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Micrograph images on the front cover - by Steven Eamegdool, Jia Hao Yeo, Mustafa (Steve) Kassem, Claude Dennis, Samson Dowland, Peter Newman, Rachel Shparberg, Michael Lovelace and Richard Sarafian, Iman Roohani, and Jia Hao Yeo.
DIRECTOR’S WELCOME

Dear colleagues,

It is my privilege to welcome you to the 2015 Annual Scientific Meeting of the Bosch Institute, *Canceromics: From Molecular and Cellular Biology to Breakthrough Therapeutics*. This Meeting is combined with our annual *Bosch Young Investigators Meeting*.

Cancer remains perhaps the most feared cause of early death, and it has become clear that there can be no simple cure for the uncontrolled growth of body tissues. For the biologist, study of cancer is a search for understanding of the mechanisms that control the development and stability of complex tissues – perhaps the most challenging of biological problems. For investigators seeking ways to assert control over malignantly proliferating tissues, there is the added challenge of understanding the biology of tumours, and the excitement of successful intervention.

Fundamental understanding of cancer and innovative interventions are the themes of this year’s joint ASM – the major intellectual event of our year. As understanding of the biology of tissues has grown, therapeutic opportunities have multiplied – prevention by management of lifestyle factors, regulation of hormonal influences, pre-emptive surgery where predisposing (often genetic) factors are identified, intervention in the biology of tumours by the control of growth factors; attacks on the vasculature of tumours; regulation of the immune system, and many more.

This Meeting will hear of the latest work on cancer, from laboratories in the Bosch Institute and well beyond. In addition, there will be a strong presentation of the work done by our Young Investigators, from laboratories working in all the Institute’s research themes. I am very proud to be able to welcome you to so rich a program.

For this Meeting, warmest thanks go to Professors Des Richardson, Steve Assinder and Frank Lovicu who conceived the theme and developed the program, and to the Institute’s COO, Charean Adams, Wannit Tongkao-on and the Bosch Young Investigator’s and Facility Officers who have dealt with all that is needed to present such a meeting with a professionalism that makes itself invisible. Important thanks go to our sponsors, whose support makes the Meeting possible.

I wish you all a meeting of interest, collegiate interchange and increased understanding of this important field.

Jonathan Stone DSc FAA
Executive Director
BOSCH YOUNG INVESTIGATORS COMMITTEE - WELCOME

The Bosch Institute was formed with the aim of enhancing the collaborative capabilities of basic science and clinical research laboratories within Sydney University and the Sydney South West Area. Thus, over the past years the Young Investigators have endeavoured to bring together undergraduate/postgraduate students and postdoctoral trainees with the aim of fostering relationships between different research themes.

This is achieved through a range of both social and scientific events held throughout the years, including the Young Investigator Seminar series, the Kioloa Annual Retreat, the Bosch trivia night and games night, as well as the Harbour Cruise.

This year the annual Bosch Young Investigators Symposium has been combined with the Bosch Annual Scientific Meeting. This allows for a showcase of the research carried out by young scientists within the Institute, University and wider community as well as to provide the opportunity to meet and talk with our peers in the field. We hope that you will find the conference both an interesting and enjoyable experience. Special thanks go to Frank Lovicu and Charean Adams for their help and support throughout the year.

Thank you to the membership of the Bosch Institute (both academics and students), for your participation and support over the past year, and we look forward to seeing you all at many more Bosch events in the remainder of the year and in 2016.

Sincerely,
Bosch Young Investigators Committee 2015

Vicki Xie
David Clarke
Ben Harris
Rosita Pang
Kevin Danastas
Sadaf Kalam

Miranda Mathews
Leyla Fouani
Hannah Glover
Sharleen Menezes
Victoria Tung
Chau Kim Le
Canceromics: From Molecular and Cellular Biology to Breakthrough Therapeutics

Scientific Program

Thursday, 16th July, 2015

REGISTRATION  8.00am – 8.45am

WELCOME
8.50 AM  Professor Jonathan Stone
Executive Director, Bosch Institute

OPENING REMARKS
8.55 AM  Professor Christopher Murphy
Associate Dean and Head, School of Medical Sciences

SESSION-1 (9.00 – 10:30 AM)

Chairs:
Professor Des Richardson, A/Professor Stephen Assinder

Plenary Speaker
9:00 AM  Professor Shinya Toyokuni, Nagoya University, Graduate School of Medicine, Japan
Cancer as a ferrotoxic disease: what we have learned from animal studies toward its prevention

Keynote Speakers:
9:30 AM  Professor Gary Halliday, Dermatology, Bosch Institute, Royal Prince Alfred Hospital, The University of Sydney
Translating chemoprevention of skin cancer with oral nicotinamide from the laboratory to the clinic

9:50 AM  A/Professor Rachel Codd, Chemical Biology in Drug Discovery Laboratory, Pharmacology, Bosch Institute, The University of Sydney
Methods in chemical biology for improved access to known and new anticancer agents

Young Investigator Talks:
10:10 AM  Dr. Daniel Johnstone, Bosch Institute, University of Sydney
Imaging large intact biological specimens

10:20 AM  Pearl Lee, University of Sydney
Novel cell adhesion regions on tropoelastin that mediate integrin-directed cell surface interactions

TEA BREAK & INTERACTION WITH SPONSORS (10:30 – 11:00 AM)
SESSION-2 (11.00 AM – 12:30 PM)

Chairs:
Professor Joy Ho, Dr. Kellie Charles

Plenary Speaker
11:00 AM Professor Ricky Johnstone, Peter MacCallum Cancer Centre, Melbourne
Targeting the epigenome to treat cancer

Keynote Speakers:
11:30 AM Professor Des Richardson, The University of Sydney
Why not target the major killers in cancer in one package? The development of the metastasis and multi-drug resistance inhibiting drug, DpC

11:50 AM A/Professor Guy Lyons, Royal Prince Alfred Hospital, The University of Sydney
Visualising clonal evolution, and the role of cooperation between clones, in carcinomas

Young Investigator Talks:
12:10 PM Chan Colonne, Disciplines of Physiology, Anatomy and Histology, and Bosch Institute, The University of Sydney
Stress anaemia leads to loss of growth restriction signalling in red blood cell progenitors: a new link in haematological malignancies?

12:20 PM Luan Vu, Department of Pathology, The University of Sydney
The fate of monocytes: a tale of two viruses

LUNCH BREAK / POSTER SESSION (12:30 – 01:30 PM)

SESSION-3 (1:30 – 3:00 PM)

Chairs:
Professor Christopher Chitambar, Dr. Zaklina Kovacevic

Plenary Speaker
01:30 PM Professor Gail Risbridger, Monash University, Melbourne
Identifying high risk features of prostate cancer

Keynote Speakers:
02:00 PM Professor Rebecca Mason, Physiology, Bosch Institute, The University of Sydney
Photoprotection By Vitamin D And Related Compounds

02:20 PM A/Professor Stephen Assinder, Andrology Research Group, Physiology, Bosch Institute, The University of Sydney
Oxytocin in prostate pathology. Is there a sinister side to the hormone of love?
Young Investigator Talks:

02:40 PM  Sharleen Menezes, Molecular Pharmacology and Pathology Program, Pathology, Bosch Institute, The University of Sydney
Role of the Metastasis Suppressor, NDRG1, in Regulating EGFR and ErbB Family Members and its Effects on Key Signaling Pathways in Pancreatic Cancer

02:50 PM  Fadi Gurdis, Pharmacology, The University of Sydney
Cytotoxic activity of the MK2 inhibitor CMPD1 in glioblastoma cells is independent of MK2

TEA BREAK & INTERACTION WITH SPONSORS (03:00 – 03:30 PM)

SESSION-4 (03.30 – 05:00 PM)

Chairs:
Professor Douglas Joshua, Dr. Tara Speranza

Plenary Speaker
03:30 PM  Professor Guillaume Lessene, The Walter & Eliza Hall Institute for Medical Research, Melbourne
Development of BCL-X<sub>L</sub> inhibitors: drug discovery and evaluation on cancer and normal cells

Keynote Speakers:
04:00 PM  Dr. Danuta Kalinowski, Molecular Pharmacology and Pathology Program, Pathology, Bosch Institute, The University of Sydney
Differential Anti-Cancer Activity of Copper Bis(Thiosemicarbazones): Reactive Oxygen Species Generation and Lysosomal Membrane Permeabilisation

04:20 PM  Dr. Emma Collinson, Anatomy and Histology, Bosch Institute, The University of Sydney
A role for Nox4 in TGF-beta-dependent EMT in the lens

Young Investigator Talks:

04:40 PM  Sukriti Krishan, Molecular Pharmacology and Pathology Program, Pathology, Bosch Institute, The University of Sydney
Novel Thiosemicarbazones mediate their anti-cancer effects via the AMPK-dependent pathway

04:50 PM  Irina Kondyurina, The University of Sydney
Polyurethane medical implants modified by ion implantation

05:00 PM  Presentation of H & PO Bishop Fellowship in Neuroscience - members of the Bishop family will be present

DAY ONE CLOSE

05.20 PM  Professor Jonathan Stone
Executive Director, Bosch Institute
Friday, 17th July, 2015

SESSION-1 (9.00 – 10:30 AM)

Chairs:
Dr. Parvin Ataie, Professor Shinya Toyokuni

Plenary Speaker
9:00 AM Professor Christopher Chitambar, Wisconsin College of Medicine, USA
Development of Iron-Mimetic Metallodrugs for the Treatment of Cancer: The journey from the laboratory to the clinic

Keynote Speakers:
9:30 AM Dr. Andrew Hoy, The University of Sydney
Adipose triglyceride lipase controls MDA-MB-231 breast cancer cell fatty and acid metabolism and influences progression

9:50 AM Dr. Angelica Merlot, Molecular Pharmacology and Pathology Program, Pathology, Bosch Institute, The University of Sydney
Induction of Endoplasmic Reticulum Stress using Thiosemicarbazone Dp44mT in Tumour Cells: Activation of PERK/eIF2α, IRE1α, ATF6 and Calmodulin Kinase

Young Investigator Talks:
10:10 AM Leigh Nicholson, Anatomy and Histology, The University of Sydney
Alpha-Parvin, Focal Adhesions and Metastasis Models

10:20 AM Nehan Munasinghe, Pharmacology, Bosch Institute, The University of Sydney
ORL1 and µ-opioid receptor agonists as therapeutics for chronic pain

TEA BREAK & INTERACTION WITH SPONSORS (10:30 – 11:00 AM)

SESSION-2 (11.00 AM – 12:30 PM)

Chairs:
A/Professor Qihan Dong, Dr. Patric Jansson

Plenary Speaker
11:00 AM Professor Judith Clements, Australian Prostate Cancer Research Centre, Queensland
Determination of novel KLK7-regulated molecular pathways in ovarian cancer

Keynote Speakers:
11:30 AM Dr. Sumit Sahni, Molecular Pharmacology and Pathology Program, Pathology, Bosch Institute, The University of Sydney
N-Myc Downregulated Gene 1 (Ndirg1) Suppresses Stress-Induced Autopathy In Cancer Cells
11:50 AM  
Dr. Dinny Graham, Centre for Cancer Research, The Westmead Millennium Institute for Medical Research, The University of Sydney  
Remodelling the genomic landscape: the role of the progesterone receptor, its cofactors and DNA structure in progesterone response in the normal and malignant breast

Young Investigator Talks:
12:10 PM  
Leyla Fouani, Molecular Pharmacology and Pathology Program, Pathology, Bosch Institute, The University of Sydney  
Targeting Zinc Finger E-Box Binding Homeobox 1 (ZEB1) to Reverse Metastasis via N-Myc Downstream Regulated Gene-1 (NDRG1)

12:20 PM  
Rachel Shparberg, Physiology, Bosch Institute, The University of Sydney  
L-proline-mediated neurogenesis using mouse embryonic stem cells

LUNCH BREAK / POSTER SESSION (12:30 – 02:00 PM)

SESSION-3 (2.00 – 3:30 PM)

Chairs:  
Professor Richard Christopherson, Dr. Andrew Hoy

Plenary Speaker  
02:00 PM  
Professor Leigh Ackland, Deakin University, Melbourne  
Zinc mediates breast cancer progression through the epithelial to mesenchymal transition

Keynote Speakers:  
02:30 PM  
Dr. Delphine Denoyer, Deakin University, Melbourne  
Investigating copper aberrations in prostate cancer: from basic science to therapeutic enquiry

02:50 PM  
Dr. Parvin Ataie, St. George Hospital, Sydney  
The developing story of Monepantel in cancer treatment

Young Investigator Talks:  
03:10 PM  
Bercovici Prize Recipient – Sam Merlin  
Deletion of Ten-m3 induces the formation of eye dominance domains in mice

03:20 PM  
Rebecca L Cooper Prize Recipient – Michael Lovelace  
P2X7 Receptors mediate innate phagocytosis by human neural precursor cells and neuroblasts

TEA BREAK & INTERACTION WITH SPONSORS (03:30 – 04:00 PM)
SESSION-4 (04.00 – 05:30 PM)

Chair & Adjudicator:
Dr. Paul Austin

3 Minute Thesis Presentations
04:00 PM  Sam Adamson
          David Clarke
          Benjamin Harris
          Sadaf Kalam
          Ji Yeon Kim
          Chau Le
          Miranda Mathews
          Diana Shinko

3 Minute Thesis Summary
4.45 PM  Professor Arthur Conigrave
          Deputy Dean, Sydney Medical School

Prize Ceremony  Prizes sponsored by AMP, GeneSearch, John Morris Scientific and Lastek
4.50 PM  Young Investigator Talk Prizes
          Young Investigator Poster Prizes
          Three Minute Thesis Prizes

CLOSING REMARKS
5.00 PM  Mr. Paul Fegan
          Chair, Bosch Institute Advisory Board

5.05 – 7.00 PM  Social Drinks in the Anderson Stuart Building
Cancer as a ferrotoxic disease: what we have learned from animal studies toward its prevention

Department of Pathology and Biological Responses, Nagoya University Graduate School of Medicine, Nagoya, Japan

1985 M.D. Kyoto University, Kyoto, Japan
1991 Ph.D. Kyoto University Graduate School of Medicine
1990-92 National Research Council Research Associate, Food and Drug Administration, Rockville, MD
1993-98 Assistant professor: Department of Pathology and Biology of Diseases, Kyoto University Graduate School of Medicine
1998-2008 Associate professor: Department of Pathology and Biology of Diseases, Kyoto University Graduate School of Medicine
2008-present Professor: Department of Pathology and Biological Responses, Nagoya University Graduate School of Medicine, Nagoya, Japan
President, Society for Free Radical Research (SFRR) Japan and Japanese BioIron Society (JBIS); president-elect SFRR Asia and SFRR International
Editor-in-chief: Nagoya J Med Sci
Cancer as a ferrotoxic disease: what we have learned from animal studies toward its prevention

Shinya Toyokuni, M.D., Ph.D.

