APG
Adelaide Protein Group

2024 APG AWARDS FEST

JULY 10 2024 | BRADLEY BUILDING, UNISA

EVENT DETAILS:

July 10th, 2024 9:30-5pm

Level 8, Bradley Building, Uni SA

Schedule

09:30 | Registration

10:00 | Keynote 1 - Prof. Brett Collins

11:00 | Student Talks

12:30 | Lunch & Poster Session

14:00 | Keynote 2 - Dr. Luke Isbel

15:00 | ECR Talks

16:30 | Awards Presentation, AGM, Refreshments

Door Prize for ASBMB Members!

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KEYNOTE SPEAKERS

Prof. Brett Collins

"Travelling through the cell: Structural biology of the protein machinery controlling endosomal trafficking"

Dr. Luke Isbel

"Transcription factor sensitivity to histones can drive specificity in genome regulation"

Stay tuned for announcement of student & ECR presenters:

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SOUTH AUSTRALIAN iMMUNOGENOMICS CANCER INSTITUTE

Adelaide Protein Group Awards FEST 2024 Programme

Level 8, Bradley Building, UNISA

Wednesday	9:30 – 10:00	Registration
10th July 24	10:00 – 10:50	**Cytiva Keynote 1: Prof. Brett Collins**
	11:00 – 12:20	**Student Talks**
	12:30 – 13:50	Poster Session
	14:00 – 14:50	**Metagene Keynote 2: Dr. Luke Isbel**
	15:00 – 16:20	**ECR Talks**
	16:30	Refreshments, AGM and Award Presentations Including: • **Metagene** ECR Travel Bursary • **Cytiva** Best Student Talk • **SAGC** People's Choice Award • **UNISA** Best Poster Award • **BMG Labtech** Best Poster Award
	After event	Dinner with Keynote Speakers

Contents

APG Committee 2024

Chair	Alison Roennfeldt, *The University of Adelaide*
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members	Manuela Klingler-Hoffmann, *The University of South Australia*
	Susie Grigson, *Flinders University*

APG AGM

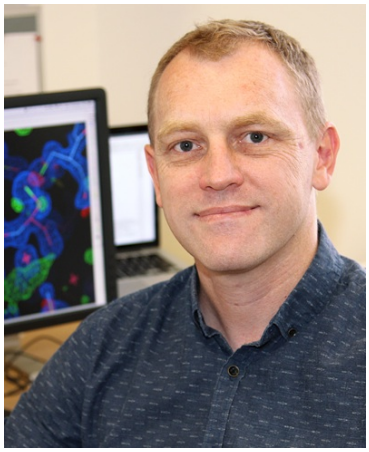
APG Annual General Meeting Minutes **Meeting called by Secretary Beth Vandborg**	**Committee Member Owner**
1. Present	
2. Apologies	
3. Treasurers/Sponsorship Report	Kim/Ash

Open Actions

Item	Action	Owner
1		

CYTIVA Keynote 1

10:00 – 10:50



Travelling through the cell: Structural biology of the protein machinery controlling endosomal trafficking

Prof. Brett Collins

Centre for Cell Biology of Chronic Disease, Institute for Molecular Bioscience, The University of Queensland

Our lab is interested in how proteins and lipids are transported between cellular organelles. Using structural biology, we are attempting to understand how some of the key protein machineries control this process in health and disease. My career in structural biology started with my PhD studies with Bridget Mabbutt and Paul Curmi at Macquarie University and the University of New South Wales, studying proteins involved in mRNA splicing. I then moved into the field of membrane trafficking under the mentorship of David Owen at the Cambridge Institute for Medical Research and have continued in this area since moving to the Institute for Molecular Bioscience at the University of Queensland in 2006. In this seminar I will focus on our recent work on the Commander complex, which is required for a process called endosomal recycling. This process maintains the cell surface levels of diverse transmembrane proteins including the amyloid precursor protein linked to Alzheimer's disease. Commander mutations also cause a rare neurodevelopmental disorder called Ritscher-Schinzel syndrome. I will talk about how we combined X-ray crystallography, cryoelectron microscopy, *in silico* structural predictions, and extensive cellular validation to determine a complete structural model of Commander [1]. The structure allows mapping of disease-causing mutations and reveals the molecular features required for the function of this evolutionarily conserved trafficking machinery. I will also discuss some of the technical challenges to solving this structure, compare our work with other cryoEM structures recently published, and describe how AlphaFold2 predictions are providing insights that are now guiding our future studies of Commander structure and function.

[1] Healy MD, McNally KE, Butkovič R, Chilton M, et al.,...Collins BM, Cullen PJ. Structure of the endosomal Commander complex linked to Ritscher-Schinzel syndrome. Cell. 2023 May 11;186(10):2219-2237.e29. doi: 10.1016/j.cell.2023.04.003. PMID: 37172566.

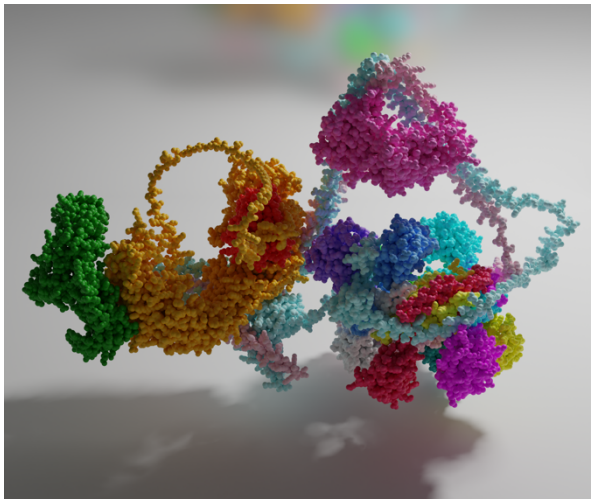


Figure 1. Structure of the sixteen subunit Commander protein complex.

Prof. Collin's travel to Adelaide is sponsored by **ATA Scientific.**

Biographical snapshot – Prof. Brett Collins

Institute for Molecular Bioscience, The University of Queensland, St. Lucia, Queensland, 4072, Australia.

