disease however safety was questioned due to the observation of pale masses, suspected tumours, when AAV encoded for the sham protein. The study also suggests that disease onset may be accelerated by sRAGE.

INFLUENCE OF SURFACE FUNCTIONALITY OF SILICA NANOPARTICLES ON CYTOTOXICITY AND CELL UPTAKE

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The general perception for delivering drug/peptides to cancer cells is to have a positively charged nanocarrier system as they would electrostatically interact with negatively charged cancer cells. Earlier we reported that encapsulation of hydrophobic anticancer drug, Curcumin in MCM-41 enhances its solubility and cytotoxicity compared to the pure curcumin when tested in SCC25 cell line. The MCM-41 under this study is negatively charged. To investigate the effect of surface functionalization, we synthesized pristine MCM-41 (hydrophilic, negatively charged), amino functionalised MCM-41 (MCM-41-NH2, hydrophilic, positively charged) and methyl functionalised MCM-41 (MCM-41-CH3, hydrophobic, negatively charged) followed by curcumin encapsulation. The above MCM-41 materials were subjected to in vitro release, in vitro cytotoxicity, confocal microscopy, Si content analysis, cell cycle analysis and Annexin V-FITC/PI assay to evaluate their performance. MCM-41-NH2-CUR and MCM-41-CUR displayed similar dose and time dependent in vitro cytotoxicity which was at par with curcumin-DMSO organic solution signifying the advantage of MCM-41 materials. MCM-41-CH3-CUR displayed negligible in vitro cytotoxicity due to its hydrophobic nature. Cell cycle analysis showed different mode of action for MCM-41-CUR and MCM-41-NH2-CUR materials by predominantly arresting S and G2/M phase of cell cycle respectively. Moreover, Annexin V-FITC/PI assay demonstrated similar efficacy which was at par compared to curcumin-DMSO solution. The findings indicate that MCM-41-CUR and MCM-41-NH2-CUR showed similar activity which could be attributed to the effects of in vitro release and cellular uptake leading to enhanced anticancer activity in SCC25 cells. Thus, it was found that negatively charged nanoparticles has similar efficacy compared to positively charged nanoparticles.

FUNCTIONAL ANALYSIS OF THE KLK3 VARIANTS, FOUND TO BE ASSOCIATED WITH PROSTATE CANCER RISK

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Introduction: Kallikrein-related peptidase 3 (KLK3)/prostate specific antigen (PSA) is the current biomarker for prostate cancer. Our recent genetic fine-mapping studies identified two KLK3 non-synonymous single nucleotide polymorphisms (SNPs), rs61752561:G>A (D102N), rs17632542:T>C (I179T) as significantly associated with prostate cancer risk. We aim to delineate the molecular consequences of the rs61752561 and rs17632542 SNPs.

Methods: In-silico tools were employed to analyze the effect of the SNPs on mRNA splicing, protein stability and glycosylation. Mini-gene assays verifying the differential splicing effect induced by the SNPs were performed. Proteomic assays: differential scanning fluorimetry (DSF) and substrate activity assays utilizing the recombinant PSA harboring wild-type and variant isoforms were undertaken. Proliferation, migration and invasion experiments using the PC3 cell line stably expressing the two SNP variants and wild-type isoforms were also performed using Incucyte.

Results: In-silico analysis suggests an alteration in splice site and protein stability for the risk allele of the rs17632542 SNP. Protein stability and glycosylation are altered for the risk allele of the rs61752561 SNP. Mini-gene assay results herein provide evidence for differential allele-specific mRNA splicing for the rs17632542 SNP. DSF and activity assays showed the SNPs to affect the stability and activity of the KLK3 protease.

Conclusions: Our results indicate the rs17632542 and rs61752561 SNPs to have a biological effect on the expression and function of the KLK3/PSA protein suggesting that genetic variation in the PSA gene may be a useful contributor to the PSA prognostic profile in prostate cancer.