Department of Pathology and Biological Responses, Nagoya University Graduate School of Medicine, Nagoya, Japan

Iron is abundant universally. However, iron works as a double-edged sword, and its excess can be a risk for cancer, presumably via generation of reactive oxygen species. Thus far, diseases such as hemochromatosis, chronic viral hepatitis B/C, exposure to asbestos as well as endometriosis have been recognized as iron overload-associated risks for human cancer. Indeed, iron is carcinogenic in animal experiments. We used a rat renal carcinogenesis model with repeated intraperitoneal injections of ferric nitrilotriacetate (NTA) and rat mesothelial carcinogenesis models by intraperitoneal administration of asbestos fibersor multiwalled carbon nanotubes (MWCNT). The obtained tumors were analyzed with array-based comparative genome hybridization. During the carcinogenesis, iron accumulation in each target cell were evident. Furthermore, these studies unexpectedly revealed that there are common target genes in these iron-induced carcinogenesis (e.g. homozygous deletion of CDKN2A/2B, etc.) with massive genomic amplifications and deletions. MWCNT also induced malignant mesothelioma, with 50 nm diameter MWCNT most carcinogenic. The fact that massive genomic alterations were observed for the first time in the iron overload-associated animal models of WILD-type animals suggests that iron overload may be a major mechanism in various human carcinogenesis. These genetic changes would be helpful for diagnosing early stage of cancer in pathology specimens. Recent epidemiological studies reported that iron reduction by phlebotomy decreased cancer risk in the apparently normal population. These results warrant reconsideration of the role of iron in carcinogenesis and suggest that fine control of body iron stores would be a wise strategy for cancer prevention. References: 1) Akatsuka S et al. PLoS One 7:e43403, 2012. 2) Toyokuni S. Cancer Sci 100: 9-16, 2009. 3) Toyokuni S. Front Pharmacol 5: 200, 2014. 4) Chew SH et al. Free Radic Biol Med 2015.
ABSTRACTS, JULY 16 SESSION 1: KEYNOTE SPEAKER

Professor Gary Halliday

*Translating chemoprevention of skin cancer with oral nicotinamide from the laboratory to the clinic*

Dermatology, Bosch Institute, Royal Prince Alfred Hospital, University of Sydney

Prof Halliday is a professor of Dermatology at the University of Sydney. He obtained his PhD from Monash University and was awarded a Doctor of Science in 2001 from the University of Sydney. He has made large contributions towards understanding the role of sunlight in skin carcinogenesis; particularly ultraviolet radiation suppression of immunity, induction of gene mutations, skin cancer cell biology and development of effective prevention. He has published 222 manuscripts, mainly on the topic of skin cancer. He is currently vice-president of the International Union of Photobiology, IUPB. He is a member of the Cure Cancer Australia Foundation Medical Grants Advisory Board, on the board of the Asia and Oceana Society for Photobiology, and is past-president of The Australasian Society for Dermatology Research (remaining on the Board). He is also currently the Associate Dean for Postgraduate Research for the Faculty of Medicine.
Translating chemoprevention of skin cancer with oral nicotinamide from the laboratory to the clinic

Gary M. Halliday and Diona L. Damian

Dermatology, Bosch Institute, Royal Prince Alfred Hospital, The University of Sydney

Ultraviolet (UV) radiation induces skin cancer by causing genetic damage and suppressing the immune response. If unrepaired prior to cell division, genetic damage can result in mutations. Both UVA and UVB cause genetic damage and immunosuppression in humans. Effective oral chemoprevention of skin cancer requires an agent that can enhance DNA repair and prevent immunosuppression caused by both UVB and UVA. UV caused a glycolytic blockade, reducing ATP levels in the skin. Nicotinamide, an amide form of vitamin B3, is the precursor of nicotinamide adenine dinucleotide, an essential cofactor for ATP production. We found that nicotinamide prevented the UV-induced glycolytic blockade and reduction in ATP levels in human skin. Subsequent laboratory based studies showed that nicotinamide enhanced repair of UV induced DNA damage. This was demonstrated by higher levels of unscheduled DNA synthesis, as well as enhanced rates of repair of both cyclobutane pyrimidine dimers and 8-oxo-7,8-dihydro-2′-deoxyguanosine. In humans we also showed that both topical and oral nicotinamide prevent both UVA and UVB induced immunosuppression. These laboratory-based studies were then translated into two phase II double blind randomized clinical trials in humans which demonstrated that oral nicotinamide significantly reduced the incidence of premalignant actinic keratosis and showed promise for skin cancer chemoprevention. Skin cancer places a huge burden on our health due to its high incidence in many countries, including Australia. The combination of sun avoidance, protective clothing and sunscreen, while important protective strategies, are not sufficient, as the incidence of skin cancer remains too high. Chemoprevention with oral nicotinamide, to inhibit the UV-induced energy crisis, thus enabling optimal DNA repair and immunity, appears promising for prevention of skin cancer. It is safe, non-toxic, inexpensive and widely available making it an ideal chemopreventive agent.
ABSTRACTS, JULY 16 SESSION 1: KEYNOTE SPEAKER

Associate Professor Rachel Codd

Methods in chemical biology for improved access to known and new anticancer agents

Chemical Biology in Drug Discovery Laboratory, School of Medical Sciences (Pharmacology) and Bosch Institute, The University of Sydney

Rachel completed a PhD in inorganic chemistry (1998) at the University of Sydney and undertook periods of postdoctoral research at the University of New South Wales and the University of Arizona before returning to the School of Chemistry to a research fellowship and an academic position. In 2007, she relocated to the School of Medical Sciences (Pharmacology) to lead the Chemical Biology in Drug Discovery laboratory. Rachel and her team conduct research in inorganic chemical biology focused on the development of new methods (microbiology, chemistry, chemical biology) for the discovery of new anticancer and anti-infective agents and ligands for PET.
Methods in chemical biology for improved access to known and new anticancer agents

Jiesi Gu, Isla Nakano, Tulip Lifa, William Tieu and Rachel Codd

Chemical Biology in Drug Discovery Laboratory, School of Medical Sciences (Pharmacology) and Bosch Institute, University of Sydney, NSW 2006, Australia

Our laboratory has developed a method in separation science designed to streamline access to structurally complex bacterial secondary metabolites used in the clinic for cancer. The method has utility for the selection of bleomycin from *Streptomyces verticillus* [1] and of doxorubicin from *Streptomyces peucetius* var. *caesius* [2]. The method is simple to use, aqueous compatible, and could support more efficient and green pathways for pharmaceutics processing, and the local production of these off-patent agents to secure supply. In a different project, we have used a metal-templated synthesis approach to access an unusual class of macrocyclic compounds produced in nature related to bisucaberin (NSC 619796) that have shown promise as anticancer agents. The method has enabled access to macrocyclic analogues with expanded molecular diversity, which could support future structure-activity relationships for drug optimization [3].

ABSTRACTS, JULY 16 SESSION 1: YOUNG INVESTIGATOR ORAL PRESENTATION

Dr. Dan Johnstone

Imaging large intact biological specimens

Dan Johnstone, Gary Xu, Jonathan Stone, Louise Cole

Bosch Institute, University of Sydney

A major limitation of traditional immunohistochemistry of large biological samples (e.g. mouse brain tissue) is the need to cut the tissue into thin sections prior to imaging, in order to overcome the inherent opacity of tissue and allow the penetration of stains and antibodies. This process hinders our ability to accurately trace large 3D anatomical structures, such as neuronal projections in the brain, and quantify complete cell populations in anatomically heterogeneous tissues. Recently, advances in tissue processing and microscopy have delivered protocols that facilitate the imaging of whole intact tissue. These advances involve (i) methods to render tissues optically transparent, while retaining normal morphology and being permeable to antibodies, and (ii) light-sheet microscopy, which allows imaging of 3D specimens while avoiding physical sectioning and photobleaching of fluorescence. We have now established an aqueous-based method of tissue clearing (PACT) that suitably ‘clears’ thick (>2mm) slices of mouse brain. We have also successfully labelled various structures (e.g. neurons, astrocytes, amyloid plaques) within cleared intact tissue using antibodies and fluorescent histochemical stains. Such tissue preparations are compatible with both confocal microscopy and the new Ultramicroscope in the Bosch Institute Advanced Microscopy Facility (AMF). For example, using the ultramicroscope, we have successfully generated a 1000-image z-stack of amyloid plaques through 4mm of tissue. An early pilot study has indicated that the PACT method can also clear fibrous tissue (e.g. the heart), suggesting that it could be applied to clear almost any tissue of interest. In summary, we have successfully used PACT as an aqueous-based method of tissue clearing for large intact tissue samples. This method is far cheaper and less labour-intensive than previous methods (e.g. CLARITY). Together this clearing and imaging approach is likely to be of interest to any researcher studying structure or pathology in heterogeneous tissues.
ABSTRACTS, JULY 16 SESSION 1: YOUNG INVESTIGATOR ORAL PRESENTATION

Pearl Lee

Novel cell adhesion regions on tropoelastin that mediate integrin-directed cell surface interactions

Pearl Lee, Anthony S. Weiss

The University of Sydney

Although tropoelastin protein monomers assemble to form elastin, tropoelastin does not contain a classic integrin-binding RGD sequence, so for some time it was under-explored for potential integrin-binding ligands. Subsequently we found that integrin αVβ3 on human dermal fibroblasts recognizes the extreme C-terminal RKRK motif of human tropoelastin. However this interaction does not account for the full cell-binding activity of tropoelastin. More recently, we have used recombinant tropoelastin constructs to identify a new cell binding site in tropoelastin and identify the major receptor involved in this novel region. We probed cell interactions with tropoelastin by deleting the C-terminal RKRK sequence in order to unmask other cell binding interactions within tropoelastin. Through this path, we found a novel human dermal fibroblast attachment and spreading site on tropoelastin. This site is located centrally in the molecule. Inhibition studies demonstrate that this cell adhesion is not mediated by either elastin binding protein or glycosaminoglycans. Cell interactions are divalent cation-dependent, indicating integrin dependence. Function blocking monoclonal antibodies reveal that αV- integrin(s), and specifically integrin αVβ5, are critical for cell adhesion to this part of tropoelastin. These data reveal a common αV integrin binding theme for tropoelastin: αVβ3 at the C-terminus and αVβ5 at the central region of tropoelastin. Each αV region contributes to fibroblast attachment and spreading but they differ in their effects on cytoskeletal assembly.

This early work shows that integrin interactions are driven by multiple sites in tropoelastin, however precise locations are yet to be elucidated. In the current work, to help understand where this additional binding is occurring, 15 tropoelastin constructs spanning the length of WT tropoelastin were made. Studies that compare the cell adhesion between each of the 15 constructs have narrowed down an upstream sequence involved in the binding to the integrin αVβ5. The divalent cation-dependence of this site confirms the interaction being driven by integrins.
ABSTRACTS, JULY 16 SESSION 2: PLENARY SPEAKER

Professor Ricky Johnstone

*Targeting the epigenome to treat cancer*

Peter MacCallum Cancer Centre, Melbourne

Professor Ricky Johnstone received his PhD from the University of Melbourne in 1993 and after a postdoc at Harvard Medical School returned to Melbourne to establish the Gene Regulation Laboratory at the Peter MacCallum Cancer Centre in 2000. Professor Johnstone is the Assistant Director of Research at the Peter MacCallum Cancer Centre and plays a key role in defining the strategic direction of the research division. He was awarded an NHMRC Senior Principal Research Fellowship in 2015 and in 2011 was promoted to Full Professor in the Department of Pathology at the University Of Melbourne. He is a cancer researcher who has utilized genetic mouse models of hemopoietic malignancies and solid tumors to decipher the molecular events underpinning cancer cell death by new targeted anti-cancer agents such as histone deacetylase inhibitors. In 2008 Dr Johnstone and Dr Grant McArthur established the Cancer Therapeutics Program within the Peter MacCallum Cancer Centre to bring together a critical mass of researchers with the aim to translate fundamental research findings into clinical outcomes that will benefit cancer patients.
Targeting the epigenome to treat cancer

Ricky Johnstone

*Peter MacCallum Cancer Centre, East Melbourne, Victoria 3002, Australia*

Altered expression, function or localisation of epigenetic enzymes and/or their partner proteins can play a crucial role in cancer onset and progression. Histone writers and erasers that regulate histone acetylation or methylation, or histone readers that recognise specific histone marks, play important roles in tightly regulating gene expression through the remodeling of chromatin and these proteins are promising targets for therapeutic interventions intended to reverse aberrant epigenetic states associated with cancer. I will outline our current understanding of altered epigenetic regulation in cancer onset and progression, the development of small molecule inhibitors of epigenetic enzymes and/or key partner proteins and the molecular, biological and clinical consequences of inhibiting these proteins.
ABSTRACTS, JULY 16 SESSION 2: KEYNOTE SPEAKER

Professor Des Richardson

Why not target the major killers in cancer in one package?
The development of the metastasis and multi-drug resistance inhibiting drug, DpC.

Molecular Pharmacology and Pathology Program, Department of Pathology, The University of Sydney

Des R. Richardson (B.Sc., M.Sc., Ph.D., D.Sc. (UWA), F.F.Sc. (RCPA)) holds the Chair of Cancer Cell Biology at the University of Sydney and is a National Health and Medical Research Council of Australia Senior Principal Research Fellow.

Prof Richardson’s major contributions to cancer research are focussed on understanding the role of iron in cancer cell proliferation and the development of novel anti-tumour agents known as iron chelators. Indeed, these studies have resulted in highly promising and potent anti-cancer drugs and are subject of a suite of active national patents.

He has published over 250 articles, chapters and books (94% as first or senior author) and in the last 5 years has published 106 articles (91% as first or senior author). His publication statistics include a h-index of 47 and has >7000 citations.

He current leads the Bosch Institute Cancer Cell Biology and Development Theme and Prostate Cancer Focus Group and is Director of the Iron Metabolism and Chelation Program. He holds academic appointments as Adjunct or Visiting Professor/Scientist at McGill University, Montreal, Lady Davis Institute for Medical Research, Montreal, Shanghai Jiao Tong University and Beijing Academy of Sciences.
Why not target the major killers in cancer in one package?
The development of the metastasis and multi-drug resistance inhibiting drug, DpC.

Richardson D.R.

The University of Sydney

Novel chemotherapeutics with marked and selective antitumor activity are essential to develop, particularly those that can overcome resistance to established therapies. Iron (Fe) is critical for cell-cycle progression and DNA synthesis and potentially represents a novel molecular target for the design of new anticancer agents.

Our studies over the last 30 years have led to the development of a new class of Fe chelators for cancer therapy. These compounds have recently been commercialised to enable their entrance into clinical trials resulting in the companies, Oncochel Therapeutics LLC (San Francisco, USA) and Oncochel Therapeutics Pty Ltd (Australia).

These agents show broad antitumor activity and could overcome resistance to established antitumor agents. The in vivo efficacy of the most effective chelator identified, di-2-pyridylketone-4,4,-dimethyl-3-thiosemicarbazone (Dp44mT), was assessed by using a panel of human xenografts in nude mice. After 7 weeks, net growth of a melanoma xenograft in Dp44mT-treated mice was only 8% of that in mice treated with vehicle. In addition, no differences in these latter animals were found in hematological indices between Dp44mT-treated mice and controls. No marked systemic Fe depletion was observed comparing Dp44mT and vehicle-treated mice, probably because of the very low doses required to induce anticancer activity.

Dp44mT caused up-regulation of the Fe-responsive tumor growth and metastasis suppressor NDRG1 in the tumor but not in the liver, indicating a potential mechanism of selective anticancer activity (Richardson et al. PNAS USA 2006;103:14901-6). The potent and selective anti-metastastic activity of Dp44mT in animal models have been independently verified by others (EMBO Mol. Med. 2012;4:93-108).

Collectively, these results indicate that the novel Fe chelators have potent and broad antitumor activity and can overcome resistance to established chemotherapeutics because of their unique mechanism of action. Moreover, they can inhibit metastasis through up-regulation of NDRG1. This latter effect is crucial, as 90% of deaths due to cancer are because of metastasis to vital organs.
Associate Professor Guy Lyons

Visualising clonal evolution, and the role of cooperation between clones, in carcinomas

The University of Sydney & Royal Prince Alfred Hospital

Guy Lyons did his PhD in the Department of Pathology at the University of Sydney, followed by postdoctoral studies at the University of Alabama at Birmingham, USA, and the Institute of Biological Chemistry in Strasbourg, France. He subsequently worked at the Kanematsu Laboratories, RPA Hospital, and NIH before joining the Dermatology Department and Bosch Institute 10 years ago. He began his research career investigating the cellular and molecular causes of epithelial cancers, and is still trying to figure them out. The most recent efforts involve interdisciplinary approaches combining molecular, cellular, animal and mathematical models.
Visualising clonal evolution, and the role of cooperation between clones, in carcinomas

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Cancer progression is an evolutionary process. The adaptation of cancer cells to their environment can be influenced by interactions with each other, analogous to the influence of symbiotic relationships in the evolution of whole organisms. This can occur when distinct mutations in different cancer cells are mutually beneficial, enabling them to cooperate with respect to proliferation, dissemination or a combination of both.