The Collins lab is interested in understanding how molecular interactions between proteins and lipids control intracellular membrane trafficking, using structural biology and biophysical approaches. Current interests include (i) endosomal trafficking by the Retromer complex and the sorting nexin protein family, (ii) the formation of plasma membrane structures called caveolae, and (ii) the mechanisms of synaptic vesicle fusion controlled by SNARE proteins. After training at Macquarie University in Sydney with Prof. Bridget Mabbutt and a postdoctoral period at the Cambridge Institute for Medical Research with Prof. David Owen, Prof. Collins returned to Australia in 2006 to take up an NHMRC RD Wright Career Development Award at the University of Queensland Institute for Molecular Bioscience. He currently holds an NHMRC Investigator Grant and is Director of the Centre for Cell Biology of Chronic Disease at the IMB. UQ. Career highlights include determining the structure of the heterotetrameric AP2 complex that regulates receptor endocytosis at the plasma membrane (*Cell*, 2002, 2010), providing key insights into the Retromer transport assembly (*Nature*, 2018, *Sci. Adv.* 2021) and the related Commander

complex (*eLife* 2018, *Cell* 2023) defining a novel family of sorting nexin proteins that regulate endosomal sorting (*Nat. Cell Biol.* 2021, *Nat. Struct. Mol. Biol*, 2016), and providing the molecular insights into the assembly of caveolar membrane coat structures (*Dev. Cell* 2014; *EMBO Rep*, 2018; *Nat. Comms*, 2021).

Student Talks

11:00 – 12:20

1. Novel RNA binding protein (ZCCHC24) regulates breast cancer cell plasticity
   Shruti Deshpande

2. Exploring ceramide metabolism as an avenue for novel therapeutic approaches in chronic lymphocytic leukaemia
   Alana M White

3. CD47 regulates cellular and metabolic plasticity in glioblastoma
   Ruhi Polara

4. Comparative structural analysis of Estrogen receptor γ reveals sequence constraint in evolution and human disease
   Daniel McDougal

Metagene Keynote 2

14:00 – 14:50

Dr. Luke Isbel

Transcription factor sensitivity to histones can drive specificity in genome regulation

Dr. Luke Isbel

South Australian immunoGENomics Cancer Institute (SAiGENCI) & The Adelaide Centre for Epigenetics (ACE), The University of Adelaide

There is a remarkable degree of specificity in the establishment and maintenance of cellular identity, given that our cells have the same DNA but vastly different gene expression profiles. The basis of this 'epigenetic' regulation is a complex network of gene activation and repression events that takes place in the context of our chromatinized genomes - DNA wrapped around histones that make up nucleosomes. While we know specific gene activation is driven by DNA-binding proteins called transcription factors, our knowledge of how they engage naked DNA vastly outstrips our insights into how they interact with chromatinized DNA in vivo. This prompts the critical question: how do transcription factors read or interpret chromatin?

We previously developed an in vitro genomics pipeline to enable the first structural characterization of transcription factors binding to nucleosomal DNA (Michael/Grand/Isbel et al., 2020, Science), which we now apply to p53. This reveals that p53 engages motifs at key points along the nucleosome, which we provide evidence for in stem cells by profiling binding with Cut and Run. Further cellular characterization using proteomics and precision mutants with functional genomics measurements (i.e ChIPseq/ATACseq/RNAseq) reveals that p53 utilizes a repressive cofactor protein called Trim24 to interpret chromatin. We reveal the mechanistic basis of this activity: Trim24 utilizes a histone binding domain to engage unmethylated lysine 4 of histone H3 at closed chromatin, while it is blocked at open chromatin by lysine 4 methylation.

These findings demonstrate how chromatin restricts the ability of p53 to activate genes that are initially off, so-called 'pioneering activity'. Additionally, as we demonstrate that the majority of p53 targets in human tissues are in closed chromatin, this suggests a means to specifically amplify its activity at repressive chromatin by targeting Trim24 in cancer. Notably, these results underscore the considerably variability in transcription

factors ability to navigate chromatin states, due to their intrinsic properties and cofactor interactions.

Bio – Dr. Luke Isbel

Dr Luke Isbel is a group leader, holding a joint appointment at The South Australian immunoGENomics Cancer Institute (SAiGENCI) and The Adelaide Centre for Epigenetics (ACE), located within the University of Adelaide. He runs an inclusive and creative team aiming to understand how epigenetic forces (i.e. on chromatin) serve as a guiding force to direct gene regulatory machinery in cells, and how such interactions might be exploited in cancer therapeutic strategies.

Luke competed his PhD in 2016 at La Trobe University, under the supervision of Professor Emma Whitelaw, whereupon he embarked on a postdoctoral position in Basel, Switzerland in the lab of Professor Dirk Schübeler at the Friedrich Miescher Institute for Biomedical Research. He was supported by two prestigious Fellowships - the Marie Sklodowska-curie Fellowship (EU) and CJ Martin Early Career Fellowship (NHMRC). He returned to Australia in late 2023 to establish his own laboratory. He is the recipient of the 2019 Chiquet-Ehrismann Originality prize and the 2024 Lorne Genome Mid-Career Millennium Prize.

ECR Talks

15:00 – 16:20

1. Mimicking nature to disrupt Staphylococcus aureus cysteine metabolism
   Dr. Jordan Pederick

2. Nucleosome eviction at TET/TDG-targeted enhancers occurs independent of the removal of the chromatin mark Oxidized-Methylcytosine
   Dr. Marion Turpin

3. Next Generation Image Analysis: Artificial Intelligence (AI)-Driven Analysis of Rhoptry Dynamics and Genome Remodelling in Malaria Parasite, Plasmodium falciparum
   Dr. Sonja Frölich

4. Tuning the antigenic profile of a whole cell inactivated pneumococcal vaccine
   Dr. Erin B. Brazel

Student Talk Abstracts

Novel RNA binding protein (ZCCHC24) regulates breast cancer cell plasticity

Shruti Deshpande* [1], Daniel P. Neumann [1], Caroline A. Phillips [1], B. Kate Dredge [1], Rachael Lumb [1], Millicent GA Bennett [1], Andrew G. Bert [1], Katherine A. Pillman [1], John Toubia [1], Cameron P. Bracken [1,2], Gregory J. Goodall [1,2], Philip A. Gregory [1,2]

[1] Centre for Cancer Biology, University of South Australia and SA Pathology, Adelaide, SA 5000, Australia.
[2] Faculty of Health and Medical Sciences, The University of Adelaide, Adelaide, SA 5000, Australia.