IDENTIFICATION OF NOVEL HYPOMORPHIC AND NULL MUTATIONS IN KLF1 DERIVED FROM A GENETIC SCREEN FOR MODIFIERS OF A-GLOBIN

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Position-effect variegation of transgene expression is sensitive to the chromatin state. We previously reported a forward genetic screen in mice carrying a variegated α-globin GFP transgene to find novel genes encoding epigenetic regulators. We named the phenovariant strains "Mommes" for Modifiers of murine metastable epi-alleles. Here we report positional cloning of mutations in two Momme strains which result in suppression of variegation; i.e. an increased percentage of GFP+ circulating red blood cells. Both strains harbour point mutations in the erythroid transcription factor, Klf1. One (D11) generates a stop codon in the zinc finger domain and a homozygous null phenotype identical to the Klf1-/- phenotype. The other (D45) generates an amino acid transversion (H350R) within a conserved linker between zinc fingers two and three. Homozygous MommeD45 mice have mild compensated microcytic anaemia which models the phenotype in a recently described human family. We crossed Klf1-H350R mice with Klf1+/- mice and interbred these. Klf1H350R/- mice have severe perinatal haemolytic anaemia and marked splenomegaly, which proves the MommeD45 phenotype is due to the mutation in Klf1. There is loss of CD71 and TER119 on the red blood surface as seen in Klf1-/- mice. We will present updated data on our experiments to determine the mechanism by which this novel linker mutation disrupts function, including in vitro assays with recombinant mutant zinc finger domains expressed in E.coli. We will discuss the implications with respect to Klf1 regulation of the normal α-globin gene locus and the α-globin transgene. This is the first genetic evidence that the linkers between the zinc fingers of transcription factors have a function beyond that of a simple spacer.

AIRWAY MICROBIOTA COMPOSITION PROMOTES CLINICALLY-RELEVANT, ALTERED COLLAGEN PROTEASE LEVELS IN BRONCHIECTASIS

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Non-cystic fibrosis bronchiectasis is a chronic inflammatory lung disease with increasing prevalence and represents an escalating healthcare burden. Matrix metalloproteinases (MMPs) are implicated in airway remodelling while bacterial infections, such as Pseudomonas aeruginosa, are associated with worse disease course. However, relationships between airway infections and MMP production are not understood. We determined concentrations of nine MMPs and four tissue inhibitors of metalloproteinases (TIMPs) in induced sputum from 86 bronchiectasis patients and 8 healthy controls by Luminex protein assay. Concentrations were then assessed in relation to lung function and airway microbiota composition. Airway microbiota composition was classified as P. aeruginosa-dominated, Haemophilus influenzae-dominated or dominated by another species. MMP-8 and MMP-9 activity levels were also measured in a subset of patients. Five MMPs and two TIMPs were significantly higher in bronchiectasis patients compared to healthy controls, supporting the role of MMPs in bronchiectasis. Ratios of MMP-8/TIMP-1 and MMP-9/TIMP-1 were higher in bronchiectasis and correlated with decline in lung function. Within bronchiectasis, MMP-8 and MMP-1 levels significantly correlated with decline in lung function and were highest in patients with P. aeruginosa and H. influenzae dominant infections. MMP-2 levels and MMP-8 activity were significantly higher in H. influenzae-dominant patients compared to P. aeruginosa. In conclusion, increased MMP levels (particularly MMP-8 and MMP-1) and MMP/TIMP ratios in bronchiectasis patients compared to healthy controls suggests a mechanistic role for these enzymes in airway disease. Further, MMP profiles differ with infective airway microbiology, potentially providing mechanistic insight into why certain pathogens are associated with worse clinical outcomes.

