Contents

Winners

The APG committee would like to thank Andrew Hill for a fantastic talk, as well as the judges, attendees and our supporters for helping make this a very successful event. We would like to thank all student finalists and poster presenters, and we congratulate the Student Awards 2019 winners!

Best Talk

Rebecca Frkic, University of Adelaide

Best Posters

Juan Balbin, University of Adelaide

Rhys Hamon, University of South Australia

Stephanie Nguyen, University of Adelaide

Student talks

Inhibition of sphingosine kinase 2 resensitises bortezomib-resistant multiple myeloma

Melissa Bennett (1,2,3)*, Melinda Tea (1,2,3), Briony Gliddon (1,2,3), John Toubia (1,2,3,4), Robert Orlowski (5), Craig Wallington-Beddoe (1,2,3,6), and Stuart Pitson (1,2,3)
1. Centre for Cancer Biology, Adelaide, South Australia
2. University of South Australia, Adelaide, South Australia
3. SA Pathology, Adelaide, South Australia
4. ACRF Cancer Genomics Facility, Adelaide, South Australia
5. MD Anderson Centre, Houston, Texas
6. Flinders Medical Centre, Bedford Park, South Australia
*. Presenting Author

Multiple myeloma, a haematological malignancy of the plasma cell, is still largely incurable. The introduction of proteasome inhibitors such as bortezomib has significantly improved patient survival, however bortezomib resistance is still a major hurdle which requires new treatments. One option may be inhibitors to sphingolipid metabolism. Sphingolipids are a family of lipids which can act as signalling molecules, influencing cellular pathways such as apoptosis. Sphingosine kinase is an especially attractive target in cancer, as it converts pro-apoptotic ceramide and sphingosine to pro-survival sphingosine-1-phosphate. We have previously shown that inhibition of sphingosine kinase 2 with K145 causes synergistic cell death when combined with bortezomib in bortezomib-naïve myeloma cells, and thus we decided to test this combination in the bortezomib-resistant setting. To do so, bortezomib-resistant myeloma cell lines were generated and analysed. A 5TGM1 cell line generated to be bortezomib-resistant was also found to be carfilzomib-resistant, demonstrating cross-resistance that has also been seen in the clinic. Notably, these cells were found to possess a clinically-relevant mutation in the bortezomib-binding site of proteasomal subunit PSMB5. K145, when combined with either bortezomib or carfilzomib, caused synergistic cell death in the bortezomib-resistant cell lines tested. This was preceded by a significant increase in ER stress markers, as measured by Western blot, suggesting that this cell death may be induced by enhanced ER stress. When tested in vivo in a highly aggressive, bortezomib-resistant myeloma model, the combination of bortezomib and K145 decreased disease burden and increased survival, suggesting this combination may be effective in bortezomib-resistant patients.

Structural basis of antagonism of PPARg as a model for structure-driven T2DM drug design

Rebecca L. Frkic (1)*, John B. Bruning (1)
1. School of Biological Sciences, University of Adelaide, Adelaide, Australia
*. Presenting Author

The previous failures of PPARg agonists treating type two diabetes have highlighted the need for improved drugs that lack transcriptionally-promoting activity on PPARg. It has been shown that high-affinity non-activating ligands of the nuclear receptor promote potent insulin sensitivity to combat type 2 diabetes, but without the harmful or even fatal side effects associated with full activation of the receptor. A robust understanding of the underlying structural mechanisms determining the level of receptor activation induced by the ligand has long evaded researchers. We have solved the crystal structure of an antagonist and an inverse agonist in complex with the ligand binding domain of PPARg, which reveal the first instance of a global structural change in PPARg. These non-activating ligands of PPARg attract helix 12 (the activation helix) away from the coactivator binding surface of the LBD, which in turn enables the formation of a larger binding pocket which can accommodate binding of transcriptionally-repressing corepressors. This mechanism is distinct from other nuclear receptors such as estrogen receptor and PPARa, where current structural data has shown that their non-activating ligands clash with helix 12 to force it out of the “active” conformation and in to the antagonist conformation. We complemented our crystal structures with native mass spectrometry to verify the corepressor-recruiting capabilities of both ligands. This work highlights a major development in the field of PPARg research, as well as the wider nuclear receptor field for its unique mechanism and clinical relevance to the treatment of type 2 diabetes.