Cancer cell plasticity, which is commonly referred to as epithelial to mesenchymal transition (EMT), plays a significant role in facilitating cancer cell invasion, tumor metastasis, and therapy resistance. Understanding and treating cancers that have progressed to metastatic and therapy resistant states is a crucial challenge for human health. Our lab has previously shown that EMT is regulated by a feedback loop between the epithelial-specific miR-200 family and the EMT transcription factor ZEB1.
In a screen for novel regulators of EMT, we uncovered an uncharacterized CCHC type Zinc finger protein (ZCCHC24) that appears to regulate cellular plasticity. ZCCHC24 is repressed by miR-200 in epithelial cells, and it is highly induced in mesenchymal cells that lack miR-200. Unlike well-known EMT-driving transcription factors, we identified ZCCHC24 as a cytoplasmic RNA binding protein (RBP) that operates through post transcriptional modes of action. Knockout of ZCCHC24 caused cancer cells to lose mesenchymal features including a reduction in migratory and invasive capacity, while overexpression of ZCCHC24 caused the opposite phenotypes. Using CLIP-seq we identified many RNA targets of ZCCHC24, but no specific binding motif was observed. However, in ~50% of cases ZCCHC24 bound near a consensus motif for the Pumilio (PUM) RBP and we demonstrated it directly interacted with PUM using IP-MS and co-localized with PUM within cytoplasmic condensates. When PUM is removed from the cell it prevented ZCCHC24s ability to regulate features of EMT. These studies uncover a ZCCHC24-PUM axis as a novel regulator of cancer-associated EMT and gaining an understanding of how it operates may lead to new treatment avenues for epithelial-derived cancers.

Exploring ceramide metabolism as an avenue for novel therapeutic approaches in chronic lymphocytic leukaemia

Alana M White [1], Oliver G Best [1], Bryone J Kuss [1], Lauren A Thurgood [1]

[1] College of Medicine and Public Health, Flinders Health and Medical Research Institute, Flinders University, Adelaide, SA, Australia.

Chronic lymphocytic leukaemia (CLL) is characterised by the accumulation of malignant B lymphocytes in the peripheral blood, bone marrow, and lymph nodes. CLL is widely considered to be incurable, and management of patients is challenging due to a high rate of drug resistance and clinical heterogeneity. We and others have shown that lipids may represent an important fuel source for CLL cells. However, their role in disease pathogenesis has not been fully elucidated. The aim of the current study was to compare the lipid composition of CLL cells and their healthy counterpart and determine whether the differences might be exploited for novel therapeutic approaches.

Liquid chromatography mass spectrometry (LC-MS) was used to define the lipid composition of peripheral blood B-cells isolated from CLL patients and healthy individuals. The effects of exogenous lipids and 1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP), a glucosylceramide synthase inhibitor, on the viability of CLL cell lines and primary CLL cells were assessed by flow cytometry. Further experiments are currently underway to elucidate the functional effects and mechanisms of action of the lipids and PDMP.

We identified 16 lipid species that were significantly differentially expressed between CLL cells and their healthy counterpart, including ceramide and glucosylceramide. However, PDMP, which targets ceramide metabolism, had no effect on the viability of either CLL cell lines or primary CLL cells. While addition of exogenous C2 ceramide also had no effect on the viability of the CLL cell lines, addition of C6 ceramide reduced CLL cell viability by 60-70% after 72h.

Regulation of lipid metabolism, including ceramides, is known to play crucial roles in cancer cell survival and mediate resistance to therapeutics. A better understanding of the metabolic processes that are dysregulated in cancer cells will be instrumental in developing more effective, targeted, and tolerable therapeutics.

CD47 REGULATES CELLULAR AND METABOLIC PLASTICITY IN GLIOBLASTOMA

Ruhi Polara [1], Briony Gliddon [1], Melinda Tea [1], Raja Ganesan [1], Kelly Lim [2], Paul Moretti [1], John Toubia [1], Sakthi Lenin [1], Chloe Shard [1], Guillermo Gomez [1], Daniel Thomas [2], Stuart Pitson [1] and Nirmal Robinson [1]

[1] Centre for Cancer Biology, University of South Australia, Adelaide, SA, Australia
[2] Adelaide Medical School, University of Adelaide, Adelaide, SA, Australia

Glioblastoma (GBM) is the most common and aggressive form of primary brain tumor with an average incidence of 3.19 per 100,000 population. The median survival time for GBM patients post diagnosis is only 14-15 months. The current standard of care for GBM patients includes maximal surgical removal, followed by concurrent chemo-radiotherapy with temozolomide. While these therapies extend median patient survival, recurrence within 6-9 months is common with no effective therapy. Thus, novel treatment strategies that have greater specificity and efficacy are warranted. Analyzing the Ivy Glioblastoma Atlas Project's transcriptomics data based on anatomic structures (http://www.brain-map.org/), we identified that CD47, a ubiquitously expressed receptor known to block phagocytosis by interacting with SIRPα on myeloid cells, is abundantly expressed on the leading invasive edge of GBM patient tumours. Increased expression of CD47 is also associated with poorer survival in GBM patients. To date, various CD47-blocking antibodies have been developed to target several solid and hematological cancers with limited efficacy. Importantly, the role of CD47 in GBM besides promoting immune escape is not well understood. In our study, we have found that loss of CD47 in GBM cells reduces cell migration and proliferation in the absence of myeloid cells, suggesting a cell autonomous function of CD47. Consistently, gene set enrichment following transcriptomics analysis in control and CD47 knock out GBM cells indicates that cell polarity, axonal guidance, cell differentiation and cell proliferation pathways are driven by CD47 signaling. Furthermore, our data shows that CD47 regulates metabolic plasticity. We found that CD47 knock out cells exhibit elevated levels of several glycolytic metabolites which correlates with increased mitochondrial spare respiratory capacity and mitochondrial biogenesis. Thus, we have identified a novel role of CD47 in linking cellular and metabolic plasticity.

Comparative structural analysis of Estrogen receptor γ reveals sequence constraint in evolution and human disease

Daniel McDougal [1], Jordan Pederick [2], Linda Shearwin-Whyatt [2], Frank Grutzner [2], John Bruning [1]

[1] Institute for Photonics and Advanced Sensing (IPAS), School of Biological Sciences, The University of Adelaide, South Australia, Australia
[2] School of Biological Sciences, The Robinson Research Institute, The University of Adelaide, Adelaide, South Australia, Australia

The author(s) have requested for the abstract to not be shared in the programme booklet.