We have developed in vivo and in vitro models for investigating the clonal evolution of squamous cell carcinomas (SCCs) and the influence of cooperation on it. One model uses a non-neoplastic line of keratinocytes, the cells that give rise to SCCs, to identify cancer-related genes that can interact when expressed in separate clones of cells. We have focused on the interactions between genes that induce proliferation with genes that cause an epithelial-mesenchymal transition. The cells expressing each gene are tagged with different-coloured fluorescent proteins, enabling us to identify each subpopulation after they have been mixed. We then determine the rates of proliferation, motility and invasion in vitro and tumour growth and dissemination in vivo, comparing the mixtures of clones with each clone individually and with clones expressing both genes, to determine whether cooperation occurs.

Another in vivo model uses a genetically modified mouse strain that enables us to trace cell lineages over time in the oral mucosa by monitoring fluorescent protein expression with a specially assembled microscope and stage. Space is always limiting in a tissue, which provides a selective pressure for clones, even in non-carcinogenic tissues. By imaging the tongue over time, we can follow the growth and shrinkage of individual epithelial clones and the effects that a chemical carcinogen has on them.
Stress anaemia leads to loss of growth restriction signalling in red blood cell progenitors: a new link in haematological malignancies?

Chanukya K. Colonne and Stuart T. Fraser

Disciplines of Physiology, Anatomy and Histology, and Bosch Institute, The University of Sydney

Acute anaemia induces red blood cell (erythroid) progenitors to migrate from the bone marrow to the spleen, a secondary site of erythrocyte production. This is termed stress erythropoiesis and results in the production of vast numbers of erythrocytes and subsequent splenomegaly. Similar expansion of erythrocyte number and splenomegaly is seen in the myeloproliferative disease, Polycythaemia Vera (PCV). Activating mutations in the Janus kinase 2 (JAK2) has been identified in many PCV patients. JAK2 is known to be involved in the JAK-2-STAT5 signalling pathway which plays an essential role in erythropoiesis promoted by erythropoietin. Acute anaemic stress can be modelled in mice by injection of the haemolytic compound phenylhydrazine. We have performed transcriptome analyses on normal and stress erythroblasts to identify similarities and differences at the mRNA level between these two erythroid progenitor populations. We have found that CD45 mRNA and surface protein expression is reduced in stress erythroblasts. CD45 is a surface tyrosine phosphatase and is a known inhibitor of JAK2. Expression of CD22, an activator of CD45, is also reduced at the mRNA and surface protein level on stress erythroblasts. We propose that the reduced expression of the JAK2 inhibitor CD45 and its activator CD22 enables the JAK2-STAT5 pathway unfettered activation thus resulting in enhanced erythroblast proliferation similar to that seen in PCV. Delineating the molecular pathways functioning during stress erythropoiesis will enhance our understanding of the processes regulating erythropoiesis during stress. We believe this in turn will aid the development of novel therapeutics into the treatment of myeloproliferative neoplasms such as PCV.
ABSTRACTS, JULY 16 SESSION 2: YOUNG INVESTIGATOR ORAL PRESENTATION

Luan D Vu

The fate of monocytes: a tale of two viruses

Luan D Vu, Nicholas J.C King

Department of Pathology, The University of Sydney

Introduction: Of the Flavivirus genus, mosquito-born neurotropic West Nile virus (WNV) and viscerotropic Dengue viruses (DENV) are associated with significant neurological complications. In WNV we have shown that infiltrating Ly6Chi monocytes is directly correlated with disease development and mortality, but precise differentiation and function of these remains contentious.

Materials and Methods: In two different experimental models of encephalitis, lethal intranasal WNV (i.n WNV) and non-lethal intracranial DENV (i.c DENV), we analysed leukocytes infiltrating into the brain by multiparametric flow cytometry, as well as mRNA transcripts by openarray and qPCR.

Results: We found two distinct differentiation patterns of bone marrow–derived infiltrating monocytes (BM-M). In the WNV model, infiltrating monocytes gave rise to inflammatory macrophages (WNV-iMϕ) (Ly6ChiCD11clowMHCIIint) in an infection-route independent manner, while in the DENV model these cells differentiated into Ly6ChiCD11chiMHCIIhi dendritic cells (DENV-DC). Adoptive transfer of BM-M from d6 p.i. i.n WNV mice into i.c DENV and vice versa confirmed this. Intriguingly, in DENV model, the differentiation of BM-M into DENV-DC was abrogated when donor cells were injected intracranially rather than intravenously. Furthermore, there is significantly higher proportion of WNV-iMϕ in vivo producing TNF than that of DENV-DC. The reduction of DENV-DC either by CCL2 neutralization or immune-modifying microparticle treatment did not alter the pathophysiological outcome.

Conclusions: The milieu of the inflamed brain, not the bone-marrow environment, drives the fate of the infiltrating monocytes. We hypothesise that the differentiation of infiltrating monocytes is controlled by the balance between pro and anti-inflammatory immune responses. The differentiation of BM-M into DC in DENV-infected brains may also require leukocyte extravasation. The disparity of mRNA expression patterns suggests that manipulation of inflammatory factors may be of potential therapeutic benefit.
ABSTRACTS, JULY 16 SESSION 3: PLENARY SPEAKER

Professor Gail Risbridger

Identifying high risk features of prostate cancer

Monash University

Professor Gail Risbridger is an NH&MRC Research Fellow, career academic and researcher who has >25 years’ experience in prostate cancer research and Men’s Health. She graduated from and taught at Monash University until becoming a founding member of the Monash Institute of Medical Research (MIMR) now known as the Hudson Institute of Medical Research. As head of an internationally recognised research team of scientists and clinicians working on prostate cancer and andrology related projects within the Department of Anatomy and Developmental Biology at Monash University, she is one of Australia’s leading prostate cancer researchers. Prof Risbridger has particular expertise in the biology of stromal-epithelial cell interactions in normal and tumour tissue using tissue recombination, animal and human specimens and she pioneered the use of stem cells for recombination studies combining stem cell biology with endocrinology. She currently holds the positions of Deputy Dean, Strategic Projects, Research Director of Monash Partners Comprehensive Cancer Consortium (MPCCC) and Chair, Faculty Research Centres & Institutes Committee as well as advisory roles in Andrology Australia and the Freemasons Foundation Centre for Men’s Health. She has authored over 210 publications and received more than $22.9 million in National and International grant funding since 2003. Her awards include an International Fulbright Senior Scholar Award, British Endocrine Society Asia-Oceania Medal and Honorary Life Member of Endocrine Society of Australia.
ABSTRACTS, JULY 16 SESSION 3: PLENARY SPEAKER

Identifying high risk features of prostate cancer

Gail P Risbridger
Monash University

Family history is a well-established risk factor for prostate cancer (PCa) and patients with BRCA germline mutations resulting in genomic instability, exhibit highly aggressive tumours with poor prognosis. Standard clinical features and/or outcome prediction models fail to accurately predict outcomes and histological features may be more useful.

Intraductal carcinoma of the prostate (IDC-P) is a distinct clinico-pathologic entity associated with aggressive PCa, although not routinely reported. The goal of these studies was to investigate the presence and implications of IDC-P in men with a familial PCa due to BRCA2 pathogenic mutation. Patient-derived xenografts (PDXs) were generated from germline BRCA mutation-carriers. The incidence of IDC-P and association with overall survival for BRCA2 patients was determined using Kaplan-Meier analysis.

Whole Genome Characteriation (WG-CNA) was performed on microdissected pathologies (normal prostate, PIN, adenocarcinoma and IDCP) from primary and PDX specimens.

IDC-P in patients with familial PCa identified the most aggressive tumours with poor survival (even when the stage and grade of cancer at diagnosis was similar), and is associated with the measurably high level of genomic instability in this pathology.
ABSTRACTS, JULY 16 SESSION 3: KEYNOTE SPEAKER

Professor Rebecca Mason

Photoprotection By Vitamin D And Related Compounds

The University of Sydney

Rebecca Mason, a medical graduate from Sydney University, has research interests in vitamin D, bone and skin. She is on the Editorial Board of the Journal of Bone and Mineral Research and the journal Endocrinology. She has been a member of the Cancer Councils of Australia Working Party on Sun and Health and consults on vitamin D with these groups, was a member of the International Commission on Illumination’s technical committee on Sunlight, Health and Vitamin D (reported in 2011) and now serves on a new technical committee for the Commission. In 2009, she received an award from the 14th International Workshop on Vitamin D for “career contributions to vitamin D research” and was a founding member of the Executive Committee for a new series of International Vitamin D Workshops. She is Head of Physiology and Deputy Director of the Bosch Institute for Medical Research, University of Sydney, a Board member of Osteoporosis Australia and a Past President of the ANZ Bone and Mineral Society.
Photoprotection By Vitamin D And Related Compounds

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5. Veterinary Pathology, University of Sydney 
6. Dermatology & Bosch Institute, University of Sydney

The production of vitamin D in skin from 7-dehydrocholesterol by UVB is well known. Less well appreciated is that vitamin D is metabolized in skin to the active hormone, 1,25-dihydroxyvitamin D (1,25D), as well as other metabolites, and appears to have important actions locally, including protection from UV-induced DNA damage and photocarcinogenesis. Topical application of 1,25D and other analogs reduce several types of UV-induced DNA damage in mice and human subjects. The mechanism of the photoprotective effect is not fully understood, but enhanced expression of p53 in the presence of D compounds, which would facilitate DNA repair, and reduced production of reactive nitrogen species, which would otherwise inhibit repair, may contribute. We have recent evidence that expression of key DNA repair proteins are enhanced with 1,25D. Analog studies as well as experiments with variant vitamin D receptors and knockout of ERp57 protein, indicate that both the vitamin D receptor and ERp57 are required for photoprotection, but results are consistent with a non-genomic mechanism. The studies to date support the hypothesis that the vitamin D system in skin contributes to photo-adaptation and suggest that vitamin D compounds may be usefully incorporated into a topical application such as a sunscreen or after sun lotion to further reduce DNA damage.
ABSTRACTS, JULY 16 SESSION 3: KEYNOTE SPEAKER

Associate Professor Stephen Assinder

Oxytocin in prostate pathology. Is there a sinister side to the hormone of love?

Andrology Research Group, Discipline of Physiology and Bosch Institute, The University of Sydney.

Steve was awarded his PhD by the University of Bristol, UK in 1996. After 6 years as Lecturer at Otago University Steve moved to the Discipline of Physiology, University of Sydney where he leads the Bosch Institute’s Andrology Research Group. Research interests include: the roles of structural proteins in cancer development; signaling pathway integration and dysregulation in cancers; the actions of oxytocin in both benign and malignant prostate disease; identification of prognostic markers of prostate disease and treatment. He is also a founding member of the Copper Biology Research Group studying the chemotherapeutic implications of copper transporters.
Oxytocin in prostate pathology. Is there a sinister side to the hormone of love?

Vanitha Bhoopalan, Johnathan Suriya, Angela Nova, Steve Assinder

Andrology Research Group, Discipline of Physiology and Bosch Institute, The University of Sydney

Oxytocin has been dubbed the hormone of love for its physiological and psychosocial roles. Indeed, it is used to pharmacologically manage a number of disorders, including autism spectrum disorder. But are there unconsidered contraindications for such use? Our work has shown oxytocin to be implicated in both benign prostatic hyperplasia and prostate cancer. In both diseases oxytocin stimulates local steroidogenesis, whilst it has been shown to have different effects on the proliferation of normal prostate stromal, epithelial and prostate cancer cell lines. Towards understanding the reasons for the differential effects we used a transcriptome analysis of PC-3 and DU 145 prostate cancer cell lines treated with or without oxytocin. Both cell lines displayed increased expression of genes for enzymes involved in the cholesterol biosynthetic pathway. This was unexpected, as we have previously hypothesised that the response to oxytocin is determined by the localisation of the oxytocin receptor to caveolae. It is known that DU 145 form caveolae whereas PC-3s do not due to their lack of the protein PTRF (cavin-1). This was further investigated by determining the effect of oxytocin on the transcriptome of PC-3 cells in which PTRF is re-expressed (PC-3/PTRF). In these cells oxytocin also increased the expression of genes involved in cholesterol biosynthesis. In PC-3, PC-3/PTRF and DU 145 oxytocin was shown to increase genes expression of DHC7 (7-dehydrocholesterol reductase), ACAT (acetyl-CoA acetyltransferase 2) and HSD17B7 (hydroxysteroid 17-beta dehydrogenase 7), key enzymes in cholesterol synthesis and metabolism. In conclusion, oxytocin appears to up-regulate expression of genes involved in cholesterol biosynthesis irrespective of the presence of PTRF.
Sharleen V. Menezes

Role of the Metastasis Suppressor, NDRG1, in Regulating EGFR and ErbB Family Members and its Effects on Key Signaling Pathways in Pancreatic Cancer

Sharleen V. Menezes, Zakiha Kovacevic, Des R. Richardson

Molecular Pharmacology and Pathology Program, Department of Pathology, Bosch Institute, The University of Sydney

N-myoc downstream regulated gene-1 (NDRG1) is a potent metastasis suppressor that has been shown to affect numerous signaling pathways that control oncogenesis.

In this study, the role of NDRG1 was investigated on a key upstream effector, namely epidermal growth factor receptor (EGFR) and other members of the ErbB family, namely human epidermal growth factor 2 (HER2) and human epidermal growth factor receptor 3 (HER3). This is of interest, as the ErbB family of receptor tyrosine kinases are involved in regulating multiple cell responses, being key regulators of down-stream oncogenic-signaling.

We demonstrate that NDRG1 is able to significantly reduce the expression, localisation and activation of EGFR, HER2 and HER3, while also inhibiting the formation of EGFR/HER2 and HER2/HER3 heterodimers. This investigation has also shown that a novel class of anti-cancer agents, namely di-2-pyridylketone 4,4-dimethyl-3-thiosemicarbazone (Dp44mT) and di-2-pyridylketone 4-cyclohexyl-4-methyl-3-thiosemicarbazone (DpC), were able to markedly up-regulate NDRG1. These agents were found to inhibit EGFR, HER2 and HER3 expression and phosphorylation in PANC-1 pancreatic cancer cells in vitro. Moreover, these compounds led to a significant reduction of the expression of these proteins in PANC-1 tumour xenografts in vivo.

Due to the limitations of current anti-cancer therapeutics, these agents were also compared to a clinically used EGFR inhibitor, Erlotinib. Our study showed that in comparison to Erlotinib, both Dp44mT and DpC displayed higher anti-proliferative activity in pancreatic cancer cells. This could be significant, as DpC is due to enter clinical trials this year for the treatment of aggressive solid tumours (http://www.colmeddev.com/oncochel/).