RACK1, flexible storage for all your Flavivirus replication needs

Byron Shue (1)*, Nicholas Eyre (1), Thu-Hien To (2), Stephen Pederson (3), and Michael R Beard (1)

1. Department of Molecular and Cellular Biology, The University of Adelaide, SA 5005, Australia
2. Centre for Integrative Genetics, Norwegian University of Life Sciences, Norway
3. Bioinformatics Hub, The University of Adelaide, SA 5005, Australia
*. Presenting Author

Flaviviruses, such as Dengue virus (DENV), West Nile Virus (WNV) and Zika virus (ZIKV) are classified as major human pathogens that inflict a significant burden on society. Cellular proteins play important roles in all facets of the flavivirus life cycle. Therefore, understanding viral-host protein interactions essential for the flavivirius lifecycle can lead to development of effective antiviral strategies.

A CRISPR/Cas9 genome-wide KO screen was employed to isolate novel host factors critical for ZIKV replication. Bioinformatics analysis identified previously characterised host factors (EMC1/EMC6) as well as a novel candidate, RACK1. RACK1 plays multiple roles in homeostatic cellular processes as a scaffold protein and acts as an indispensable hub for signalling transduction of multiple pathways. Interestingly, RACK1 was previously identified as essential for replication of several viruses (HCV, Pox virus).

siRNA knockdown of RACK1, followed by infection with flaviviruses ZIKV, DENV and WNV confirmed that RACK1 has a critical role in viral replication. Next, RACK1 was shown to interact with multiple non-structural (NS) viral proteins, indicating a multifaceted role in ZIKV replication. More specifically, interaction of NS1 with RACK1 was shown via confocal microscopy to localise to the ER while knockdown of RACK1 prior to infection showed that NS1 localisation is significantly altered compared to infected wildtype cells. Collectively, these experiments suggest RACK1 is important for replication complex formation, a critical step in establishing flavivirus replication. Further understanding of the intricate steps in viral replication establishment may potentially aid development of broadly acting antivirals against multiple flaviviruses.

Poster presentations

Functional characterisation of a stage-specific *Plasmodium falciparum* protein and its role in erythrocyte invasion

Juan M. Balbin (1)*, Benjamin Liffner (1), Paul Gilson (2), Danny Wilson (1,2)

1. Research Centre for Infectious Diseases, School of Biological Sciences, University of Adelaide
2. Burnet Institute, Melbourne, Victoria
*. Presenting Author

The apicomplexan parasite Plasmodium falciparum is the causative agent of malaria and is responsible for >400,000 deaths per year, mainly of children under the age of five. The onset of drug resistance against our frontline antimalarials is becoming a significant threat and is further exacerbated by the lack of an effective licensed vaccine. We must therefore prioritize the identification of novel proteins which are targetable by new vaccines. In pursuit of this, we have begun detailed functional characterisation of a zinc-finger protein of P. falciparum (Pf3D7_1468400) which is expressed during the invasive merozoite stage of the parasite lifecycle. Pf3D7_1468400 is highly conserved across P. falciparum isolates, has previously been localised to the merozoite surface and was reported to be susceptible to growth inhibitory vaccine induced antibodies; characteristics typical of leading vaccine candidates. However using gene editing approaches, our knock-out and knock-down parasite models demonstrated that Pf3D7_1468400's role in red blood cell invasion and blood stage growth is not essential. We explored Pf3D7_1468400's cellular localization in the merozoite using biochemical assays which indicated that the protein was unlikely to be on the merozoite surface, in contrast to the findings of the earlier study. Immunofluorescence microscopy studies are ongoing to determine Pf3D7_1468400's accessibility to antibodies, however the protein's limited signal has made it difficult to confirm its localisation within the merozoite. This study is the first detailed attempt to determine the function of the zinc-finger protein Pf3D7_1468400, with early results calling into questions its suitability as a vaccine target.

The role of Arrdc4 and ubiquitination in extracellular vesicle biogenesis and protein trafficking

Ammara U. Farooq (1)*, Kelly Gembus (1), Natalie Foot (1) and Sharad Kumar (1)