ECR Talk Abstracts

Mimicking nature to disrupt Staphylococcus aureus cysteine metabolism

Jordan Pederick [1], Bethiney Vandborg [1], John Bruning [1]

Institute for Photonics and Advanced Sensing (IPAS), School of Biological Sciences, The University of Adelaide, Adelaide, South Australia, Australia

The pathway of bacterial cysteine biosynthesis is gaining traction for the development of antibiotic adjuvants. Bacterial cysteine biosynthesis is facilitated by two enzymes possessing O-acetyl-L-serine sulfhydrylase (OASS) activity, CysK and CysM. CysK can also form functional complexes with other proteins that regulate cysteine metabolism. In Staphylococcus aureus there is a single OASS homologue, herein termed SaCysK. Knockout of SaCysK has been found to increase sensitivity to oxidative stress, making it a relevant target for inhibitor development. SaCysK forms two functional complexes via interaction with the preceding enzyme in the pathway serine acetyltransferase (CysE) or the transcriptional regulator of cysteine metabolism (CymR) in S. aureus. These interactions occur through insertion of a C-terminal peptide of CysE or CymR into the active site of SaCysK, inhibiting OASS activity. Hence, this interaction therefore an excellent starting point for developing SaCysK inhibitors. Here we detail the characterization of CysE and CymR C-terminal peptides as inhibitors of SaCysK. First, interactions between CysE or CymR C-terminal decapeptides and SaCysK were assessed by X-ray crystallography. While both peptides occupy the active site of SaCysK the CymR decapeptide formed more extensive interactions. Surface plasmon resonance binding assays and SaCysK inhibition assays revealed that the CymR decapeptide bound to SaCysK with nanomolar affinity (KD = 25 nM) and inhibited SaCysK activity (IC50 = 180 nM). To understand the determinants of this interaction the structure-activity relationships of 16 rationally designed peptides were also investigated. This identified that the CymR pentapeptide motif facilitates the high affinity interaction with SaCysK, and that subtle structural modification of the pentapeptide can be tolerated. Ultimately, this work has identified CymR pentapeptides as a promising scaffold for the development of antibiotic adjuvants targeting SaCysK.

Nucleosome eviction at TET/TDG-targeted enhancers occurs independent of the removal of the chromatin mark Oxidized-Methylcytosine

Marion Turpin [1], Thierry Madigou [1], Rachael Acker [1,2], Maud Bizot [1], Katie Sawvell [1,3], Stephane Avner [1], Audrey Laurent [1], Gaëlle Palierne [1], Christine Le Péron [1], Gilles Salbert [1]

[1] Université de Rennes, CNRS, Institut de Génétique et Développement de Rennes, UMR 6290, 35000 Rennes, France; [2] University of Pennsylvania, Philadelphia, Pennsylvania, U.S ; [3] Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, U.S

Genome-wide CpG methylation patterns reflect the competing action of DNA methyltransferases (DNMTs) and demethylation mechanisms, relying in part on 5-methylcytosine (5mC) oxidation by Ten-Eleven-Translocation (TET) enzymes. CpG methylation patterns can reflect cell-specific transcriptional programs and besides depending on DNMTs and TETs, these patterns can also be regulated locally by the engagement of transcription factors (TFs). This can be observed at small genomic regulatory regions called enhancers which undergo 5mC oxidation upon TF binding and recruitment of TETs. However, it remains unclear how 5mC oxidation functionally relates to enhancers, and what are the mechanisms underlying the successive oxidative steps. In this respect, priming is linked to the first step of 5mC oxidation into 5-hydroxymethylcytosine (5hmC), and further oxidation into 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) correlate with enhancer activation. However, it is unknown whether removal of oxidized bases (oxi-mCs) is required for enhancer activation. Elimination of oxi-mCs in cycling cells can either occur through a passive mechanism of cell division, or the recognition of 5fC/5caC as DNA damages and their active removal by the T:G mismatch DNA glycosylase (TDG)/base excision repair (BER) machinery. Here, we identified enhancers undergoing a TET/TDG-dependent full demethylation process during differentiation of pluripotent embryonal carcinoma cells into neural progenitor-like cells (NPCs), through genome-wide mapping of 5caC in TDG null cells. The impact of oxi-mC persistence on the chromatin structure of these enhancers was analyzed by MNase-seq and -qPCR assays. We identified hundreds of enhancers undergoing TDG-dependent oxi-mC removal during differentiation but observed that these regions undergo nucleosome eviction even without oxi-mC removal. Hence, we conclude that neural enhancer activation is linked to oxi-mC occurrence but does not require their removal by TDG.

Next Generation Image Analysis: Artificial Intelligence (AI)-Driven Analysis of Rhoptry Dynamics and Genome Remodelling in Malaria Parasite, Plasmodium falciparum

Sonja Frölich and Danny Wilson

Department of Molecular and Biomedical Science, School of Biological Sciences, Research Centre for Infectious Diseases, University of Adelaide, Adelaide, Australia. Institute for Photonics and Advanced Sensing (IPAS), University of Adelaide, Adelaide, Australia.

Malaria, infecting over 200 million people and claiming 600,000 lives annually, relies on a critical 48-hour blood stage life cycle where Plasmodium falciparum invade red blood cells (RBCs). Interactions between the RBC surface and invasion logands from merozoite rhoptries are essential for RBC invasion and parasitophorous vacuole (PV) formation. Within this PV, the parasite replicates via “closed” mitosis and unique cytokinesis, culminating in a multinucleated schizont, with each progeny merozoite inheriting the cellular machinery to function independently. To accelerate studies on parasite development within RBCs, we developed a machine learning (ML)-driven image analysis pipeline. Leveraging random forest algorithms, we automated the detection of parasite nuclei and rhoptries in super-resolved 3D volumes of immune-labelled infected RBCs. Our ML approach excels in accurately identifying nuclei in various mitotic stages and quantifying rhoptry structure, providing further insights into schizont maturation. The pipeline was strained on images of parasites at different developmental stages, enabling automated differentiation between pre- and post-mitotic nuclei and structural rhoptry features associated with merozoite maturation. This novel ML-based method outperformed traditional techniques in speed and accuracy, particularly for proteins PfCERLI1, PfRAP1 and PfRON4, identifying additional organelles that were missed by traditional approaches. This ML-driven pipeline enhances analysis, enabling deeper exploration of nuclear replication and rhoptry function. It surpasses traditional methods and has potential for broader parasite research, demonstrating the value of ML in malaria research, expediting functional studies and drug discovery.