Together, these findings reveal the molecular mechanisms that underlie the anti-cancer effects of NDRG1, and in turn, demonstrate the potential origin of the extensive down-stream effects attributed to this molecule.
ABSTRACTS, JULY 16 SESSION 3: YOUNG INVESTIGATOR ORAL PRESENTATION

Fadi Gurgis

Cytotoxic activity of the MK2 inhibitor CMPD1 in glioblastoma cells is independent of MK2

Fadi Gurgis and Lenka Munoz

Discipline of Pharmacology, The University of Sydney

Glioblastoma is among the most lethal and least successfully treated solid tumors. With solely one chemotherapeutic agent available in clinic, novel therapies for glioblastoma are urgently needed. MAPK-activated protein kinase 2 (MK2) is a checkpoint kinase involved in the DNA damage response. MK2 inhibitors enhance efficacy of conventional chemotherapeutic agents, but their effectiveness as single agents has not been investigated. CMPD1 was reported as a non-ATP competitive p38 MAPK inhibitor that selectively inhibits MK2 phosphorylation without affecting the activation of other p38 MAPK substrates (Boehringer-Ingelheim; Davidson, W., et. al., Biochemistry 2004 Sep 21;43(37):11658-71). To assess whether MK2 inhibition using CMPD1 could result in anti-cancer activity, we compared the activity of CMPD1 with a structurally unrelated MK2 inhibitor III (MK2i) in a panel of glioblastoma cell lines. While CMPD1 exhibited anti-proliferative activity in glioblastoma cells at sub-micromolar concentrations, MK2i was at least 45 times less potent. Furthermore, CMPD1 treatment induced a significant G2/M arrest but MK2i-treated cells only minimally arrested at G1 phase. Intriguingly, at doses that were cytotoxic to U87 glioblastoma cells, CMPD1 did not inhibit phosphorylation of MK2 and of its downstream substrate Hsp27. Genetic knock-down of MK2 did not alter the survival of glioblastoma cells or anti-proliferative activity of CMPD1. These results suggested that CMPD1 exhibits anti-proliferative activity independent of MK2. Indeed, we identified CMPD1 as a tubulin-depolymerising agent causing microtubule disruption similarly to vinblastine. CMPD1 promoted proteasomal degradation of MCL-1 and induced significant apoptosis in glioblastoma cells. Importantly, CMPD1 displayed minimal toxicity to non-malignant cells including primary human astrocytes. In summary, our study demonstrates that the MK2 kinase is dispensable for glioblastoma survival. In addition, we show that the MK2 inhibitor CMPD1 is a novel microtubule depolymerising agent with potential anti-cancer efficacy for glioblastoma therapy.
ABSTRACTS, JULY 16 SESSION 4: PLENARY SPEAKER

Professor Guillaume Lessene

Development of BCL-X\textsubscript{i} inhibitors: drug discovery and evaluation on cancer and normal cells

ACRF Chemical Biology Division, The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia
Department of Medical Biology, The University of Melbourne, Melbourne, Australia
Department of Pharmacology and Therapeutics, The University of Melbourne, Melbourne, Australia

A/Prof Guillaume Lessene jointly heads the ACRF Chemical Biology Division at the Walter & Eliza Hall Institute of Medical Research. The division assembles expertise in medicinal chemistry, biochemistry and molecular biology; and applies chemical biology approaches to validating therapeutic targets, and elucidating the biological pathways that drive disease.

A/Prof Lessene trained as an organic chemist, completing his PhD at the University of Bordeaux, before undertaking postdoctoral work with Prof Feldman at Penn State. Since moving to WEHI in 2003, his major research focus has been developing small molecules that target the apoptotic and necroptotic cell death pathways.

His work on targeting the Bcl-2 family proteins for cancer therapy formed the basis of a major collaboration between WEHI and two biopharmaceuticals, Genentech and AbbVie. Moreover, his ground breaking work developing a potent and selective inhibitor of the Bcl-2 protein, Bcl-x\textsubscript{i}, has been recognised by the RACI and WEHI; where he was awarded the Biota Award in 2009, the Burnet Prize in 2013 and the inaugural Sir John Dixon Hughes Medal for Medical Research Innovation from the NFMRI in 2014. This research has also afforded a number of licenced patents, and publications in high impact journals like Nature Chemical Biology.
Development of BCL-X\textsubscript{L} inhibitors: drug discovery and evaluation on cancer and normal cells

Guillaume Lessene

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Department of Medical Biology, The University of Melbourne, Melbourne, Australia  
Department of Pharmacology and Therapeutics, The University of Melbourne, Melbourne, Australia

Apoptosis, or programmed cell death, is a highly conserved biological process required for the removal of unwanted, damaged or infected cells. The central regulators of apoptotic programmed cell death belong to the BCL-2 family of proteins. A delicate interplay between members of this family that promote cell survival (e.g. BCL-2, BCL-X\textsubscript{L}, MCL-1) and those that induce cell death (e.g. BIM, BAD, BAX, BAK) dictates whether a cell will live or die. Down-regulation of apoptosis is thought to contribute to cancer and autoimmune diseases. Due to their central role in the regulation of apoptosis, the BCL-2-family proteins represent highly attractive targets both for chemical biology studies and drug discovery. ABT-199/Venetoclax, a potent and selective inhibitor of BCL-2 has reached the clinic for the treatment of chronic lymphocytic leukemia. Inhibitors of BCL-X\textsubscript{L} may present an equally promising application against solid tumours and against malignancies that have developed resistance to chemotherapies.

This presentation describes the discovery and development of the first potent and selective inhibitors of the pro-survival protein BCL-X\textsubscript{L} using a combination of classical medicinal chemistry strategies as well as structure-guided drug discovery. We show that these BCL-X\textsubscript{L} inhibitors induce apoptosis by specifically engaging BCL-X\textsubscript{L} in cells. We also show that these compounds potently inhibit the growth of tumour cells \textit{in vitro} and \textit{in vivo}. Furthermore, we demonstrate the impact of BCL-X\textsubscript{L} pharmacological inhibition on normal hematopoietic cells. Our data provides support for the use of BCL-X\textsubscript{L} inhibitors in the treatment of cancer to complement the current set of BH3-mimetics currently in the clinic.
ABSTRACTS, JULY 16 SESSION 4: KEYNOTE SPEAKER

Dr. Danuta Kalinowski

Differential Anti-Cancer Activity of Copper Bis(Thiosemicarbazones): Reactive Oxygen Species Generation and Lysosomal Membrane Permeabilisation

Molecular Pharmacology and Pathology Program, The Department of Pathology and Bosch Institute, The University of Sydney

Dr Kalinowski is currently appointed as a NHMRC RD Wright Fellow in the Department of Pathology at the University of Sydney. She obtained a B.Sc. in Chemistry from Macquarie University and was awarded her Ph.D. in November of 2007 at the University of Sydney. She has published a total of 78 peer-reviewed publications (H-index: 21; >1600 citations), including a book, book chapter, and a number of invited review articles.
Differential Anti-Cancer Activity of Copper Bis(Thiosemicarbazones): Reactive Oxygen Species Generation and Lysosomal Membrane Permeabilisation

Danuta S. Kalinowski, Christian Stefani, Zaynab Al-Eisawi, Patric J. Jansson, Des R. Richardson

Molecular Pharmacology and Pathology Program, The Department of Pathology and Bosch Institute, The University of Sydney

Bis(thiosemicarbazones) and their copper (Cu) complexes possess unique anti-neoplastic properties. However, their mechanism of action remains unclear. We examined the structure-activity relationships of twelve bis(thiosemicarbazones) to elucidate factors regarding their anti-cancer efficacy. Importantly, the alkyl substitutions at the diimine position of the ligand backbone resulted in two distinct groups, namely, unsubstituted/monosubstituted and disubstituted bis(thiosemicarbazones). This alkyl substitution pattern governed their: (1) Cu$^{II/III}$ redox potentials; (2) ability to induce cellular $^{64}$Cu release; (3) lipophilicity; and (4) anti-proliferative activity. The potent anti-cancer Cu complex of the unsubstituted bis(thiosemicarbazone) analog, glyoxal bis(4-methyl-3-thiosemicarbazone) (GTSM), generated intracellular reactive oxygen species (ROS), which was attenuated by Cu sequestration by a non-toxic Cu chelator, tetra(thiomolybdate), and the anti-oxidant, N-acetyl-L-cysteine. Fluorescence microscopy suggested that the anti-cancer activity of Cu(GTSM) was due, in part, to lysosomal membrane permeabilisation (LMP). For the first time, the role of LMP in the anti-cancer activity of bis(thiosemicarbazones) is demonstrated.
ABSTRACTS, JULY 16 SESSION 4: KEYNOTE SPEAKER

Dr. Emma Collinson

A role for Nox4 in TGF-beta-dependent EMT in the lens

The University of Sydney

Emma Collinson is a Lecturer in the Discipline of Anatomy and Histology and a member of the Bosch Institute. She trained in the UK, and undertook her PhD studies at the University of Manchester Institute of Science Technology (UMIST) under the direction of Professor Chris Grant. It was here that she developed an interest in oxidative stress and how cells respond to this stress in disease states. Her decision to pursue a career in academic science was due to the mentoring and support she received from Professor Grant during this period. Whilst undertaking her PhD she travelled to Australia in order to undertake microarray and deletion collection experiments under the direction of Professor Ian Dawes (UNSW). On completion of PhD, she completed post-doctoral training in the laboratories of Professor Ian Dawes (UNSW) and Professor Roland Stocker (University of Sydney). She was appointed Lecturer the Discipline of Anatomy and Histology in 2013 and has developed a fruitful collaboration with Professor Frank J Lovicu. She is particularly interested in the role of growth factor dependent reactive oxygen species (ROS) generation in the development of diseases of the lens and growth factor dependent reactive oxygen species (ROS) production in general.
A role for Nox4 in TGF-beta-dependent EMT in the lens

Collinson, Emma J¹; Das, Shannon J¹; Lovicu, Frank J.¹,²

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2. Save Sight Institute, Sydney, NSW, Australia.

TGF-β can induce an epithelial to mesenchymal transition (EMT) in lens, which arises from the aberrant growth and differentiation of lens epithelial cells. Studies in other models of EMT have shown that TGF-β-driven EMT is dependent on the expression of the reactive oxygen species (ROS) producing enzyme NADPH oxidase 4, Nox4. We investigated the role of this enzyme in TGF-β-induced lens EMT and determined whether it was required for this process. Here we demonstrate for the first time in rat lens epithelial explants, that TGF-β treatment induces both Nox4 expression and activity. Increased Nox4 expression was first detected at 6-8 hours following TGF-β treatment and was maintained in explants at 48 hours. At 8 hours post TGF-β treatment, Nox4 was observed in cell nuclei, while at later stages in the EMT process, at 48 hours, Nox4 was predominately co-localized with α-smooth muscle actin. The inhibition of Nox4 expression and activity using VAS2870, inhibited EMT progression, as determined by reducing cell loss, abolishing α-smooth muscle actin expression, as well as the abrogation of lens capsular wrinkling. TGF-β drives the expression of the ROS-producing enzyme Nox4 in the rat explant system. Nox4 contributes to the development of TGF-β-induced EMT, as Nox4 inhibition impairs the EMT process. Experimental findings here may facilitate the prevention of the development of posterior capsular opacification in patients undergoing cataract surgery.
Sukriti Krishan

Novel Thiosemicarbazones mediate their anti-cancer effects via the AMPK-dependent pathway

Sukriti Krishan, Sumit Sahni, Des R. Richardson

Molecular Pharmacology and Pathology Program, Department of Pathology and Bosch Institute, The University of Sydney

Due to rapid growth and division, cancer cells generally have higher demand for energy than their normal counterparts. Metals such as iron are known to play significant role in the energy-generating pathways (e.g., electron transport chain). AMP-activated protein kinase (AMPK) is a cellular energy sensor that regulates intracellular energy homeostasis. Hence we hypothesised that metals could play an important role in the regulation of the AMPK-dependent pathways. This study investigated the effects of the metal-ion chelator and potent anti-cancer agent, Dp44mT, on the AMPK-mediated energy homeostasis pathway. We observed that Dp44mT significantly ($p< 0.05$) activated the AMPK-dependent pathways. Studies with metal complexes of Dp44mT demonstrated that its effect on AMPK was due its ability to chelate metal ions and the generation of reactive oxygen species. We also observed that activation of AMPK by Dp44mT was mediated by an upstream kinase, namely liver kinase B1 (LKB1). Furthermore, Dp44mT was also shown to regulate acetyl CoA carboxylase 1 (ACC1), raptor, and unc 51-like kinase (ULK1), which are known regulators of fatty acid synthesis and autophagy, respectively. The regulation of ACC1 and ULK1 by Dp44mT was demonstrated to be due to its ability to activate AMPK. In conclusion, this study demonstrates metal-binding anti-cancer agents (such as Dp44mT) can target the AMPK-dependent energy homeostasis pathway and this can be one of the potential mechanisms through which they exert their anti-cancer activity.
Polyurethane medical implants modified by ion implantation

Irina Kondyurina, S Bao, A Kondyurin, M Bilek

The University of Sydney

Polyurethane is widely used in medicine for permanently implanted devices due to its elasticity, mechanical strength, biostability, biocompatibility and hemocompatibility. However, the immune system recognises polyurethane as foreign and initiates an immune response that can result in a range of negative consequences including foreign body rejection, inflammation, bacterial infection, pain and dysfunction of the implant. We use plasma immersion ion implantation for activation of the polyurethane surface to facilitate covalent binding of a biologically active protein layer and report on the results in application relevant assays.

A polyurethane composition with mechanical properties adjusted to soft tissue was developed to match the mechanical properties of the vasculature and ensure suitability for surgical suture. Plasma immersion ion implantation was performed to active the surface. Mechanical properties of the structures were characterised with tensile testing whilst the chemistry and morphology of the surfaces were characterised by AFM, FTIR and XPS spectroscopy. The activated surface was then used to covalently immobilise bioactive protein molecules directly from solution. The immobilised protein layers were characterised with ELISA. The effects on cell adhesion in-vitro and on cellular responses in a mouse model of the ion treated materials both with and without protein immobilised will be reported.
ABSTRACTS, JULY 17 SESSION 1: PLENARY SPEAKER

Professor Christopher Chitambar

Development of Iron-Mimetic Metallodrugs for the Treatment of Cancer: The journey from the laboratory to the clinic

Division of Hematology and Oncology, Department of Medicine (CRC, MY, JPW) and Department of Radiology (KMS), Medical College of Wisconsin, Milwaukee, Wisconsin, USA

Christopher R. Chitambar, MD, FACP, is Professor of Medicine in the Hematology and Oncology Division of the Medical College of Wisconsin, Milwaukee, Wisconsin, USA. He is the Breast Cancer Program Leader in the Froedtert and Medical College of Wisconsin Clinical Cancer Center and is the Chair for the Faculty Research Committee in Breast Cancer. He Chairs the Faculty Appointment and Promotions Committee in the Department of Medicine and is a member of the Brain Tumor Translational Research Program and the Free Radical Research Program. Dr. Chitambar obtained his medical degree from Christian Medical College in India and completed a residency in internal medicine at Brackenridge Hospital in Austin, Texas. He joined the faculty of the Medical College of Wisconsin after completing a clinical and research Fellowship in Hematology and Oncology at the University of Colorado Health Science Center in Denver, Colorado. His research focuses on the role of iron proteins in tumor biology with a particular emphasis on the development of novel metallodrugs as therapeutic agents. He has published extensively on the development of gallium metallodrugs as iron-targeting agents for cancer therapy. His clinical work focuses on breast cancer and clinical trials for this disease.
Development of Iron-Mimetic Metallodrugs for the Treatment of Cancer: The journey from the laboratory to the clinic

Christopher R. Chitambar, MD, Meiying Yang, PhD, Janine P. Wereley, BS, and Kathleen M. Schmainda, PhD

Division of Hematology and Oncology, Department of Medicine (CRC, MY, JPW) and Department of Radiology (KMS), Medical College of Wisconsin, Milwaukee, Wisconsin, USA

Cellular iron homeostasis is tightly regulated by the complex interplay of proteins involved in the cellular import, storage, and export of iron. Numerous processes essential for cell viability and function are iron-dependent. Malignant cells have a far greater requirement for iron than normal cells and may have altered expression of iron proteins. Thus, the role of iron in tumor biology provides an opportunity to perturb iron-dependent processes in cancer cells as a therapeutic strategy.