THE ROLE OF BONE MARROW MACROPHAGES IN ACUTE MYELOID LEUKAEMIA

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Tumour associated macrophages have been linked to many types of cancers but this relationship has never been properly investigated in a leukaemic model. Macrophages develop within the BM in the same area that Leukeamia cells originate and spread from. There is a population of macrophages that remain within the bone marrow (BM) called bone marrow macrophages. This proximity of the BM macrophages and the leukaemic cells means that there is an increased potential for the two cell types to interact with each other. Our aim is to determine whether these BM macrophages are altered in leukaemia and whether targeting them could lead to novel treatment options. We are using a mouse model of two different types of acute myeloid leukaemia, MLL-AF9 and AML-Eto9a. Using flow cytometry we have identified several changes in the cell surface markers of non-leukaemic BM macrophages of mice with MLL-AF9 leukaemia. These results indicate that these macrophages may be modified by the leukaemia cells to shift away from an antigen presenting phenotype. This would greatly affect the immune system's ability to recognise and fight the leukaemic cells. These changes will next be investigated in AMLEto9a leukaemia to determine if these results are seen across multiple models. The results will also be confirmed using real time PCR. Transgenic mouse models of macrophage depletion/inactivation, including CD169DTR/+ mice, CD11bDTR/DTR mice, and LysMCreHif1a mice will then be used. Finally we will use these mouse models in conjunction with chemotherapy to determine if macrophage alteration sensitises leukaemic cells to treatment.

TARGETING YKL40: A NOVEL APPROACH TO CURB ANGIOGENESIS IN METASTATIC PROSTATE CANCER

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Angiogenesis plays a fundamental role in promoting tumour growth and hence anti-angiogenesis therapy has provided a promising approach for treating patients with metastatic castration resistant prostate cancer (CRPC) disease. Despite clinical benefits in some cancers, the results of anti-angiogenic (mainly anti-VEGF) therapy in prostate cancer (PCa) are relatively modest, possibly due to alternative escape routes employed by tumour cells. YKL40 has been identified as a tumour angiogenic factor and a potent inducer of carcinogenesis in glioblastoma, breast, and colon cancers. We have recently shown for the first time in PCa that YKL40 expression increases with progression to CRPC phenotype in PCa cell lines and clinical samples [1]. We also show that YKL40 affects cell invasion, migration, and clonogenicity of PCa cells [1]. However, the therapeutic value of targeting YKL40 and its role in tumorigenic pathways remains unknown in PCa. Methods: Effect of YKL40 on the expression profile of angiogenic and proinflammatory targets was analysed by quantitative PCR and immunoblotting. Gene expression microarrays were performed to delineate the network biology of YKL40. Results: Our preliminary results show that YKL40 significantly increased the expression of vascular endothelial growth factor (VEGF) and interleukin 6 (IL6) both at the gene and protein levels. Gene expression microarray analysis is underway to further explore the relationship of YKL40 with other molecules in the angiogenesis cascade. Conclusion: The novel findings from this study indicate that YKL40 may serve as a potential anti-angiogenic target for the treatment of PCa as well as a contributor to key tumorigenic pathways.

1. Jeet, V. et al., Elevated YKL40 is associated with advanced prostate cancer and positively regulates invasion and migration of prostate cancer cells. Endocr Relat Cancer, 2014.

AN IMMUNODIAGNOSTIC ASSAY FOR SPECIFIC IGE TO THE MAJOR POLLEN ALLERGEN COMPONENT, PAS N 1, OF THE SUBTROPICAL BAHIA GRASS

Victoria L Timbrell[1], Lindsay Riebelt[2], Claire Simmonds[2], John W Upham[1], Peter Smith[3], Sheryl van Nunens[4], William Smith[5], Andrew Mclean-Tooke[6], Graham Solley[7], Anita Kober[8], Daman Langguth[2], Janet M Davies[1]

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Background: Grass pollen is a major global cause of hayfever contributing to the financial and medical burden of allergic asthma. A standardised assay for Bahia grass (Paspalum notatum) pollen (BaGP) is needed for better immunodiagnosis of subtropical grass pollen allergy.

Methods: A high throughput test based on serum IgE to the major allergen Pas n 1 (patented in Australia, continuing in USA) purified from BaGP was established on the ImmunoCAP platform (Thermofisher, Sweden) and validated by Sulivan Nicolaides Pathology (Taringa) with 316 subjects recruited with ethical approval.