Tuning the antigenic profile of a whole cell inactivated pneumococcal vaccine

Erin B. Brazel* [1,2], Chloe J. Gates [1], Eve V. Kennedy [2], Carla R. Gallasch [2], Giuseppe Ercoli [3], Jeremy S. Brown [3], Shannon C. David [2], Timothy R. Hirst [2], Mohammed Alsharifi [1,2], James C. Paton [1,2].

[1] Research Centre for Infectious Diseases and Department of Molecular and Biomedical Sciences, The University of Adelaide, SA, Australia
[2] GPN Vaccines, Yarralumla, ACT, Australia
[3] Centre for Inflammation and Tissue Repair, UCL Respiratory, Division of Medicine, University College Medical School, Rayne Institute, London UK

The bacterial pathogen Streptococcus pneumoniae kills more than 1 million people each year. Although pneumococcal vaccines exist, current vaccines only protect against a small subset of the 100+ known serotypes. We have developed a new serotype-independent whole cell-inactivated pneumococcal vaccine. Central to the success of this vaccine approach is the expression of antigenic surface proteins that reflect those expressed during disease. During pneumococcal infection, nutrients are restricted by host binding proteins. In the present study, we aimed to investigate whether we could mimic host-imposed nutrient limitation during vaccine manufacture to improve the immunogenicity of this whole cell vaccine. The S. pneumoniae Rx1ΔlytAPdT strain was cultured with or without a metal chelator (EDTA) and the metal content was assessed by inductively coupled plasma-mass spectrometry. RNA sequencing was performed to understand the broad changes in gene expression induced by EDTA. Bacteria were inactivated by gamma irradiation and mice were immunised by intramuscular injection. Antibody responses were extensively profiled by ELISA, flow cytometry, and against a multi-antigen microarray of pneumococcal proteins. S. pneumoniae cultured with EDTA displayed reduced manganese and zinc abundance, which was associated with changes in the corresponding metal-responsive antigenic genes. EDTA-treated vaccines showed improved immunogenicity in mice. Antibodies from mice immunised with the EDTA-treated vaccine displayed increased binding to the bacterial surface and exhibited differences in antigen recognition. This work highlights the potential to apply molecular knowledge of host-pathogen interactions to improve vaccine antigen expression and immunogenicity via simple changes to the manufacturing process.

Poster Abstracts

1.

Mapping the dengue virus NS1 protein microenvironment in infected cells.

Siena Centofanti [1], Alex Colella [2], Nusha Chegeni [2], Gustavo Bracho [2], Jillian M. Carr [1], Tim Chataway [2], Nicholas S. Eyre [1].

[1] College of Medicine and Public Health and Flinders Health and Medical Research Institute, Flinders University, Bedford Park, Adelaide, South Australia, Australia; [2] Flinders Proteomics Facility, College of Medicine and Public Health, Flinders University, Bedford Park, Adelaide, South Australia, Australia; [3] CellScreen SA, Flinders Centre for Innovation in Cancer, Flinders University, Bedford Park, Adelaide, South Australia, Australia.

Dengue virus (DENV) is a rapidly-spreading mosquito-borne (+)RNA virus that is currently endemic in over 100 countries and is responsible for a major public health burden. Despite this, there are no approved antiviral therapeutics available. The DENV non-structural protein 1 (NS1) plays critical roles in viral RNA replication, infectious virus particle production and viral pathogenesis and has emerged as a major target in the development of vaccines and antivirals. Towards the identification of DENV non-structural protein 1 (NS1)-host factor interactions that can serve as antiviral drug targets, we have mapped the proteomic composition the NS1 microenvironment in live infected cells using an APEX2 proximity labelling-coupled quantitative proteomics approach in conjunction with a APEX2-tagged reporter virus (DENV2-NS1-APEX2). An siRNA screen targeting the top 50 identified NS1-proximal host factors is underway to identify a panel of host factors that are required for DENV infection. It is hoped that the ongoing characterisation of a selection these validated hits using CRISPR/Cas9-mediated host gene knockout, protein-protein interaction assays and high-resolution confocal imaging analysis will reveal novel DENV NS1-host protein interactions that are essential to the viral replication cycle.

2.

The novel TR-compound, TR-107, is a highly effective agent against multiple myeloma cells in vitro.

Olivia Burling [1], Lauren Thurgood [1], Bryone Kuss [1], Giles Best [1]

[1] Flinders University, College of Medicine and Public Health, Flinders Health and Medical Research Institute, Adelaide, Australia.

Introduction
Multiple myeloma (MM) is an incurable haematological malignancy characterised by clonal expansion of plasma cells in the bone marrow. MM is associated with a five-year survival rate of approximately 55%, with most patients experiencing relapse or developing refractory disease. This highlights the currently unmet need for novel, targeted therapies. The TR-compounds are a novel class of drugs that activate the mitochondrial protease, ClpP, and have shown efficacy against triple negative breast and prostate cancer and diffuse large B-cell lymphoma. The current study was conducted to explore the potential of one compound of this class, TR-107, as a novel therapy for MM.

Methods
Dose responses of TR-107 against a panel of 3 MM cell lines were conducted by flow cytometry and IC50 values calculated. Work is on-going to further assess the functional effects of the drug and its mechanisms of action against MM cells. Effects on cell proliferation and accumulation of mitochondrial reactive oxygen species will be presented. Proteomic analyses will be completed to elucidate signaling pathways targeted by TR-107; changes in protein and mRNA expression will be confirmed using immunoblotting and RT-QPCR, respectively.

Results
The IC50 values for TR-107 against each cell line were 30.7 ± 6.8nM, 32.9 ± 10.6nM and 23.1 ± 4.3nM for LP-1, KMS-18 and RPMI-8226 cells, respectively. These values are consistent with published data in other cancers showing half-maximal inhibitory action of TR-107 in the low nanomolar range.

Conclusions
Multiple myeloma is an incurable malignancy and the high proportion of patients who relapse highlights the pressing need for novel therapeutic approaches. Our data suggests TR-107 is highly effective at inducing cell death of MM cells in vitro. Further studies are warranted and underway to determine whether TR-107 may represent an effective treatment for MM patients, particularly those who are resistant to current therapies.

3.