1. Centre for Cancer Biology, University of South Australia, Adelaide, South Australia, Australia.

Extracellular vesicles (EVs) are small membrane-bound vesicles secreted by cells to control many cellular processes, both in normal and diseased cells. Although first described over 30 years ago, the mechanisms behind how contents are selectively targeted to EVs are only now being investigated. The ubiquitin machinery has been implicated in this process, however the exact mechanism remains unclear. Ubiquitin is linked to protein substrates by ubiquitin ligases, which bind either directly to the substrate, or indirectly through adaptor proteins. Arrdc4 is an adaptor protein involved in the regulation of the divalent metal ion transporter DMT1 by mediating its ubiquitination and targeting it to EVs. Proteomic analysis revealed five lysine residues that were ubiquitinated in Arrdc4. By mutating these lysines to arginine, we found two residues, K131 and K270, that were critical for Arrdc4 stability and localization. Using a fluorescence quenching assay to measure the relative activity of DMT1, $Arrdc4_{K270R}$ significantly decreased DMT1 activity, suggesting a decrease of transporter at the cell surface. We also found that EVs from cells expressing $Arrdc4_{K270R}$ had lower expression of DMT1, suggesting that ubiquitination at this site is important for protein trafficking to EVs. Together, this data suggests that K270 is a critical residue within Arrdc4 responsible for its proper function in protein trafficking and EV biogenesis.

Role of the Quaking protein isoforms in promoting epithelial-mesenchymal transition and cancer progression.

Daniel Neumann (1)*, 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. Discipline of Medicine, The University of Adelaide, Adelaide, SA 5005, Australia.
*. Presenting Author

Metastasis is the major cause of death in epithelial-cell derived cancers. Epithelial-mesenchymal transition (EMT) is an important step in metastasis that invokes morphological and functional characteristics that contribute to disease progression. Using Snail1 and ZEB1 inducible models of prostate cancer EMT, we found that the 3 protein isoforms of the RNA binding protein Quaking (QKI) are strongly upregulated during EMT concomitant with changes in alternative splicing of several genes including ADD3, NUMB and CD47. The most highly expressed isoform, QKI-5, contains a nuclear localization signal which causes it to be almost exclusively expressed in the nucleus and the most likely candidate for splicing regulation. However, QKI isoforms cross-regulate and dimerise with one another, complicating the study of the function of individual isoforms. To overcome this, we have generated a QKI CRISPR knockout model using a human mesenchymal immortalized breast cell line. Using an inducible expression system, we have reconstituted the individual QKI isoforms in this cell line to study them in an isolated context. We found that all 3 QKI isoforms were able to promote the mesenchymal-alternative splicing pattern, but neither could rescue it to the extent seen in the wild-type cell line. These data indicate that QKI acts as a major contributor to mesenchymal-associated alternative splicing and that all 3 QKI isoforms have differential roles in EMT and cancer progression.

The host-virus interplay of IFN-ε, a uniquely expressed type–I interferon in the female reproductive tract protects against Zika virus infection.

Rosa Coldbeck-Shackley (1)*, Michelle Tate (2), Paul Hertzog (2), Nicholas Eyre (1), Kylie Van der Hoek (1), and Michael Beard (1)

1. The University of Adelaide, South Australia
2. Hudson Institute of Medical Research, Victoria Australia

Type-I interferons (IFN) are crucial for immunity against viruses. These proteins incite an intracellular antiviral state by up-regulation of effector genes called Interferon Stimulated Genes (ISGs). Typically, IFNs are secreted in response to viral detection within infected cells. However, the recently discovered interferon, IFN epsilon (IFNε), is uniquely expressed at mucosal surfaces of the female reproductive tract (FRT). Distinctively, IFNε is regulated by the female sex hormone progesterone instead of by viral detection. IFNε's localisation and constitutive expression suggests a preventative role against sexually transmitted viruses. The recently re-emerging Zika Virus (ZIKV) is mainly transmitted by mosquitoes but can also be sexually transmitted. ZIKV infections are normally mild, however they can cause severe developmental defects in foetuses of infected mothers, attributed to its persistence in the FRT. Therefore, we investigated IFNε's signalling properties and its role in preventing ZIKV infections of the FRT.

In vitro analysis of IFNε's signalling properties reveals it stimulates the type-I receptor pathway, leading to anti-ZIKV activity by upregulation of ISGs. However, ZIKV infection prior to IFNε exposure or expression of the ZIKV NS5 protein potently repressed this activity via degradation of the STAT2 protein, required for IFN signalling. To test IFNε in vivo we intravaginally infected IFNε-/-, wildtype and type-I receptor knockout mice with ZIKV revealing IFNε had a significant, non-redundant role at early times post infection consistent with in vitro results.

These results affirm the significance of IFNε protecting against ZIKV infections and may lead to changes in sexual health recommendations during ZIKV outbreaks.

Increased Expression of 14-3-3 Proteins in Lung Epithelial Cells: A Potential Role in the Development of Lung Cancer.