Results: The test performance and reproducibility were outstanding. Pas n 1 IgE was highly correlated with current tests based on crude whole pollen extracts; BaGP skin prick test ($r = 0.775$, $p < 0.0001$) and BaGP IgE ($r = 0.891$, $p < 0.0001$). The diagnostic sensitivity (91.8%) and specificity (92.6%) were excellent (ROC area 0.954, $p < 0.0001$).

Conclusions: This molecular component immunoassay for Pas n 1 IgE has potential to improve the diagnosis of patients with grass pollen allergy in subtropical regions with clinical relevance for large markets including parts of Australia, India and southern USA.

HUMAN CD1C+ DENDRITIC CELLS ARE THE MAJOR PRODUCERS OF INTERLEUKIN-23 AND INTERLEUKIN-12P70 IN RESPONSE TO TOLL-LIKE RECEPTOR AG

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Dendritic cells (DC) are professional antigen presenting cells that play a crucial role in mounting immune responses. Human blood DC comprise plasmacytoid DC (pDC), CD1c+ DC and CD141+ DC. pDC excel in anti-viral defence via production of Type I interferons. CD141+ DC promote anti-tumour and CD8+ T cell responses. Whilst the function of CD1c+ DC is unknown, its putative mouse equivalent is specialised at inducing CD4+ T cell responses in particular Th17 responses via IL-23. We hypothesise that human CD1c+ DC are capable at inducing CD4+ T cell responses. IL-23 is a heterodimeric cytokine sharing a common subunit with Th1-polarising cytokine, IL-12p70 and plays an important role in driving autoimmune diseases. Cytokine production by DC is induced by recognition of pathogens and damaged host cells by specific pattern recognition receptors (PRR) expressed by DC such as the toll-like receptors (TLR). TLR8 binds single stranded RNA and has been recently linked to autoimmune diseases such as Still's disease and systemic lupus erythematosus due to the increased levels of IL-23. We demonstrated that CD1c+ DC produced significant levels of IL-23 and IL-12p70 in response to the TLR7/8 agonist, R848 alone, which was further enhanced by the addition of agonists for TLR3 (poly I:C) or TLR4 (LPS). Furthermore, direct comparisons with monocytes, CD141+ DC and pDC demonstrated that CD1c+ DC were the main producers of IL-23 and IL-12p70 under these conditions. Our results suggest that human CD1c+ DC are the major producers of IL-23 and may therefore play an important role in driving autoimmune responses upon TLR8 ligation.

INVESTIGATING THE MECHANISM OF INTRINSIC RESISTANCE TO FIBROBLAST GROWTH FACTOR RECEPTOR INHIBITION IN ENDOMETRIAL CANCER

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Background: FGFR2 mutations have been identified in 10-16% of EC patients' tumour samples. Multiple FGFR2mutant EC cell lines are highly sensitive to FGFR inhibition, while many other FGFR2mutant EC cells exhibit intrinsic resistance. Inhibition of the MAPK signalling pathway was observed in the sensitive cells after FGFR inhibition. However, activation of the MAPK signalling pathway remained in the resistant cells.

Aims: To investigate whether intrinsic resistance to FGFR inhibition is associated with reactivation of FGF/FGFR downstream signalling pathways or abnormal activation of kinase-independent signalling pathways.

Methodology: Combination studies of BGJ398 (FGFR inhibitor) with Trametinib (MEK inhibitor) and/or GDC0941 (PI3K inhibitor) were employed to investigate whether the resistant cells will be sensitised to BGJ398 treatment. Western blot analyses alongside cell proliferation and cell apoptosis assays were employed. Phospho-RTKs and phospho-kinases arrays, RNA-Seq and a genome siRNA screen will also be employed.

Results: Western blot analysis showed 30nM Trametinib has abrogated pERK expression in all the resistant cell lines. Trametiniib alone decreased cell proliferation to about 50-65% in the resistant cell lines. The addition of 30nM Trametinib to BGJ398 has not sensitised the resistant cells to BGJ398 treatment. While a combination of BGJ398 and GDC0941 resulted in more cell growth inhibition in the sensitive cell lines, it did not sensitise the resistant cell lines to BGJ398 treatment.