NOVEL PRIME EDITING APPROACH FOR THERAPEUTIC TARGETING OF AUTOSOMAL DOMINANT RETINITIS PIGMENTOSA

Lachlan Staker [1,2], Ashleigh Geiger [1,2], Jesse Kennedy [1,2], Fatwa Adikusma [1,2] and Paul Thomas [1,2]

[1] University of Adelaide, Adelaide, SA, Australia; [2] South Australian Health and Medical Research Institute (SAHMRI), Adelaide, SA, Australia

The author(s) have requested for the abstract to not be shared in the programme booklet.

4.

Taking Aim at Targeted "Whole-Gene" Insertion: A CRISPR-Prime Editing and BxbI Integrase Duet

Jesse G Kennedy [1,2], Ashleigh B Geiger [1,2,3], Lachlan G Staker [1,2], Fatwa Adikusuma [1,2], Paul Q Thomas [1,2]

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Easily programmable and highly efficient insertion of large, "whole gene sized" DNA fragments at specific genomic loci has been a longstanding but elusive goal of molecular genetics. Excitingly, recent publications have described a promising two-step strategy that may now make this a reality. Firstly, a 38bp attB BxbI integrase recognition site is incorporated into the target DNA sequence using a CRISPR/Cas9 editing technique termed Prime Editing. This "second generation" editing system uses a Cas9-Reverse Transcriptase fusion enzyme to generate specific targeted edits encoded by a dual purpose PEG RNA which functions as a gRNA and repair template. Subsequently, the desired large DNA fragment is integrated at the attB "landing pad" using BxbI.

This study aims to further improve and optimise this strategy using various Prime Editing technologies and a novel "traffic light assay". This will include in vitro Next-Generation-Sequencing data comparing attB Prime Editing efficiencies in HEK293T cells using various gRNA lengths, PEG RNA combinations and nickase versus nuclease Prime Editor versions. The most impressive Prime Editor system to date demonstrated 88% (n=3) successful attB insertion. Additionally, the development of a "traffic light assay" shall be discussed. This assay aims to demonstrate successful location specific insertion and expression of an intact entire gene via red-green fluorescence in a Flp-in HEK293T

Trex cellular platform. Results include successful cell line generation, verification and some assay red-green fluorescence microscopy and FACS outputs. Together, these efforts progress towards making targeted, large DNA insertion a valuable addition to the genome editor's toolbox.

5.

F2R as a Potential Therapeutic Target in Ovarian Cancer: Towards Personalized Nanomedicine Strategies

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Genetic heterogeneity in ovarian cancer underscores the necessity for personalized treatment strategies. Despite this, only a handful of G-protein coupled receptors (GPCRs) have been investigated for active targeting with nanomedicines, leaving a significant untapped potential. To address this gap and tailor treatments for individual patients, we propose a strategy of identifying unique GPCRs expressed in ovarian cancer biopsies and matching them with personalized GPCR-targeted nanomedicines for precise drug delivery. Our study begins with systematic analysis of public RNA-seq gene expression data, revealing 13 GPCRs as potential candidates with frequent overexpression in ovarian cancer tissues. Among these, we focus specifically on the F2R receptor due to its intriguing potential as a therapeutic target.
To validate the relevance of F2R in ovarian cancer, we will employ a multi-step approach. Initially, we will confirm its presence in ovarian cancer cell lines and a selection of patient-derived samples using advanced techniques such as flow cytometry and fluorescence imaging. This comprehensive analysis will provide valuable insights into the prevalence and distribution of F2R across different ovarian cancer probes.

Furthermore, we plan to utilize two representative ovarian cancer cell lines as in vitro models to assess F2R's role in cellular uptake and internalization processes. Specifically, we will explore peptide internalization and assess F2R's potential as a target for nanoparticle delivery systems. By elucidating the mechanisms underlying F2R-mediated internalization, we aim to develop novel strategies for targeted drug delivery.
Our findings underscore the importance of personalized targeting approaches in ovarian cancer therapy and highlight the potential of F2R as a promising therapeutic target. By integrating molecular techniques with nanomedicine strategies, we strive to pave the way for more effective and tailored treatment for ovarian cancer patients.

6.

Elucidating the mechanisms by which GATA2 regulates transcription to control lymphatic vessel valve development.

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GATA2 is a transcription factor that is essential for lymphatic vessel valve (LV) development and maintenance. Our previous work had revealed that GATA2 coordinates with three other transcriptional regulators of LV development; PROX1, FOXC2 and NFATc1, to regulate activity of a PROX1 enhancer element located 11kb upstream of the PROX1 promoter in lymphatic endothelial cells. When the GATA2 site of this enhancer element was ablated, recruitment of these four transcription factors to the enhancer region was perturbed and Prox1 mRNA levels in lymphatic endothelial cells were reduced. Mice carrying a homozygous deletion of the GATA2 binding site died soon after birth as a result of profound lymphatic vascular defects. Our discovery that GATA2 plays a pivotal role in assembling these transcription factors at the PROX1 -11kb enhancer region prompted us to investigate further the intricacies of this transcriptional complex. To this end, we are investigating protein-protein interactions between GATA2, PROX1, FOXC2 and NFATc1 and mapping the protein domains required to mediate these interactions. The impact of GATA2 variants found in Emberger syndrome, a disorder characterised by haematological abnormalities and primary lymphoedema, on the interaction between GATA2 and PROX1 is also being assessed. Together, this work will provide new insight to the transcriptional mechanisms regulating lymphatic endothelial cell identity during development and disease.

7.

Understanding Neuroblastoma Development through Single-Cell Analysis

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Introduction: Neuroblastoma is one of the most common extracranial cancers observed in infants and remains a leading cause of oncology-related paediatric deaths. It is a highly heterogeneous cancer and arises from faulty differentiation of the sympathetic nervous system. Typically, such differentiation processes are regulated by proteins called transcription factors. However,it remains unclear how these transcription factors influence differentiation and contribute to malignant behaviour.Objective: To investigate whether and how transcription factors contribute to the abnormal differentiation underlying neuroblastoma development.Method: Our wet lab collaborators induced the differentiation of healthy iPS Cells into sympathetic neurons, collected cells at key differentiation stages and subjected them to single cell RNA sequencing. I performed clustering analysis using Seurat to annotate cell-type populations at each developmental stage. I integrated the data to match shared cell populations between different timepoints and with cells from the developing human adrenal gland. To gain insights into the differentiation dynamics I performed trajectory analysis using Monocle. CellOracle is a machine learning approach that identifies gene regulatory networks and predicts cell identity changes through in silico knockout and overexpression simulations. I performed CellOracle analysis using our differentiation data and that from the fetal adrenal medulla to understand how changes in specific transcription factors affect development. I simulated knockouts and overexpression of the identified key transcription factors.Results: Single cell analysis revealed highly heterogenous cell population for each time point and integration showed shared cell populations across datasets indicating that our model closely mimics the natural differentiation process. CellOracle enabled the identification of 14 transcription factors that could be significant in differentiation.