Our research has focused on the development of gallium compounds as iron mimetic metallodrugs; it has led to clinical trials with gallium nitrate. We have shown that transferrin receptor-targeting, perturbation of iron-dependent mitochondrial function, and blockade of the iron-dependent subunit of ribonucleotide reductase (RRM2) are important steps in the antineoplastic action of gallium compounds. Interestingly cytoprotective responses such as metallothionein (MT2A), heme oxygenase-1 (HO-1), and ferritin gene expression are also induced by gallium. MT2A and HO1 gene expression appear to be secondary the increase in mitochondrial production of reactive oxygen species (ROS) possibly through an action of gallium on complex II/succinate dehydrogenase.

Whereas the antineoplastic efficacy of gallium nitrate in lymphoma and bladder cancer has been demonstrated in preclinical and clinical studies, recent research has revealed a new direction for the antineoplastic activity of gallium compounds. Our preclinical studies in a rodent brain tumor model show that gallium maltolate inhibits the growth of glioblastoma multiforme (GBM), an aggressive brain tumor. Therapeutic options for GBM are limited since very few chemotherapeutic drugs can cross the blood brain barrier and enter the brain. The antineoplastic activity of gallium in GBM is thus of great clinical relevance. Approaches to enhance the efficacy of gallium metallodrugs through improved delivery systems are being explored. The development of gallium metallodrugs in cancer and their advancement to the clinic will be discussed.
ABSTRACTS, JULY 17 SESSION 1: KEYNOTE SPEAKER

Dr. Andrew Hoy

*Adipose triglyceride lipase controls MDA-MB-231 breast cancer cell fatty acid metabolism and influences progression*

Dr Hoy is the Helen and Robert Ellis Postdoctoral Research Fellow from the Sydney Medical School Foundation and Head of the Lipid Metabolism Laboratory in the Discipline of Physiology, School of Medical Sciences and Bosch Institute. He is also an Honorary Associate with the Boden Institute of Obesity, Nutrition, Exercise & Eating Disorders. Dr Hoy received his BSc (Biomedical Sc.) and MSc (Research) at the University of Wollongong, PhD training at the Garvan Institute of Medical Research within the Diabetes and Obesity Research Program, and Post-Doctoral training at Monash University as a NHMRC Biomedical Australia Training Fellow. He returned to Sydney in 2012 to establish his independent research laboratory which has interests in lipid metabolism and how it is perturbed in obesity, type 2 diabetes and cancer, including breast and prostate.
Adipose triglyceride lipase controls MDA-MB-231 breast cancer cell fatty acid metabolism and influences progression

Lisa S. Lee¹, Seher Balaban¹, Rob Shearer³, Michelle van Geldermalsen², Harrison Shtein¹, Jeff Holst², Darren N. Saunders³, and Andrew J. Hoy¹

¹ University of Sydney, ² Centenary Institute, ³ The Kinghorn Cancer Centre, Garvan Institute of Medical Research

Obesity has adverse clinical outcomes on breast cancer. At the cellular level, breast cancer cells have increased intracellular stores of fatty acids, known as lipid droplets. Interestingly, this lipid phenotype is independent of the oestrogen-receptor status, but associated with the aggressiveness, survival, as well as the proliferative and migratory capacities of breast cancer cells. Adipose triglyceride lipase (ATGL) is the major rate-limiting enzyme in the breakdown (lipolysis) of intracellular triglyceride stores. This study aimed to elucidate the role that intracellular lipid stores, regulated by ATGL, play in cancer progression.

Increasing concentrations of fatty acids in the media elevated intracellular triglyceride (TAG) levels and resulted in increased lipolysis and oxidation of intracellular fatty acids as well as protected against palmitate-induced apoptosis. Inducible ATGL knockdown in MDA-MB-231 cells increased TAG levels by reducing lipolysis and consequently, fatty acid oxidation. Conversely, inducible ATGL overexpression lowered TAG content by increasing lipolytic activity and fatty acid oxidation.

ATGL overexpression increased cell viability (MTT assay) but did not protect against palmitate-induced apoptosis. Interestingly, palmitate-induced apoptosis was exacerbated in ATGL knockdown cells pre-incubated with 300 µM fatty acids to increase TAG levels.

These findings demonstrate that endogenous lipid stores and intracellular FA metabolism are regulated by ATGL in MDA-MB-231 breast cancer cells. Furthermore, ATGL-mediated hydrolysis of endogenous lipid stores, and the associated changes to FA metabolism, may indeed influence breast cancer cell behaviour. Ongoing studies are investigating other aspects of cancer cell biology to further elucidate the link between intracellular lipid homeostasis and cancer biology.
Dr. Angelica Merlot

Induction of Endoplasmic Reticulum Stress using Thiosemicarbazon Dp44mT in Tumour Cells: Activation of PERK/eIF2α, IRE1α, ATF6 and Calmodulin Kinase

Molecular Pharmacology and Pathology Program, Department of Pathology and Bosch Institute, The University of Sydney

Dr. Angelica Merlot is a Postdoctoral Research Associate in the Pathology Department of the University of Sydney. She is currently working under the supervision of Prof Des Richardson in the Molecular Pharmacology and Pathology Program. Her passion for cancer research was sparked during her Honours and PhD degree, awarded in November 2013, where she investigated the pharmacological mechanisms of action and membrane transport of anti-cancer thiosemicarbazones. Angelica is interested in improving the targeting and pharmacological complications of cancer therapies that cause toxicity and drug resistance. Her current research focuses on the characterization of the endoplasmic reticulum stress pathways in cancer and modulating these signals to induce cancer cell death.
Induction of Endoplasmic Reticulum Stress using Thiosemicarbazone Dp44mT in Tumour Cells: Activation of PERK/eIF2α, IRE1α, ATF6 and Calmodulin Kinase


Molecular Pharmacology and Pathology Program, Department of Pathology and Bosch Institute, The University of Sydney

The endoplasmic reticulum (ER) plays major roles in the maturation and folding of proteins and Ca$^{2+}$ regulation. Cellular stresses lead to an overwhelming accumulation of mis-folded proteins in the ER termed ER stress, leading to the unfolded protein response (UPR). Due to the unfavourable conditions of the tumour microenvironment, UPR is generally activated in cancer cells promoting cancer cell survival. A novel strategy for anti-cancer drugs is to re-sensitise cancer cells to ER stress apoptosis by altering the kinetics of the UPR. The UPR can be manipulated by either reducing survival signals or increasing apoptotic signals. Di-2-pyridylketone 4,4-dimethyl-3-thiosemicarbazones (Dp44mT) overcomes multi-drug resistance and demonstrates potent \textit{in vitro} and \textit{in vivo} anti-cancer and anti-metastatic activity (PNAS 2006;103:14901-06; EMBO Mol Med 2012;4:93-108). However, the mechanisms involved in its activity including the role of UPR and ER stress remains largely uncharacterized. Our results show the cytotoxic agent Dp44mT, which forms redox-active metal complexes, significantly leads to: (1) increased activation of ER stress-associated pro-apoptotic signalling (\textit{i.e.}, p-eIF2α, ATF4 and CHOP); (2) increased phosphorylation of IRE1α and XBP1 mRNA splicing; (3) reduced expression of molecules involved in ER stress-associated cell survival signalling (\textit{e.g.}, XBP1s and p58IPK); (4) activation of the transcription factor, ATF6, and its downstream targets (\textit{i.e.} CHOP and BiP); and (5) activated phosphorylation of CaMKII that can induce apoptosis. In contrast to Dp44mT, desferoxamine, which forms redox-inactive iron complexes, and the classical ER stress-inducing agent, tunicamycin, had some and/or no effect on the expression/phosphorylation of these proteins. In conclusion, pro-apoptotic ER stress is induced by Dp44mT, suggesting that the modulation of ER stress responses could serve as a novel approach to ameliorate the treatment of cancer.
ABSTRACTS, JULY 17 SESSION 1: YOUNG INVESTIGATOR ORAL PRESENTATION

Leigh Nicholson

Alpha-Parvin, Focal Adhesions and Metastasis Models

Leigh Nicholson, Laura A Lindsay, Christopher R. Murphy

Department of Anatomy and Histology, The University of Sydney

Our study aims to provide a novel model for studying quantifiable differences in a cell's ability to metastasize by looking at the focal-adhesion associated protein, alpha-parvin. This is the first study to look at this protein in the uterus. The phosphorylation of alpha-parvin has been shown to be associated with cell movement and focal adhesion (FA) disassembly in both normal and cancerous cells. By studying the localization of alpha-parvin and its phosphorylation during early pregnancy in rats, we have been able to research the role that this protein plays in adhesion disassembly, as during the time of implantation these adhesive complexes in the epithelium lining the lumen disassemble to facilitate blastocyst attachment. By determining the role and quantifiable differences exhibited by alpha-parvin during this time, we hope to provide a model for metastasis potential and biomarkers in endometrial cancer, which is being studied concurrently with this project. Using immunohistochemical and western blotting techniques, we looked at the amount of alpha-parvin during early pregnancy. Alpha-parvin is present at the time of fertilization, which shows its association as a FA protein. At the time of implantation, when the FA complexes are disassembled, alpha-parvin is significantly decreased. We also showed that phosphorylated alpha-parvin has an inverse relationship, in that it is significantly increased at implantation suggesting its role in FA disassembly. From additional data, we preliminarily suggest an association with MAPK/Erk1, a protein which phosphorylates alpha-parvin. We show for the first time that alpha-parvin is phosphorylated prior to FA disassembly during early pregnancy and that this phosphorylation can be potentially used as a quantificational tool for cell movement due to its association with adhesive complex loss.
ORL1 and µ-opioid receptor agonists as therapeutics for chronic pain

Nehan. R. Munasinghe, and Macdonald. J. Christie

Discipline of Pharmacology, The University of Sydney

Pain is sensed in peripheral nociceptors which transmit the signal through primary sensory neurons in the dorsal root ganglion (DRG) up the spinal cord to higher brain centres. Within these DRG neurons there are a range of voltage gated calcium (CaV) channels. These channels are regulated through G-protein coupled receptors linked to opioid like receptor 1 (ORL1) and µ-opioid receptors. Both µ-opioid and ORL1 receptors have been long implicated in pain pathways. As calcium is important for the regulation of excitability in DRG neurons, a reduction in calcium influx would help decrease the hyperexcitability of sensory neurons in chronic pain states. Recently, a combined ORL1 and µ-opioid agonist named Cebranopadol was discovered. It was effective in multiple models of chronic pain with no major side effects. The current project explores a similar compound to cebranopadol identified as [Dmt1]N/OFQ(1-13)-NH₂. In order to determine changes in CaV channel kinetics, whole cell patch clamp electrophysiology was conducted on acutely isolated DRG neurons. Types of DRG neurons were discriminated based on presence of neuropeptides and cell size. Initial results show that the activation of ORL1 or µ-opioid receptors by a selective agonist cause a decrease in CaV channel currents of both peptidergic and non peptidergic DRG neurons of wild type rats. Further study will be conducted to determine the potency of [Dmt1]N/OFQ(1-13)-NH₂ in DRG neurons isolated from rats that undergo sciatic nerve ligation to develop chronic pain.
ABSTRACTS, JULY 17 SESSION 2: PLENARY SPEAKER

Professor Judith Clements

Determination of novel KLK7-regulated molecular pathways in ovarian cancer

Distinguished Professor Judith Clements AC
Australian Prostate Cancer Research Centre-Queensland, Institute of Health & Biomedical Innovation, Queensland University of Technology (QUT) at the Translational Research Institute, Queensland, Australia

Distinguished Professor Judith Clements is a prostate and ovarian cancer researcher in the Institute of Health and Biomedical Innovation, QUT based at the Translational Research Institute on the Princess Alexandra Hospital Biomedical Precinct. She is also Scientific Director of the Australian Prostate Cancer Research Centre-Queensland located on this campus. The focus of her research is the role of the prostate-specific antigen (PSA) (the current test for prostate cancer)-related enzymes and their utility as biomarkers or therapeutic targets for prostate and ovarian cancer. She helped establish, and since 2005, has directed the Australian Prostate Cancer BioResource, the national prostate cancer tissue bank which is a key resource that underpins prostate cancer research nationally. She is also co-leader of the Queensland node of the international genetic consortium for prostate cancer, PRACTICAL, which is another key consortium that has discovered 100 new genetic regions that are associated with cancer risk. She is Chair of the Queensland Board of the Prostate Cancer Foundation of Australia (PCFA) and a member of the PCFA National Board. She is one of three elected academic representatives currently serving on QUT Council. She was awarded the Queensland Women in Technology Biotech Outstanding Achievement Award for 2012, and the prestigious title of Distinguished Professor, the only woman in QUT’s history, in 2013. She was awarded the Companion of the Order of Australia in 2015.
Determination of novel KLK7-regulated molecular pathways in ovarian cancer

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Kallikrein-related peptidase 7 (KLK7) is a serine protease that is highly expressed in high grade serous ovarian cancer (HGSOC), particularly the proliferative/C5/Stem-A molecular subtype. It is also associated with various aggressive features of progression such as increased invasion and migration \textit{in vitro} and \textit{in vivo} tumour growth and peritoneal metastases. To further understand the KLK7 directed molecular pathways involved in these processes, we have used an unbiased proteomic and transcriptomic analysis approach. KLK7 protease targets were identified using two platforms that screen for novel N-termini generated by the test protease (Terminal Amine Isotopic Labelling of Substrates/TAILS) platform, and a decrease in molecular weight of newly cleaved products on SDS PAGE (PROtein TOpography and Migration Analysis Platform/PROTOMAP). For transcriptome analysis, HGSOC cell lines, grown in 3D-suspension to mimic the ascites microenvironment of patients, were treated with active KLK7 and RNA-Seq performed followed by edgeR quantification, relative to controls.

Eighteen putative novel KLK7 substrates were identified by both the TAILS and PROTOMAP platforms in HGSOC cell secretions along with one established target (fibronectin), whose detection served to validate our proteomics approach. These included the collagens (COL12A1, COL5A1), fibrillin 1, matrix metalloproteases (MMP1, MMP10), and thrombospondin 1, all of which are involved in matrix degrading processes required for peritoneal invasion and metastasis. KLK7-treated HGSOC cell lines expressed elevated transcript levels many of which were similar to that of the ascites isolated patient cells. Interestingly, levels of several genes that modulate inflammation, such as interleukin 1\(\beta\) (IL1\(\beta\)), interleukin 6 (IL6) and vascular endothelial growth factor A (VEGFA) were elevated significantly by KLK7 treatment. These data suggest that KLK7 in HGSOC mediates peritoneal metastasis directly or indirectly via the above cleaved substrates, and potentially angiogenesis by inducing an inflammatory reaction.
ABSTRACTS, JULY 17 SESSION 2: KEYNOTE SPEAKER

Dr. Sumit Sahni

*N-Myc Downregulated Gene 1 (Ndrg1) Suppresses Stress-Induced Autophagy In Cancer Cells*

Molecular Pharmacology and Pathology Program, Department of Pathology and Bosch Institute, Blackburn Building (D06), University of Sydney, Sydney, New South Wales, 2006 Australia.