Conclusion: Inhibition of the MAPK and or PI3K/AKT pathways has not sensitised the resistant cell lines to BGJ398 treatment. The combination of BGJ398 and GDC0941 induce more cell death than BGJ398 alone in sensitive cells.

GLUTATHIONE TRANSFERASES ARE VARIABLY CONTAINED WITHIN EXOSOMES OF PROSTATE CANCER CELLS

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Background and Aims: Prostate Cancer (PCa) is responsible for almost 30% of new cancer cases and most advanced PCas are androgen independent. Exosomes are involved in contributing to PCa progression and the protein profile of exosomes differs from cytosolic protein profiles. Glutathione transferases (GSTs) play critical roles in carcinogenicity and cancer progression as part of the chemical defence system and in the apoptosis signalling pathway. For example, GSTP1-1 increased cell viability, growth rate and migration particularly in breast cancer cells. GSTs are expressed in both normal prostate and PCa cells. Therefore, this study determines which GSTs are expressed in various PCa cell lines and whether they are packaged into their exosomes.

Methodology: Prostate cancer cell lines (DU145, PC3, LNCaP, 22Rv1) were cultured for preparation of cell lysates. Exosomes were isolated from culture media with fractionated ultracentrifugation. Samples were subjected to Western blotting and probed with specific exosome markers and GST antibodies.

Results: All cell lysates and exosome preparations were positive for the exosome marker TSG101. GSTT1-1 was expressed in all cell lines. GSTP1-1 and GSTM3-3 were expressed in DU145 and PC3 but not in LNCaP or 22Rv1 cells. So far, only GSTP1-1 was found in exosome preparations of DU145 and PC3.

Conclusions: GSTs are variable expressed in PCa cells. Interestingly, both cell lines expressing GSTP1-1 and GSTM3-3 are androgen independent. GSTP1-1 was found in exosomes indicating a role in PCa progression, which will be addressed in future experiments.

DEVELOPMENT OF A HUMANISED MOUSE MODEL TO EVALUATE HUMAN VACCINES

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Dendritic cells (DC) are the key initiators of adaptive immune responses and comprise multiple subtypes with specific functions. In mice, CD8+ DC subtypes is specialised in the induction of cytotoxic T cells that are critical for the induction of anti-viral and anti-tumor immune responses. Delivering antigen to the CD8+ DC subset in vivo using antibodies (Ab) specific for CD8+ DC receptors, such as Clec9A, is an effective vaccine strategy in mice. However, translating this to humans has been confounded by their rarity and lack of models to study the human CD141+ DC equivalent of mouse CD8+ DC. To overcome this limitation, we developed a model of human haematopoietic stem cell (HSC) engraftment in NOD/SCID/IL2rgnull/HLA-A2 (NSG-A2) mouse strain. We demonstrated robust multilineage differentiation of human CD34+ cord blood HSC after transplantation into NSG A2 neonatal mice via intrahepatic injection. Human B cells, T cells, monocytes and all dendritic cells subsets, including CD141+ DC, develop in the peripheral blood, bone marrow, spleen, liver and lung by 10-14wk post transplantation. As few as 2x10^4 human HSC facilitate engraftment of up to 30% of human cells in peripheral blood. Human CD141+ DC in the mice specifically take up anti-human Clec9A Ab in vivo and effectively prime naive CD8+ T cells in vitro. Our data suggests this humanised mice model will be a powerful model to understand human DC function and evaluate new human vaccine strategies in vivo.

CHK1 INHIBITOR: A PROMISING TARGET IN MELANOMA FOR SINGLE-AGENT THERAPY

ZY Oo[1], S Daignault[1], J Chen[1], K Brooks[1], A Stevenson[1], L Spoerri[1], B Gabrielli[1]

1. The University of Queensland Diamantina Institute, Translational Research Institute, Princess Alexandra Hospital, Brisbane, Queensland.