8.

Development of Protein-based Gold Biosensor

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The author(s) have requested for the abstract to not be shared in the programme booklet.

9.

The RNA binding protein Quaking regulates prostate cancer cell plasticity by influencing widespread changes in alternative splicing.

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One of the greatest challenges in the treatment of metastatic prostate cancer (PCa) is the development of therapeutic resistance to androgen targeted therapies (ATT). Cell lineage plasticity is increasingly being implicated as a part of adaptive response to ATT, however, the mechanisms that drive this plasticity are not well understood. Our lab has previously identified an RNA binding protein, Quaking (QKI), that promotes breast cancer cell plasticity by regulating alternative mRNA splicing which produces alternative protein isoforms. In PCa clinical samples, we find QKI is induced during PCa progression, increases upon ATT, and is further elevated in castrate resistant PCa (CRPC). In LNCaP cells, QKI is markedly upregulated by treatment with the ATT Enzalutamide (ENZ) and modulates ENZ-induced changes in cell plasticity and alternative splicing. Furthermore, knockout of QKI in the ENZ-resistant LNCaP derived cell line MR42D re-sensitises these

cells to ENZ. In highly metastatic PC-3 cells, QKI modulates hallmark features of metastasis including changes in cell morphology, migration, and invasion with changes in alternative splicing. CLIP-seq studies suggest that QKI binds directly to many mRNAs where it drives alternative splicing of cytoskeletal protein-encoding transcripts. These studies reveal a QKI-regulated alternative splicing program influences PCa progression and the development of resistance to ATT. Overall, findings from this project will help distinguish a distinct splicing signature in different stages pf PCa and pave the way for novel alternative splicing targeting therapies to treat resistant PCa.

10.

BNC2, A NOVEL REGULATOR OF THE EXTRACELLULAR MATRIX

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Epithelial-mesenchymal transition (EMT) is a reversible process crucial for early development, wound healing in adults, and cancer progression. During EMT, several biological features are altered such as cell motility, cytoskeletal organization and structure of the extracellular matrix (ECM), in addition to extensive changes in the transcriptome. ECM, a core aspect of EMT, comprises a complex environment of extracellular proteins that serve as anchorage points for cells and enable signaling responses to chemical and biochemical stimuli. Dysregulation of ECM is often associated with diseases such as cancer, fibrosis, and chronic wounds. Our published study has demonstrated that Basonuclin-2 (BNC2), a mesenchymal-expressed zinc-finger protein, serves as a novel regulator of ECM production and degradation. Consequently, our data indicate that BNC2 modulates the motility and invasiveness of cancers and links high BNC2 expression with increasing cancer grade and poor patient prognosis. Several Genome-Wide Associated Studies (GWAS) have associated BNC2 with multiple cancer types and developmental defects. Despite numerous reported functional roles for BNC2, its molecular mechanisms remain mostly unknown, with limited publications demonstrating its DNA-binding activity as a transcription factor. This study aims to investigate the mechanisms by which BNC2 operates through determining the protein binding partners of BNC2. Furthermore, we aim to characterize

other phenotypical effects of BNC2 expression, such as cytokine secretion and drug resistance, in cancer cells and cancer-associated fibroblasts.

11.

Uncovering the functions of alternative splicing using novel RNA-targeting CRISPR technology

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Almost all human genes undergo alternative splicing to produce multiple protein isoforms. Protein isoforms often have different biological activities; however, the functions of most protein isoforms have not been characterised. To address this gap in understanding, we are developing RNA-targeting CRISPR technology to precisely manipulate alternative splicing of individual genes and study their functions in high-throughput screening formats. We are utilizing catalytically inactive “dead” (dCasRx) protein that binds to target RNA sequences, blocks the access to splice machinery and causes exon skipping. In parallel, we are developing a dCasRx fusion protein with the RNA binding protein QKI to facilitate exon inclusion when targeted to QKI biding sites. QKI is a major regulator of alternative splicing during epithelial-mesenchymal transition (EMT), a differentiation process that promotes cell migration and cancer progression. We aim to precisely manipulate alternative splicing events that are regulated during EMT, and by QKI, at scale to uncover functions of splicing events in cell proliferation, migration and in EMT transitions. As proof of principle, we have used dCasRx to effectively block the inclusion of exon 3 in the transcription factor NFYA and shown this represses migration of mesenchymal cells. This strategy will be applied with pool single cell screening, to assess the functional consequences of hundreds of individual splicing alterations on cell proliferation, migration, EMT, and drug resistance.

12.

The effects of single nucleotide variants in trace amine associated receptor 1 (TAAR1) structure-function

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Trace Amine Associated Receptor 1 (TAAR1) is an emerging drug target for treating several neuropsychiatric conditions. TAAR1 single nucleotide variants (SNV) have been identified in patients with mental health conditions and metabolic conditions, but they remain poorly characterised. To investigate this, we designed a multilevel study to identify and assess the effects of SNVs reported in existing databases. In our study, we leveraged the publicly available Database of Genotypes and Phenotypes and discovered all the missense SNVs in human TAAR1. A critical computational analysis was performed using experimental structures to identify SNVs affecting TAAR1 ligand activated mechanisms. Our analysis shows 19 orthosteric, 9 signalling and 16 micro-switch SNVs hypothesised to critically influence agonist induced TAAR1 activation. Notably, our dataset presented with orthosteric SNVs D103N (found only in the South-East Asian Region and Western Pacific Region) and T194A (found only in South-East Asian Region), and 2 signalling SNVs (V125A/T252A, found in African Region and commonly, respectively), all of which have previously demonstrated to influence ligand induced functions of TAAR1. These SNVs may non-proportionally influence populations from discrete regions and differentially influence the activity of TAAR1-targeting therapeutics in genetically and geographically diverse populations. Our future studies aim to functionally validate these SNVs in HEK293T cell lines, by establishing their response to endogenous and exogenous agonists. Moreover, our studies will provide novel insights into the role of TAAR1 in neuropsychiatric disorders associated with SNVs, and how they may influence the response to new TAAR1 therapeutics.