Dr Sahni did his PhD in Medicinal Chemistry from University of Illinois at Chicago. He then moved to Prof. Des Richardson’s lab at the University of Sydney to undertake his postdoctoral research. Since his PhD, he has focused his research on understanding the mechanisms via which metastatic suppressor protein NDRG1 exert its effects.
N-Myc Downregulated Gene 1 (Ndrg1) Suppresses Stress-Induced Autophagy
In Cancer Cells

Sumit Sahni\textsuperscript{1}, Dong-Hun Bae\textsuperscript{1}, Danuta S. Kalinowski\textsuperscript{1}, Patric J. Jansson\textsuperscript{1}, Des R. Richardson\textsuperscript{1}

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\textit{N-myc downstream regulated gene 1 (NDRG1)} is a well established stress response gene. Autophagy, which is also involved in stress response, is an evolutionary conserved pro-survival pathway, which involves lysosomal degradation of damaged molecules and mis-folded proteins. Autophagy is also known to be regulated via PERK/eIF2\textalpha mediated endoplasmic reticulum (ER) stress pathway. In the current study, we investigated the role of the \textit{NDRG1} gene in stress-induced autophagy. As iron chelators are known to induce stress and up-regulate NDRG1, we studied their effect on ER stress response and autophagy in pancreatic cancer cells. First, we compared the effect of the redox-inactive iron chelator, desferoxamine (DFO; 250 \(\mu\)M), to our novel redox-active iron chelator, Dp44mT (5 \(\mu\)M). We observed that both these ligands were effective in inducing ER stress as shown by activation of the PERK/eIF2\textalpha pathway (increased levels of p-PERK or p-eIF2\textalpha). Interestingly, we observed a concomitant increase in the levels of the autophagic marker, LC3-II, which was much more marked with redox-active Dp44mT compared to DFO. Furthermore, these studies also demonstrated that Dp44mT was an effective inducer of autophagic initiation. Importantly, we showed that NDRG1 over-expression inhibits ER stress via the PERK/eIF2\textalpha pathway, which results in suppression of Dp44mT-induced autophagic initiation. Notably, NDRG1 over-expression led to increased susceptibility of the cells towards stress induced apoptosis, potentially due to the ability of NDRG1 to suppress pro-survival autophagic pathway. Collectively, this investigation demonstrates that NDRG1 is a major regulator of ER stress-induced autophagy, and hence, plays an important role in the cellular stress response. The observed suppression of the pro-survival autophagic pathway by NDRG1 in presence of stress stimulus (iron depletion and redox stress) will aid in further elucidating the mechanisms involved in the anti-metastatic effects of this protein.
Dr. Dinny Graham

Remodelling the genomic landscape: the role of the progesterone receptor, its cofactors and DNA structure in progesterone response in the normal and malignant breast.

Centre for Cancer Research, The Westmead Millennium Institute for Medical Research, The University of Sydney

Dinny Graham completed her PhD on mechanisms of transcriptional regulation by progesterone, at the University of Sydney, before travelling to the United States as a NHMRC CJ Martin postdoctoral fellow to complete a postdoctoral fellowship at the University of Colorado, Denver, in the laboratory of Professor Kathryn Horwitz. There she investigated the role of nuclear receptor coregulators in endocrine resistance. Dr Graham joined Professor Christine Clarke in the Breast Cancer Group at the Centre for Cancer Research in 2001. She leads the molecular genomics program in the group, examining molecular mechanisms of nuclear receptor action. Her research focuses on mechanisms of cell specific hormone action in the breast, and how the altered nuclear environment of cancer cells leads to aberrant genomic interactions and altered transcriptional outcomes.
Remodelling the genomic landscape: the role of the progesterone receptor, its cofactors and DNA structure in progesterone response in the normal and malignant breast.

J Dinny Graham, Audrey Silvestri, Nicole Santucci, Heidi N Hilton, Christine L Clarke

Centre for Cancer Research, The Westmead Millennium Institute for Medical Research, The University of Sydney

The ovarian hormone progesterone plays a key role in regulating a diverse range of female reproductive functions, which include development of lobular alveolar structures of the breast, decidualization of the endometrium, maintenance of bone density and regulation of inflammatory and immune functions. Large randomized trials of exposure to progesterone analogues in hormone replacement therapy have established its role in increasing breast cancer risk, yet the mechanisms that confine this adverse influence to the breast, and likely underlie its diverse tissue specificity, are incompletely understood. Progesterone effects are mediated via the nuclear progesterone receptor (PR), which binds to specific sequences in genomic DNA to regulate transcription of target genes. Ligand activation of PR results in nuclear repositioning into transcriptional hotspots, which are dependent on PR interactions with both the nuclear matrix and chromatin for their fidelity. In the disrupted nuclear environment of breast cancer, aberrant PR nuclear localisation is observed, and results in an altered PR-regulated transcriptional programme compared to non-malignant breast. PR is expressed as two isoforms, PRA and PRB, which act as both heterodimers and homodimers. There is considerable in vitro and in vivo evidence that PRA and PRB have distinct transcriptional activities. In normal tissues the isoforms are equivalently expressed, but their expression is disrupted resulting in predominance of one form in breast cancer and an aberrant progesterone response. We explored PR genomic interactions genome wide to understand the mechanisms underlying altered transcriptional responses to progesterone in cancer cells. We discovered striking differences between PR binding patterns in normal and malignant cells, and this was reflected in non-overlapping transcriptional responses to progesterone. Progesterone regulated overlapping but different transcriptomes with PRA and PRB and this was mirrored by the cistromes of the two isoforms. Analysis of PR cistromes revealed that cell type-specific chromatin structure, regulated by specific pioneer factors, is a critical driver of PR genomic interaction patterns.
**Targeting Zinc Finger E-Box Binding Homeobox 1 (ZEB1) to Reverse Metastasis via N-Myc Downstream Regulated Gene-1 (NDRG1)**

Leyla Fouani, Zaklina Kovacevic and Des R. Richardson

*Molecular Pharmacology and Pathology Program, Discipline of Pathology, and Bosch Institute, The University of Sydney*

Metastasis is the least effectively treated aspect of cancer, making it the most lethal and feared prospect. Unfortunately, metastasis is a likely outcome of pancreatic cancer. The epithelial to mesenchymal transition (EMT), is a process known to facilitate metastasis. The focus of the current study was to examine the capacity of the metastasis suppressor gene, *N-myc downstream regulated gene 1 (NDRG1)*, to reverse this process through its effects on an epithelial gene repressor, zinc finger E-box binding homeobox 1 (ZEB1). Here, we report that NDRG1 can negatively regulate this latter protein through attenuating oncogenic signalling pathways, namely, transforming growth factor beta (TGF-β) and nuclear factor kappa B (NF-κB).

This study examined how NDRG1 is able to attenuate canonical NF-κB signalling through inhibiting: (i) the IκB kinase (Iκκ) complex and its activation; (ii) the phosphorylation and subsequent degradation of the inhibitory IκBα protein bound to the NF-κB complex; and (iii) the nuclear expression of down-stream molecules, such as, claudin-1 and ZEB1.

Importantly, the advantageous effects of NDRG1 can be up-regulated using a novel class of anti-cancer agents, namely the thiosemicarbazones, Dp44mT and DpC. Interestingly, these agents were shown to potently attenuate NF-κB signalling and were also found to reduce ZEB1 levels in cancer cells. The effect of these agents on NF-κB signaling could be important to consider in terms of their marked anti-tumour activity.
L-proline-mediated neurogenesis using mouse embryonic stem cells

Rachel Shparberg, Timothy Mason, Michael Morris

Discipline of Physiology, and Bosch Institute, The University of Sydney

To build a functional nervous system, cells of the embryo must differentiate into four distinct cell populations before becoming terminally-differentiated neurons. However, the molecular mechanisms by which these populations arise are still poorly understood due to the complexity of *in vivo* neurogenesis. Previous data from our lab has shown that when cultured in the presence of the amino acid L-proline, mESC recapitulate neural lineage development, but do so heterogeneously. We therefore aim to optimize this protocol to drive the *sequential* and *homogenous* differentiation of mESC to neural cells, via populations similar to those that arise during embryonic development.

In this work, D3 and 46C-Sox1-GFP mESC were cultured as embryoid bodies (EBs) for 9 days using concentration and time-dependent additions of L-proline and nodal (mesendoderm) inhibitor (SB431542). EBs were seeded in serum-free conditions from day 9 and allowed to differentiate for a further 6 days, after which they were assessed for the presence of neural cells. By day 15, a significant increase in the number of neural EBs was observed in samples cultured in L-proline (~65%) compared to basal medium controls (~10%), as confirmed by the expression of the neural markers Nestin, BLBP and NeuN. qPCR indicated sequential differentiation of the L-proline-treated EBs through primitive ectoderm (*Dnmt3b* + and *Fgf5* +), definitive ectoderm (*Pard6b* +) and neurectoderm (*Sox1* +) intermediates. By day 8, 80% of cells cultured in L-proline expressed Sox1-GFP, as determined by flow cytometry. Fluorescence time-lapse imaging indicated that this expression was first evident in the outer neurepithelium of the EBs and switched off in more mature neural cell types. Taken together, we have developed an *in vitro* model of embryonic neurogenesis using the unique growth factor-like properties of L-proline. Using this protocol, we can begin to investigate the molecular mechanisms driving early neural development.
Zinc mediates breast cancer progression through the epithelial to mesenchymal transition

Centre for Cellular and Molecular Biology, Deakin University, Victoria Australia

Prof Ackland holds a personal chair in Molecular Biosciences at Deakin University where she is the Director of the Centre for Cellular and Molecular Biology. Her research has focused on the role of trace metals, in particular zinc, in human health and disease. Her team developed a novel in vitro cell culture model that mimics the human mammary epithelium for investigating the epithelial to mesenchymal transition in relation to breast cancer metastasis. Prof Ackland is a member of the Australian Society for Medical Research, the Cancer Council Victoria, Medical and Scientific Committee and was a founding member of TEMTIA, the Epithelial to Mesenchyme Transition International Association. She is on the editorial board of several journals and has been a member of the organising committee of international metal-related conferences including the International Society for Zinc Biology 2014. Prof Ackland established the Chinese-Australian Consortium for Environmental and Human Health and the Chinese-Australian Joint Research Centre for Environmental Remediation and Health, to foster Australia-China research collaborations in 2008. In 2014, Prof Ackland participated in the Ernst Strungmann Forum in Frankfurt, Germany as a member of a team of scientists selected to address the most important global issues in relation to metals, including metal deficiencies and toxicities.
Zinc mediates breast cancer progression through the epithelial to mesenchymal transition

ML Ackland  
Centre for Cellular and Molecular Biology, Deakin University, Victoria Australia  
Kathryn M Taylor: Department of Biosciences, Cardiff University, Wales, UK  
Christer Hogstrand: Nutritional Sciences Division, King’s College, London, UK  
Agnes A Michalczyk: Centre for Cellular and Molecular Biology, Deakin University, Victoria, Australia

Zinc is an essential trace element with multiple biochemical roles. Zinc homeostasis is regulated by cellular zinc transporters. A zinc transporter gene, Zip 6 was previously found to be essential for zebrafish gastrulation, through a process termed the epithelial to mesenchymal transition (EMT)\(^1\). Genes involved in normal developmental processes are candidate mediators of tumour progression. High levels of Zip6 were associated with metastasis of breast cancer to the lymph nodes. We investigated the role of Zip6 in EMT in cultured human breast cancer cells PMC42-LA. Zip6 transcripts were upregulated 25-fold in PMC42-LA cells undergoing epidermal growth factor-induced EMT\(^2\). STAT3 and Snail, signaling molecules that are linked to EMT and constitutively activate in breast cancer, were increased by 5-6 fold. Zip 6 was expressed as a pro-protein that was N-terminally cleaved and relocated to the plasma membrane, driven by STAT. In cells transfected with Zip6, STAT3 pSer\(^{727}\) was decreased and STAT3 pTyr\(^{705}\) was increased. Zip6 transfected cells had increased G2/M-phase and increased migration. Recombinant and endogenous Zip6 was enriched in non-adherent cells. We propose that EMT in breast cancer cells can be mediated through a zinc-signaling pathway where plasma membrane Zip6 causes an influx of zinc that inactivates GSK3b and activates Snail. Thus Zip6/zinc signaling may play a significant role in breast cancer metastasis.

References
ABSTRACTS, JULY 17 SESSION 3: KEYNOTE SPEAKER

Dr. Delphine Denoyer

Investigating copper aberrations in prostate cancer: from basic science to therapeutic enquiry.

Centre for Cellular and Molecular Biology, School of Life and Environmental Sciences, Deakin University, Burwood, Victoria, Australia.
Department of Pathology, University of Melbourne, Parkville, Victoria, Australia

Dr Delphine Denoyer is a research fellow in the Centre for Cellular and Molecular Biology at Deakin University. She obtained her PhD in Biomedical Engineering in France in 2004, where she investigated the mechanisms of uptake of new radiopharmaceuticals in cancer cells. After being awarded a prestigious fellowship from NHMRC/INSERM (Australia/France research cooperation program), she relocated to Australia in 2007 to pursue her interest in preclinical validation of radiopharmaceuticals for imaging and therapy of cancers at the Peter MacCallum Cancer Centre, Melbourne. Recently, her research focus moved to the metallomics field where she uses her expertise in translational research to investigate the role of copper in the development and growth of prostate cancers.
Investigating copper aberrations in prostate cancer: from basic science to therapeutic enquiry

Delphine Denoyer\(^1\), Sharnel A.S. Clatworthy and Michael Cater\(^1,2\)

\(^1\)Centre for Cellular and Molecular Biology, School of Life and Environmental Sciences, Deakin University, Burwood, Victoria, Australia
\(^2\)Department of Pathology, University of Melbourne, Parkville, Victoria, Australia

Elevated copper in both malignant tissue and serum has emerged as universal features of many cancer types\(^1\). We are building upon our discovery that copper-ionophores can selectively target and destroy cancerous prostate cells in vitro and significantly reduce prostate cancer burden in an orthotopic mouse model\(^2\). Ionophores transport specific metals into cells often allowing them to become bioavailable. In addition to further developing copper-ionophores as therapeutics, we aim to understand the cellular mechanisms and pathways leading to copper aberrations and ionophore sensitivity in cancer cells. Furthermore, we have recently demonstrated that a small subset of prostate cancer patients may be suitable for treatments and imaging techniques (PET) that target elevated intratumoral copper\(^3\). We will discuss the implications for developing prostate cancer therapies that target copper and our recent advances in our understanding of the role of copper-transporters in prostate cancer progression.

ABSTRACTS, JULY 17 SESSION 3: KEYNOTE SPEAKER

Dr. Parvin Ataie

The developing story of Monepantel in cancer treatment

Cancer research laboratories, Department of surgery, St. George Hospital, Kogarah, NSW, Australia.