The incidence of malignant melanoma is growing rapidly worldwide and while there are now a number of targeted therapies for treatment of metastatic disease, rapid development of resistance and restricted expression of targets means there are still a significant proportion of patients without effective treatment options. Conventional chemotherapeutic drugs damage DNA and create cellular stresses in the proliferating cells including the tumor cells in the patients and induce the cell cycle checkpoint arrest. Thus, standard chemotherapeutic treatments need to use high doses of drugs and produce low response rates with adverse side effects. Chk1 inhibitors are being investigated for their abilities to abrogate the cell cycle checkpoint arrest and, as a result, enhance the effects of conventional chemotherapy, but their activity as single agents is relatively unknown. We have investigated the activity of Chk1 inhibitor GNE323 in 40 different melanoma cell lines and normal cells in vitro. This single-agent treatment with GNE323 produces cytotoxic effects in low nanomolar range in a subset of melanoma cell lines with high levels of replicative stress, but only cytostatic effects in normal cells at the higher drug concentration. Our results also show that this cytotoxic effect in cell lines most sensitive to Chk1 inhibition is related to cells undergoing apoptosis either prior to or during an aberrant mitosis due to inhibition of S phase Chk1. This sensitivity suggests that Chk1 play an integral part in these drug-sensitive melanoma cells' adaptation to replicative stress and as the sensitivity to Chk1 inhibitor is independent of the currently targeted melanoma mutations, Chk1 inhibitors may be useful as a single-agent therapy for the currently untreated patient population or in patients with developed resistance to MAPK targeted agents.

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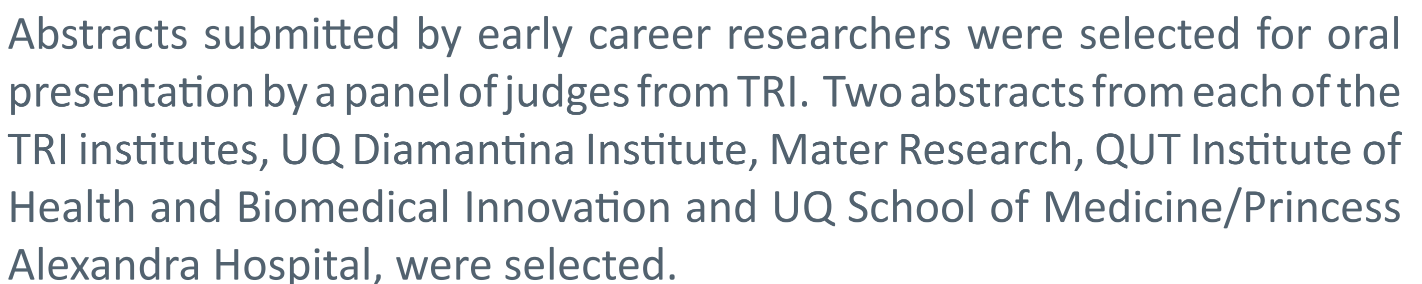

Abstracts submitted by early career researchers were selected for oral presentation by a panel of judges from TRI. Two abstracts from each of the TRI institutes, UQ Diamantina Institute, Mater Research, QUT Institute of Health and Biomedical Innovation and UQ School of Medicine/Princess Alexandra Hospital, were selected.

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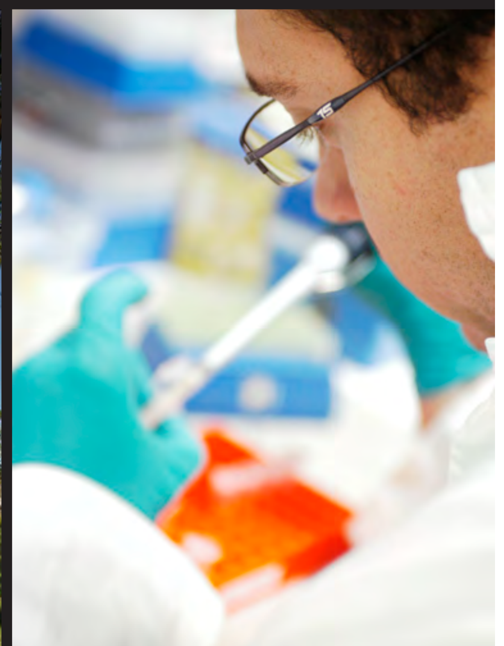