13.

The conserved sex determination gene DMRT1 has undergone independent evolution in egg-laying mammals

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Egg-laying mammals (monotremes), which include species of platypus and echidna, are the most basal surviving mammalian lineage, and provide unique insight into mammalian evolution. In addition, monotremes feature unique reproductive biology and have evolved sex chromosomes and sex determination genes independently from other mammals. DMRT1 is located on an X chromosome in monotremes and is expressed in gonadal tissues but much about the evolution and function of this gene is unknown. Doublesex and mab-3 related genes (DMRT genes) encode a family of transcription factors, that play central roles in the development of sex-specific differentiation across the metazoans. DMRT genes are characterised by the presence of a DNA binding DM domain, and the most well characterised DMRT gene, DMRT1, functions as a sexual regulator universally in metazoans. In chicken DMRT1 is located on the Z sex chromosome and acts as dosage dependent primary sex determination gene. In therian mammals DMRT1 is on an autosome but two copies are required for male development. In monotremes DMRT1 has been mapped to the X specific region of platypus X5 which means that males have only one copy of the gene. Here we identified and characterised multiple transcripts of DMRT1 which encode novel DMRT1 isoforms in both echidna and platypus through RT-PCR, sequencing, and expression analysis. This research identified unexpected changes in this extraordinarily conserved sex determination gene. Future research aims to better understand the functional and regulatory relevance of the evolutionary changes in DMRT1 of monotremes.

14.

Targeting Proliferating Cell Nuclear Antigen (PCNA) for Broad Spectrum Cancer Therapeutics

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15.

Microvascular damage and impaired LV heart rate: contractility relationship in a rat model of diabetic heart failure

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Background: There is ongoing debate about mechanisms whereby acute and subacute heart failure (HF) occur frequently in the presence of hyperglycaemia with substantial associated mortality. Some early reports have suggested that microvascular permeabilization may play a part, while more recent investigations have revealed oxidative stress, inflammatory damage to microcirculatory function, and a clinical presentation of HF with preserved ejection fraction (HFPEF) in most cases. We therefore developed a rat model of subacute diabetic/hyperglycaemic HF (DHF) to delineate relevant mechanisms.

Methods: Diabetes with severe hyperglycaemia was induced in Wistar rats (with matched controls) by diet and streptozotocin injections. After 8 weeks, echocardiography was performed, and whole heart was immediately excised for Langendorff perfusion, and then quantitation of inflammatory markers. Plasma concentrations of markers of damage to the endothelial glycocalyx (EG), which controls microvascular permeability, were also measured.
Results: Induction of DHF was associated with reductions in LV ejection fraction (EF) and E/E' ratio with severe attenuation of heart rate: EF relationship. Langendorff-perfused diabetic hearts lacked significant tachycardia-induced coronary vasodilatation and tended (p=0.06) to have impaired force-frequency relationships. Plasma concentrations of markers of EG damage were elevated, as was myocardial expression of thioredoxin-interacting protein (TXNIP), an inflammasome activator.

Conclusions: DHF is associated with (1) evidence of redox stress-induced damage to the microvascular glycocalyx (2) inflammatory activation within the myocardium (3)

incremental systolic dysfunction during tachycardia, presumably resulting from energetic impairment. All these present potentially novel approaches to therapy of DHF.

16.

UNDERSTANDING CHEMORESISTANCE MECHANISM IN TRIPLE NEGATIVE BREAST CANCER – A NOVEL CELL SIGNALLING AXIS

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Chemoresistance is the leading cause of death in cancers and its incidence in aggressive metastatic cancers like triple negative breast cancer (TNBC) poses a grave threat to its successful therapy. Being a highly heterogeneous tumour and lacking hormone receptors and HER2 amplification, there is a dearth of reliable targeted therapies since TNBC cells can escape the effects of chemotherapeutics through interaction with various cell signalling pathways. Even today, standard systemic chemotherapy is the primary mode of treatment. Identifying chemoresistance mechanisms in TNBC is crucial because merely 30 to 40 per cent of treated patients acquire pathological complete response. The remaining fraction succumb to relapse within five years of treatment due to chemotolerance. We have uncovered a novel signalling axis where PKCδ, a sub-family of protein kinase C, gets phosphorylated on tyrosine 374 (Y374) by FER and this phosphorylation of Y374-PKCδ enhances cell proliferation and drives chemoresistance in human TNBC cell lines. We identified hyperphosphorylation of Y374-PKCδ in 25 per cent of TNBC patient samples. Currently, we are developing in vivo mouse model systems of TNBC to study the effect of phospho-PKCδ on tumour sensitivity to chemotherapy in an immune-competent environment. We have observed varied degree of chemoresistance across various mouse breast cancer cell lines of TNBC to standard chemotherapeutics with respect to phosphorylation of Y374-PKCδ. The use of these mouse models will provide data on the effect of phosphorylation of PKCδ, thereby heightening the prospect of using Y374-PKCδ as a biomarker of chemoresistance and for developing targeted therapeutics.

17.

Exploring molecular features impacting raffinose metabolism in Streptococcus pneumoniae

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Streptococcus pneumoniae (the pneumococcus) is one of the most common bacterial causes of middle ear infections (otitis media) in children. In many cases, these infections can become persistent and challenging to treat. The pneumococcus is highly genetically diverse and while the molecular factors that influence disease are not fully understood, bacterial metabolic capacity is known to play an important role in infection. Previous studies in our laboratory have demonstrated that pneumococcal isolates taken from the ear displayed polymorphisms in genes encoding the uptake and regulatory pathways for raffinose-family oligosaccharides (rafK and rafR), which were associated with reduced raffinose metabolism in vitro and had a marked influence on disease tropism in animal models of infection. In the present study, we assessed serotype 3 isolates of S. pneumoniae taken from the middle ear of children with otitis media, and showed one was unable to metabolise raffinose. Serotype 3 is a clinically relevant serotype in otitis media and is a serotype of concern associated with significant vaccine escape. We next performed whole genome sequencing and variant analyses to identify potential molecular factors associated with the observed metabolic defect. These studies identified a premature stop codon in the gene encoding the raffinose ABC transporter substrate-binding protein (RafE). Together, this work provides further evidence supporting potential variation at the raffinose utilisation locus in pneumococci isolated during an episode of otitis media, which aids our understanding of these infections and may have implications for the design of new therapies and vaccines.