Dr Parvin Ataie-Kachoie is Post-Doctoral Research Associate in Surgery Lab, Cancer Research Laboratories as well as an Honorary Associate Position with Department of Surgery St. George Hospital. She received her PhD training at UNSW within Faculty of Medicine Research Program. Her PhD research involved the pharmacological evaluation of the anticancer potential of Minocycline which led to the discovery of the mechanism of action of Minocycline in ovarian cancer. Following this she is doing her Post-Doctoral training in the laboratory of Prof Morris at St. George Hospital. Her current research interest is basic and pre-clinical oncology and in particular drug development.
The developing story of Monepantel in cancer treatment


Cancer research laboratories, Department of surgery, St. George Hospital, Kogarah, NSW, Australia

Monepantel (MPL) is a new nematode-specific anthelmintic agent with proven safety in rodents and mammals. We have evaluated MPL potential as an anticancer agent for the first time. MPL showed cytotoxic effect on a wide range of human tumor cell lines, including those resistant to standard therapy. It inhibits growth, proliferation and colony formation, arrests cells at G1 and induces PARP-1 cleavage. Mechanism exploration revealed that MPL triggers autophagy through deactivation of mTOR/p70S6K signaling. MPL also showed single agent in vivo efficacy in various xenograft models in mice. Potent anti-tumor activity could be documented in models from different origin like ovarian, colorectal and pancreatic cancer. Treatment with MPL resulted in inhibition of intratumoral p-mTOR and its down-stream targets p-P70S6K and p-4E-BP1 protein levels. As emerging data indicate that mTOR inhibitors are most effective when combined with other target agents, we next evaluated whether MPL could favorably be combined with the clinically-approved chemotherapeutics to improve therapeutic efficacy. In vitro, MPL in combination with a panel of chemotherapeutics synergistically reduced survival rates of malignant cells from different origins. The in vivo efficacy of MPL was also significantly enhanced when combined with several chemotherapeutics. These pharmacology data provided the rationale for the initiation of clinical development of MPL. A first-in-human, phase 1, dose-escalation study of MPL is ongoing to investigate the safety, tolerability, pharmacokinetics, and antitumor activity of MPL in patients with advanced cancer. In preliminary safety analyses, MPL was well-tolerated at doses examined to date with no adverse events reported so far. Moreover, the inhibition of p-P70S6K and p-4E-BP1 levels was observed in blood cells of MPL-treated patients indicating the potential clinical efficacy of MPL. In conclusion, the preclinical data combined with emerging clinical safety warrants further evaluation of MPL as a potent anticancer agent with first-in-class potential to inhibit mTOR/p70S6K/p-4E-BP1 pathway.
Sam Merlin

*Deletion of Ten-m3 induces the formation of eye dominance domains in mice*

A key characteristic of the visual system is the precise retinotopic organisation of each eye, and the exquisite binocular matching of the two eyes. Ten-m3, a member of the Ten-m/Odz/Teneurin family, regulates axonal guidance in the retinogeniculate pathway. Deletion of Ten-m3 results in altered arrangement of the ipsilateral projection to the dorsal lateral geniculate nucleus, leading to a mismatch in retinotopic alignment. Here, we demonstrate the impact that this geniculate mismatch has on primary visual cortex. Transneuronal tracing and c-fos immunohistochemistry demonstrate that the geniculate mismatch is conveyed to visual cortex, and that the mismatched ipsilateral and contralateral projections segregate into distinct regions. This segregation is confirmed by intrinsic optical imaging. Single-unit electrophysiology shows that cortical cells can receive inputs from vastly different receptive fields, and that binocular stimulation leads to functional suppression in these cells. These results indicate that the misalignment of inputs from the two eyes are capable of inducing the formation of novel eye dominance regions, and can potentially lead to impaired visual function through suppression of activity in the visual cortex.
Michael Lovelace

**P2X7 Receptors mediate innate phagocytosis by human neural precursor cells and neuroblasts**

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During early human neurogenesis there is overproduction of neuroblasts and neurons accompanied by widespread programmed cell death (PCD). Whilst it is understood that CD68+ microglia and astrocytes mediate target-dependent PCD and subsequent phagocytosis that takes place much later in embryonic development, little is known of the cell identity or the scavenger molecules utilized to remove apoptotic corpses during the earliest stages of human neurogenesis. Recently, studies have found that the P2X7 receptor can serve as a scavenger receptor for apoptotic debris in the absence of extracellular ATP or serum, both of which inhibit this function¹. As the early developing human central nervous system (CNS) does not contain microglia or astrocytes, and has an established blood-brain barrier which prevents the extravasation of serum proteins across into the brain parenchyma, we hypothesized that the P2X7 receptor could play a role in removing the corpses of dying cells during neurodevelopment.

Using a combination of multiple-marker immunohistochemical staining, functional blocking antibodies and antagonists, we showed that human neural precursor cells (hNPCs) and neuroblasts express functional P2X7 purinoceptors. Utilising live-cell imaging, flow cytometry, phagocytic assays and siRNA knockdown, we showed that in a serum free environment doublecortin⁺ (DCX) neuroblasts and hNPCs can clear apoptotic cells by innate phagocytosis mediated via P2X7. We found that both P2X7⁺⁻DCX⁺⁻ hNPCs and P2X7⁺⁺DCX⁺⁺ neuroblasts, derived from primary cultures of human foetal telencephalon, phagocytosed targets including latex beads, apoptotic RenCels and apoptotic hNPC/neuroblasts. Pretreatment of neuroblasts and hNPCs with ATP, OxA TP (P2X7 antagonist) or siRNA knockdown of P2X7 inhibited phagocytosis of these targets. Our exciting results² show that P2X7 functions as a scavenger receptor under serum-free conditions resembling those in early neurogenesis. This is the first suggestion that hNPCs and neuroblasts may participate in clearance of apoptotic corpses during pre target-dependent neurogenesis and mediate phagocytosis using P2X7 as a scavenger receptor. The data support the novel concept that hNPCs and neuroblasts may regulate their own numbers by phagocytosis in early CNS development.

**References:**

ABSTRACTS, BOSCH YOUNG INVESTIGATORS THREE MINUTE THESIS COMPETITION

Sam Adamson
Dark Rearing As A Means Of Mimicking ‘Physiological Hypoxia’: A Non-Invasive Intervention For Retinopathy Of Prematurity (ROP)

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Partial genetic deletion of neuregulin 1 modulates the effects of chronic stress on dendritic morphology in adolescent mice

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Miranda Mathews
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Diana Shinko
Investigation of the Immune complexity in regulating clinical outcomes in cancer
Dark Rearing As A Means Of Mimicking ‘Physiological Hypoxia’: A Non-Invasive Intervention For Retinopathy Of Prematurity (ROP)

Samuel J Adamson, Rita Maccarone, Mark Koina, Jennifer Lau, Riccardo Natoli, Nigel Barnett, Silvia Bisti, Jan Provis, Robert A. Linsenmeier, Tailoi Chan-Ling

University of Sydney

At birth, the lungs of premature infants lack surfactants, and as a result are less effective at supplying the oxygen needs of the infant. As a consequence, premature infants are treated with oxygen to protect the developing brain from damage. The initiating event in the pathogenesis of ROP is when the infant is placed in the high oxygen tension required to protect the brain from damage. However, because the choroid has a limited autoregulatory ability, the inner retina becomes flooded with oxygen. These higher-than-normal levels of oxygen result in a down-regulation of hypoxia-induced vascular endothelial growth factor (VEGF), the stimulus for normal retinal vascular formation. Reduction in VEGF expression reduces the density and rate of formation of the retinal vasculature, such that when infants are no longer requiring oxygen supplementation for their vital function, the formation of retinal vasculature is mismatched to that required by the metabolic demand of the neurons, resulting in tissue hypoxia and pathological vaso-proliferation.

Our rationale behind dark rearing (DR) is that total darkness results in continuous depolarization of the photoreceptors, which requires an energy (oxygen) intensive repolarisation. As the energy consumption in the tissue would thus be doubled, so would the oxygen consumption and this induced “physiological hypoxia” would restore VEGF levels and normal blood vessel development. We suggest that DR precludes the initiation of ROP, offering a viable, noninvasive treatment for prevention of ROP and that DR could serve to supplement other strategies to minimise the damaging effect of retinopathy of prematurity. This is timely, given the push for clinical adoption of anti-VEGF therapy for ROP, where possible systemic effects are not fully investigated.
Partial genetic deletion of neuregulin 1 modulates the effects of chronic stress on dendritic morphology in adolescent mice

David J Clarke, Tariq Chohan, Mustafa S Kassem, Sandra Y Fok, Maxwell R Bennett, and Jonathon C Arnold

Discipline of Pharmacology, Brain and Mind Research Institute, and Bosch Institute, University of Sydney

Neuregulin 1 (Nrg1) is a neurotrophic factor and a schizophrenia susceptibility gene. Dendritic spine atrophy has been observed in the schizophrenia brain. Chronic restraint stress promotes alterations in dendritic morphology. We aimed to examine whether partial genetic deletion of Nrg1 modulated stress-induced changes in dendritic morphology. Adolescent wild-type (WT) and neuregulin heterozygous (HET) mice underwent 6 h of restraint stress per day for a total of 21 days. 24 h following the last restraint stress session the mice were sacrificed and the brains extracted for Golgi staining and analysis. Chronic restraint stress during adolescence promoted opposing effects on dendritic spine density in the prelimbic cortex, with a stress-induced increase in HET mice and a decrease in WT mice. Stress-induced reductions in dendritic complexity in the prelimbic cortex were more pronounced in HET mice relative to WT mice. Stress promoted greater dendritic spine atrophy in HET than WT mice in the CA1 region of the hippocampus. HET mice appeared to confer resilience to stress-induced dendritic spine atrophy in the infralimbic cortex, stressed WT mice displayed reduced dendritic spine density compared to controls, whereas no significant changes were observed between stressed and non-stressed HET mice. Partial genetic deletion of Nrg1 alone promoted dendritic spine density reductions in the CA3 region of the hippocampus. The results confirm that Nrg1 deficiency during adolescence modulates the effects of stress on dendritic morphology.
ABSTRACTS, BOSCH YOUNG INVESTIGATORS THREE MINUTE THESIS COMPETITION

Investigating mechanisms of chemoresistance to platinum drugs mediated by systemic inflammation

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Lung, colorectal and head and neck cancers are among the most common cancers worldwide and are often treated with platinum based drugs including cisplatin, carboplatin and oxaliplatin. However, cancer patients that develop chemoresistance to platinum drugs have poor response to chemotherapy. Chemoresistance is often caused by changes to drug transporter expression on tumours, ultimately causing reduced intracellular accumulation of drug in tumours and hence chemoresistance. Mechanisms behind this remain unclear but our lab has previously linked elevated systemic inflammation in cancer patients to altered platinum pharmacokinetics (drug distribution and metabolism), poorer response and reduced overall survival. This study aims to investigate how inflammation alters platinum drug transport and efficacy in vitro to explain platinum pharmacokinetics changes observed clinically. Changes in gene and protein expression of platinum drug transporters will be measured in human cancer cell lines in the presence of pro-inflammatory cytokines or conditioned media using qRT-PCR and Western Blots. Intracellular platinum uptake and platinum-DNA adduct formation will be analysed using GF-AAS. Additionally, changes in platinum cytotoxicity and apoptosis in the presence of inflammation will be assessed. Preliminary results suggest cytokines decrease gene expression of influx transporters and increase gene expression of efflux transporters. This altered expression may ultimately cause decreased drug accumulation in cancer cells resulting in chemoresistance.
ABSTRACTS, BOSCH YOUNG INVESTIGATORS THREE MINUTE THESIS COMPETITION

VAMP2 and Syntaxin 3 coordinate vesicle machinery in uterine epithelial cells during early pregnancy in the rat

Sadaf Kalam, Laura Lindsay and Christopher Murphy

Discipline of Anatomy and Histology, and Bosch Institute, University of Sydney

Uterine epithelial cells undergo extensive morphological and molecular remodelling to prepare for implantation; these changes are collectively termed ‘the plasma membrane transformation’. These changes are likely mediated by vesicular trafficking and indeed there is a large increase in the number of apical vesicles as well as an increase in vesicular activity at the time of receptivity.

This study examined the role of VAMP2 and Syntaxin 3 in the uterus during early pregnancy. Vesicle associated membrane protein 2 (VAMP2) is known to travel in vesicle membranes that constitutively fuse with the plasma membrane. Syntaxin 3 is a crucial protein involved in the delivery of proteins from the trans-golgi network to the apical surface of polarized epithelia.

Uterine tissues were collected from pregnant rats during early pregnancy for immunofluorescence and uterine epithelial cells were isolated for western blot analysis.

Immunofluorescence microscopy at the time of fertilisation (non-receptive) has demonstrated that VAMP2 and Syntaxin 3 are diffusely distributed throughout the cytoplasm of uterine epithelial cells. At the initial stage of implantation (apposition), VAMP2 remains diffused throughout the cytoplasm with granular staining in the perinuclear region. During adhesion, VAMP2 becomes restricted to the cytoplasm region above the nucleus but below the localisation of Syntaxin 3, which is found immediately below the apical plasma membrane. Western blot analysis of isolated uterine epithelial cells reveals an overall increase in the amount of VAMP2 and Syntaxin 3 from the non-receptive phase to the time of implantation.

This increase in VAMP2 and Syntaxin 3 as well as the more confined localisation at the apical cytoplasmic region of uterine epithelial cells suggests that these proteins are involved in vesicle regulation. This may play a role in maintaining directional vesicle traffic to the apical plasma membrane at the time of uterine receptivity.
ABSTRACTS, BOSCH YOUNG INVESTIGATORS THREE MINUTE THESIS COMPETITION

Understanding the Mechanisms of Near-Infrared Light-Induced Neuroprotection

Ji Yeon Kim, John Mitrofanis, Thomas Ashhurst, Jonathan Stone and Daniel Johnstone

Discipline of Physiology, and Bosch Institute, University of Sydney, University of Queensland

Neurodegenerative diseases are increasing in prevalence as the population ages, however currently available therapies only target symptoms, and do not prevent, slow, halt or reverse disease progression. The efficacy of near-infrared light therapy (NIR; \( \lambda = 600-1070 \text{nm} \)) as a neuroprotectant in various animal models of neurodegeneration has been well established. It is safe, convenient and noninvasive, with minimal side effects - an ideal therapeutic intervention. However, the mechanisms by which NIR improves neuronal survival are unclear. It is thought to directly stimulate mitochondrial ATP production, and may also trigger a systemic response by stimulating immune cells or bone marrow-derived mesenchymal stem cells (MSCs) to migrate to damaged sites, as well as altering gene expression to upregulate cell survival and repair mechanisms.

My project aims to clarify the mechanisms of NIR-induced neuroprotection, using both \textit{in vitro} studies on the SH-SY5Y human neuroblastoma cell line and \textit{in vivo} studies on the MPTP-induced mouse model of Parkinson’s disease. The neurotoxin MPTP is converted \textit{in vivo} to MPP+, which reproduces Parkinson’s disease pathology by killing the dopaminergic cells of the substantia nigra. A flow cytometry panel identifying MSCs has been developed, and will be used to assess whether NIR causes recruitment of MSCs in MPTP-treated mice. I will also determine how NIR affects gene expression, by analyzing microarray data from the brains of mice treated with MPTP and NIR, then confirming findings with protein studies. \textit{In vitro} experiments on SH-SY5Y cells have successfully established a dose-response relationship between MPP+ concentration and cell viability. This model will be used to determine optimal NIR doses for neuroprotection, followed by gene expression studies as above. In this way, I hope to clarify the cellular and molecular mechanisms underlying NIR-induced neuroprotection. This may help pave the way for clinical trials, and lead to future development of more targeted therapies.
ABSTRACTS, BOSCH YOUNG INVESTIGATORS THREE MINUTE THESIS COMPETITION

Diet-microbe interactions and their role in brain gut communication and feeding behaviour

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School of Molecular and Microbial Biosciences, and Bosch Institute, University of Sydney

The prevalence of obesity has rapidly increased with two thirds of Australians classified as overweight or obese. The cause of this epidemic is multifactorial, though it is widely accepted that genetics, overconsumption of nutrient poor, energy dense food and decreased energy expenditure are all key contributors. Many studies have focused on the impact of an imbalanced diet on body systems including immune function, endocrine response and the gut microbiota. However, the resulting impact of these systems on the brain and feeding behaviour, are not well known. Furthermore, the impact of diet on the gut microbiota is well established however the direct and indirect roles of microbes and their metabolites in brain gut communication requires further study. This study investigated the role of the diet-microbe interaction on brain gut axis communication outcomes for feeding behaviour. Male, C57Bl/6 mice were fed either a Standard Chow (SCD), High Fat (HFD) or High Sucrose (HSD) diet for three or six months. Mice on a HFD exhibited typical obesity-related morbidities such as weight gain, systemic inflammation, glucose intolerance and fatty degeneration in the liver. When mice were placed on a food choice experiment, SCD fed mice had a greater ability to adjust their nutrient intake to optimal caloric and macronutrient targets whereas HFD and HSD fed mice had greater caloric intake and a fixed macronutrient intake which was suboptimal. Changes in hypothalamic nuclei involved in feeding were also identified between diet groups. Lastly, preliminary analysis of the gut microbial community showed differences between diet groups, with decreased diversity in HFD fed mice. Ultimately, our whole body approach provides novel insights into the role of the gut microbes in brain gut communication and how these systems are interconnected.
ABSTRACTS, BOSCH YOUNG INVESTIGATORS THREE MINUTE THESIS COMPETITION

Jenga: Building the blocks of balance

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Discipline of Biomedical Science, Discipline of Anatomy and Histology, Bosch Institute, University of Sydney, and Department Biochemistry and Molecular Biophysics, Howard Hughes Medical Institute, Columbia University, New York

Despite the importance of our sense of balance we still know little about the central control of the peripheral balance system. While previous work has shown that activation of the efferent vestibular system results in modulation of afferent vestibular neuron discharge, the intrinsic and synaptic properties of efferent neurons themselves are largely unknown. Here we substantiate the location of the efferent vestibular nucleus (EVN) in the mouse, before characterizing the input and output properties of EVN neurons in vitro. We made transverse serial sections through the brainstem of 4-week-old mice, and performed immunohistochemistry for calcitonin gene-related peptide (CGRP) and choline acetyltransferase (ChAT), both expressed in the EVN of other species. We also injected fluorogold into the posterior canal and retrogradely labelled neurons in the EVN of Chat::tdTomato mice expressing tdTomato in all cholinergic neurons. As expected the EVN lies dorsolateral to the genu of the facial nerve (CNVII). We then made whole-cell current- and voltage-clamp recordings from visually identified EVN neurons. In current-clamp, EVN neurons display a homogeneous discharge pattern. This is characterized by a high frequency burst of action potentials at the onset of a depolarizing stimulus and the offset of a hyperpolarizing stimulus that is mediated by T-type calcium channels. In voltage-clamp, EVN neurons receive either exclusively excitatory or inhibitory inputs, or a combination of both. Despite this heterogeneous mixture of inputs, we show that synaptic inputs onto EVN neurons are predominantly excitatory. Together these findings suggest that the inputs onto EVN neurons, and more specifically the origin of these inputs may underlie EVN neuron function.