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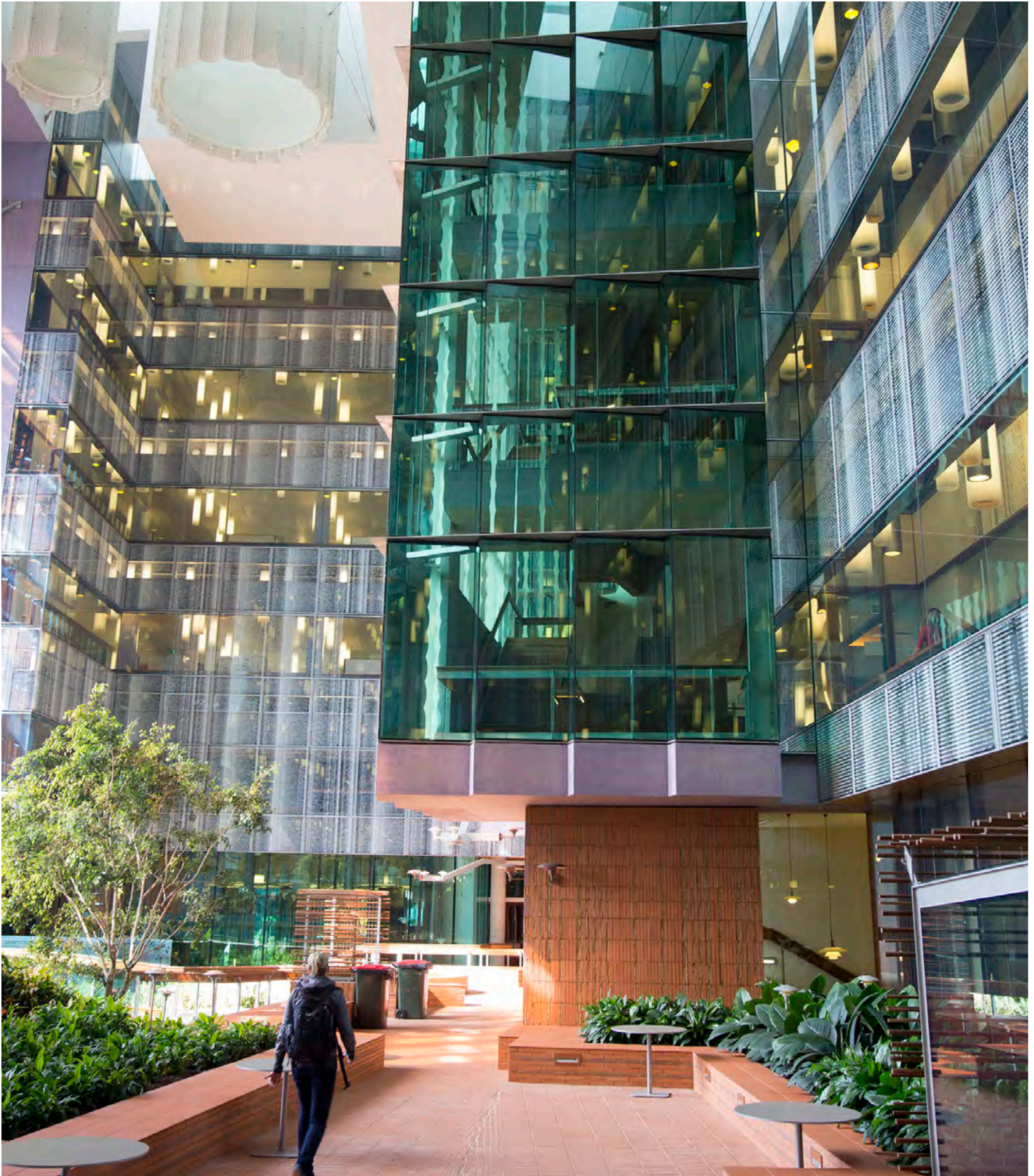

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