Rhys Hamon (1)*, Carl Coolen (1), Paul Reynolds (2), Stuart Pitson (1), Jo Woodcock (1)

1. Centre for Cancer Biology, SA Pathology and UniSA, Adelaide, Australia
2. Royal Adelaide Hospital Chest Clinic, Adelaide, Australia
*. Presenting Author

Molecularly targeted therapies are proving effective in many cancers. However, in Non-small cell lung cancer (NSCLC), effective treatment is only available for a subset of patients due to the heterogeneity in oncogenic drivers. Oncogenic driver mutation in the NSCLC manifest within cell proliferation and survival signalling pathways, inclusive of receptor tyrosine kinases (RTKs). Oncogenic signalling through RTKs is facilitated by the 14-3-3 protein family consisting of 7 isoforms: Beta, Epsilon, Eta, Gamma, Sigma, Tau and Zeta. Increased expression of 14-3-3 protein in NSCLC is associated with aggressive chemoresistance cancer with poor prognosis.

Lentivirus encoding individual 14-3-3 isoforms with GFP co-expression were used to infect the normal human bronchial cell line, BEAS-2B. Clonogenic and soft agar colony formation assays were used to assess anchorage dependent and independent growth, respectively, in BEAS-2B cells over-expressing 14-3-3 isoforms. 14-3-3 isoform over-expressing cell populations were also assessed for the expression of pro-survival proteins by immunoblot.

Clonogenic potential was increased in cells with forced expression of 14-3-3 Epsilon, Beta and Zeta isoforms, with significantly more colonies observed in 14-3-3 Epsilon expressing cells ($n=5$, $p<0.05$). BEAS-2B cells with forced expression of 14-3-3 Epsilon were able to produce significantly larger anchorage independent colonies compared to empty vector treated cells, ($n=5$, $p<0.05$). Forced expression of 14-3-3 isoforms in BEAS-2B cells resulted in increased abundance of both phospho- and total ERK and MCL-1 suggesting the cells are more resistant to apoptosis. These data warrant further study into the role of 14-3-3 proteins in tumour development.

β-casein prevents and stabilises α-lactalbumin misfolding in order to prevent amorphous and fibrillar aggregation

Henry M. Sanders (1)*, Blagojce Jovcevski (1), Michael Graetz (1), John A. Carver (2), Tara L. Pukala

1. School of Physical Sciences, University of Adelaide, Adelaide, SA 5005, Australia
2. Research School of Chemistry, Australian National University, Acton, ACT 2601, Australia
*. Presenting Author

A broad range of debilitating human diseases (including Alzheimer's and Parkinson's Disease) is connected with the failure of a specific protein to remain in its functional, folded three-dimensional state. Despite recent advances, understanding the molecular basis of protein misfolding and toxicity in such diseases remains elusive. Accordingly, there are currently no effective therapeutic approaches to prevention.

Under conditions of stress proteins can unfold and self-associate forming either amorphous or fibrillar aggregates. Bovine α-lactalbumin (α-LA) is a milk protein which is known to undergo amorphous aggregation when reduced with dithiothreitol (DTT α-LA), and fibrillar aggregation when reduced and carboxymethylated (RCM α-LA). Molecular chaperones, such as β-casein (β-CN), are important biomolecules capable of preventing both amorphous and fibrillar protein aggregation, but the mechanism is not fully understood. The aim of is this work is to provide a model for understanding misfolding protein and to probe the mechanism of chaperone activity.

Light scattering and thioflavin T fluorescence assays showed β-CN to be significantly more effective at preventing fibrillar aggregation than amorphous aggregation. Intrinsic and extrinsic fluorescence assays showed distinct structural differences between DTT α-LA and RCM α-LA. Ion-mobility mass spectrometry revealed that RCM α-LA exhibits less stability than native α-LA and that stability can be restored following addition of β-CN, suggesting a potential mechanism for fibril inhibition. Using a multi-disciplinary approach, this work explores the differences between the two misfolding pathways as well as the chaperone activity of β-CN to provide new insights that may ultimately inform new approaches to treatment.

Poster presentations (abstracts embargoed)

Jessica Heatlie

School of Pharmacy and Medical Science, University of South Australia, Australia.

Stephanie Nguyen

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

Methinee Pipatthana

Department of Biochemistry, Faculty of Science, Mahidol University, Thailand.

School of Pharmacy and Medical Science, University of South Australia, Australia.