Investigation of the Immune complexity in regulating clinical outcomes in cancer

Diana Shinko

Discipline of Pharmacology, Bosch Institute, University of Sydney

A large variability in response is found in individuals undergoing chemotherapy as a treatment for cancer. Studies have shown that inflammation reduces the overall survival rate in cancer patients. However, the underlying mechanism of how inflammation reduces overall survival is unknown. This study aims to identify the relationships between changes in inflammation and immune cell phenotypes/signalling responses during chemotherapy and clinical outcomes in cancer patients. Blood is collected from patients with stage III or IV cancer and undergoing chemotherapy. The study utilises a new technique called Mass Cytometry, using a Cytometry by Time-of-Flight (CyTOF®), to measure immune parameters in the blood as an alternative to Flow Cytometry. Mass Cytometry allows for an increased number of parameters (up to 100) used in an assay. This technique will allow us to view a more complete immune profile which allows us to identify changes in immunophenotype and function in patients undergoing chemotherapy as well as identify immune parameters to predict chemotherapy response. Furthermore, the study will enable us to identify possible new drug targets that could help improve the chemotherapy response in cancer patients.
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Bosch Institute Annual Scientific & Young Investigator’s Meeting – 16th and 17th July 2015
Chronic Neuropathic Pain: it's all about the rhythm


1 Department of Anatomy and Histology, University of Sydney, Australia, 2006
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Background and Aims: Trigeminal neuropathic pain (TNP) is an orofacial chronic pain condition characterised by sharp and shooting pain in the branches of the trigeminal nerve. TNP is thought to reflect changes to the pattern of activity within the thalamocortical (TC) circuitry. Thus, we hypothesised that the pattern of activity in TC circuitry is altered in TNP in the ventral posteromedial thalamus (VPM), the somatosensory thalamic reticular nucleus (TRN) and the somatosensory cortex. Further, we hypothesised changes in the pattern of activity at the spinotrigeminal nucleus (SpV) in TNP. Methods: Functional magnetic resonance imaging (fMRI) was used to measure resting brain activity in 16 TNP patients and 44 healthy controls. A grid comprised of 3x3x3 volumes of interest (VOI) was created within the thalamus to assess ongoing signal intensity fluctuations. A frequency versus power domain was created for each VOI. Two sample t-tests were used to determine significant differences between groups for the spectra in each thalamic VOI. Results revealed changes in the pattern of activity in 16 TNP patients and 44 healthy controls. A grid comprised of 3x3x3 volumes of interest (VOI) was created within the thalamus to assess ongoing signal intensity fluctuations. A frequency versus power domain was created for each VOI. Two sample t-tests were used to determine significant differences between groups for the spectra in each thalamic VOI. Results revealed changes in the pattern of activity in 16 TNP patients and 44 healthy controls. A grid comprised of 3x3x3 volumes of interest (VOI) was created within the thalamus to assess ongoing signal intensity fluctuations. A frequency versus power domain was created for each VOI. Two sample t-tests were used to determine significant differences between groups for the spectra in each thalamic VOI. 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Results: Significant power differences between patients and controls occurred within the rostral thalamus, specifically contralateral to the side of ongoing pain, including VPM and TRN. Whole-brain, voxel-by-voxel spectral analysis confirmed the thalamic grid analysis. In addition, the analysis revealed greater power in TNP subjects in the ipsilateral SpV within the frequency range of 0.03–0.06 Hz. Conclusions: These findings show that TNP is associated with changes in the pattern of TC activity, specifically affecting the thalamus contralateral to the ongoing pain within a discrete frequency band. Furthermore, this change in activity likely results from altered neural modulation by astrocytes and may underlie the experience of chronic pain.
ABSTRACTS, BOSCH YOUNG INVESTIGATORS POSTER PRESENTATIONS

A Role For Adipocyte Lipolysis In Breast Cancer Progression

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Obesity is defined as excessive accumulation of adipose tissue and is associated with many chronic diseases, such as cardiovascular disease and type 2 diabetes. Obesity is associated with reduced survival and increased recurrence of breast cancer. The majority of breast tissue is made up of highly dynamic adipocytes, which may be key players in promoting breast progression within the mammary microenvironment through delivering critical metabolic substrates and mediating paracrine signalling – both of which are altered in obesity.

The aim of this project was to investigate how breast cancer cells mobilise metabolic substrates (focusing on free fatty acids) from surrounding ‘lean’ and ‘obese’ adipocytes and determine the reciprocal effects of ‘lean’ and ‘obese’ adipocyte lipolysis on breast cancer (BrCa) growth.

We observed that free fatty acid release from 3T3-L1 adipocytes was enhanced by co-culturing with either MCF-7 (ER+) or MDA-MB-231 (ER-) BrCa cells. Furthermore, the rate of transfer of fatty acids from ‘obese’ adipocytes to BrCa cells was increased compared to ‘lean’ by two-fold. Interestingly, siRNA knockdown of the primary triglyceride lipase ATGL in adipocytes resulted in decreased fatty acids transfer to MCF-7 or MDA-MB-231 cells by one and two fold, respectively. We observed that ‘lean’ adipocytes promoted enhanced confluence in both BrCa cell lines, increased cell cycle progression in MCF-7 cells as well as migration rate in MDA-MB-231 cells. Confluence and migrations rates of MDA-MB-231 cells were much greater when co-cultured with ‘obese’ adipocytes. These changes are associated with altered fatty acid as well as glucose and glutamine metabolism in both MCF-7 and MDA-MB-231 cells when co-cultured with ‘obese’ adipocytes.

These data indicate that adipocyte lipolysis, which is stimulated by BrCa cells, provides fatty acids to BrCa cells, alters lipid partitioning and increases confluency and cellular migration. Future studies aim to further characterise this paracrine signalling, especially in obesity, to identify potential therapeutic strategies targeting tumour metabolism.
ABSTRACTS, BOSCH YOUNG INVESTIGATORS POSTER PRESENTATIONS

**Influence of the MuSK system in the mdx mouse model of muscular dystrophy**

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Duchenne muscular dystrophy (DMD) is a severe X chromosome-linked myopathy caused by mutations in dystrophin gene. The absence of dystrophin expression in DMD skeletal muscles caused the continuous degeneration and insufficient regeneration of muscle fibres, leading to progressive muscle weakness. Eccentric contractions (EC) occur when active muscles are stretched and such contractions are thought to exacerbate muscle fiber degeneration. *Mdx* mice have a null mutation in their dystrophin gene and are widely used as an experimental model of DMD. When subjected to EC, muscles of *mdx* mice showed a greater loss of strength and more muscle fiber damage, compare to wild type mice. *Mdx* muscles showed deficiencies in expressing muscle specific kinase (MUSK), a receptor tyrosine kinase that is essential in maintaining healthy neuromuscular junctions. Recently, Dr Sofie Trajanovska has tested the impact of transgenic MUSK-GFP delivered into *mdx* muscles via adeno-associated viral vector (rAAV). Her work suggested that, supplementing MUSK expression can reduce the acute EC damage in *mdx* muscles. Downhill running exercise represents a more physiological model for inducing EC damage. My experiments will test the effect of downhill running on muscle fiber damage of *mdx* mice and potential of MUSK-GFP to protect against such damage. I will also test whether voluntary wheel running is protective for *mdx* muscles. In my experiment, MUSK-EGFP and empty vector (control) will be injected into right and left tibialis anterior (TA) muscles of *mdx* mouse respectively. After allowing 3 weeks of expression, I will subject mice to 45 minutes of downhill treadmill running. Mice will be sacrificed 24 hours later, and transverse sections of their TA muscles will studied histologically to measure the degree of muscle damage between experimental and sedentary *mdx* mice.
Oxytocin induces up regulation of key enzymes involves cholesterol biosynthesis in PC-3 and DU-145 prostate cancer cell lines

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Prostate cancer is the most commonly occurring cancer in men and the second leading cause of cancer related deaths. Oxytocin, a nonapeptide of the neurohypophyseal hormone family, has differential effects on the proliferation of prostate cancer cell lines. Previous studies have shown that oxytocin regulates androgen levels by conversion of testosterone to dihydrotestosterone by 5-alpha reductase. This differential effect of oxytocin on prostate cancer is thought to depend on localization of oxytocin receptors (OXTR) inside and outside of caveolin rich invaginations known as caveolae. To investigate these differential effects of oxytocin induced gene expression in prostate cancer cell lines, we performed transcriptome analysis of androgen independent prostate PC-3 (without caveolae), and DU-145 (with caveolae) cancer cell lines treated with or without oxytocin. In PC-3 and DU-145, oxytocin was shown to increase the expression of three key enzymes involved in the steroid biosynthesis pathway including: dehydrocholesterol reductase (DHCR7), acetyl-CoA acetyltransferase 2 (ACAT) and hydroxysteroid 17-beta dehydrogenase 7 (HSD17B7). We also found an up regulation of major genes involved in cholesterol biosynthesis by oxytocin irrespective of caveolae presence. Of these, HSD17B7 is of interest as it is not only involved in post squalene cholesterol biosynthesis, converting zymosterone to zymosterol, but also acts as a reductase and converts androstenedione to testosterone in the prostate, and oxidizes estrone to estradiol in the breast. We will further investigate the significance of hydroxysteroid 17-beta dehydrogenase 7 as a proposed target of oxytocin antagonist treatment of steroid dependent cancers.
ABSTRACTS, BOSCH YOUNG INVESTIGATORS POSTER PRESENTATIONS

Assessing Mouse Cognition In The Intellicage

Thomas J. Burton & Atomu Sawatari

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Goal-Directed Learning and Executive Functions allow organisms to develop, apply, maintain and alter appropriate patterns of behaviour in a dynamic world and ultimately underlie informed decision making. Much is still unknown about the mechanisms that control complex and integrative higher order functions in the brain. Advancing our knowledge in this field is not only of fundamental interest to basic neuropsychology but is vital for the development of effective therapies for the debilitating cognitive symptoms associated with neuropsychiatric disorders (such as schizophrenia and Alzheimer’s disease). Developing reliable and species-relevant behavioural tests is essential for improving our understanding of healthy and disordered neural systems.

We have developed novel and fully automated methods for investigating the cognitive functions of *mus musculus* in the IntelliCage - a more naturalistic social setting for this species. Group housed adult male C57BL/6 mice were required to learn one of either two tasks for access to water in this home cage setting: a Visual cue-Dependent [VD] task or a Response-Dependent [RD] task. Once an animal reached acquisition criterion (performance of >80% correct for 2 consecutive sessions in 2 designated operant corners), the task contingencies were changed in either one of two ways: an Extradimensional Shift (EDS; between-task switch) or a Rule Reversal (RR; within-task change).

While strong goal-directed learning was observed in both discriminatory tests, animals required significantly fewer sessions to acquire the RD task compared to the VD task (p<0.0001). Moreover, all EDS and RR manipulations caused performance disturbances that were consistent with theoretical expected performances after such task changes. The automated approach in more naturalistic setting allowed for a novel and highly detailed examination of the evolution of choice behaviour during all phases of learning and adaptation to contingency changes.
The angiogenic potential of VEGF111 on microvascular endothelial cells

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Vascular endothelial growth factor A (VEGF-A) is a secreted glycoprotein that binds selectively to endothelial cells (ECs) to induce angiogenesis. The recently discovered VEGF₁₁₁, lacks the sites of proteolytic cleavage and extracellular matrix (ECM) binding, resulting in high angiogenic activity compared to other VEGF isoforms. The only known inducer of VEGF₁₁₁ in humans is DNA damage, and thus VEGF₁₁₁ may play a role in tumour angiogenesis. This work aims to determine the effects of VEGF₁₁₁ on microvascular ECs compared to the naturally produced isoforms.

In vitro studies involve primary human brain microvascular ECs stimulated by VEGF₁₁₁, VEGF₁₆₅ and VEGF₁₂₁. ECs are stimulated to invade an ECM gel and the network of tube-like structures is imaged and measured (length of the vessels, numbers of branch points). As angiogenesis is a major step in cancer development and metastasis, we applied the same approach to study the effects of cancer cells and the growth factors they produce. ECs were treated with conditioned media from breast cancer cells (MCF-7) with high metastatic potential and lung cancer cells (A549) with low metastatic potential.

In vivo studies used angioreactors, containing a matrix mixed with testing or control agents implanted into the dorsal flank of nude BALB/c mice. After invasion of the matrix by vessels, the angioreactors were removed on day 17, and the number of ECs measured.

In vitro, VEGF₁₁₁ is capable of forming a denser network of capillaries compared to other tested isoforms. In vivo, VEGF₁₁₁ triggers a higher response than VEGF₁₆₅ or PBS but lower than a combination of VEGF/FGF. Analysis of ECs treated with conditioned media indicates both cell lines secrete pro-angiogenic growth factors, however no difference was found.

So far our results show that VEGF₁₁₁ has a higher pro-angiogenic potential than the other isoforms and this warrants further investigations into its mechanisms of action.
E-cadherin and desmoglein-2 changes to the uterine epithelial cells during pregnancy in the domestic cat and the fat tailed dunnart

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The uterine luminal epithelium is the first site of contact between foetal and maternal tissues and so must undergo specialised changes for successful implantation to occur. These changes, collectively termed the ‘plasma membrane transformation’ (PMT), occur regardless of the placentation type. There are similarities in morphological and molecular changes in viviparous species during the PMT. Within eutherian species (e.g. pig, rat, and rabbit) the pre-implantation period is characterised by the loss or reduction of microvilli on the uterine epithelial cells leaving a smooth, flat surface for blastocyst attachment. Similar changes occur within a marsupial, the fat-tailed dunnart, Sminthopsis crassicaudata. Changes during pregnancy to the adhesion junctions such as desmosomes and molecules such as cadherin are common among mammals. We characterised the epithelial changes that occur in the fat tailed dunnart as well as uterine epithelium from pregnant and non-pregnant domestic cats (Felis catus). Immunofluorescence microscopy, was used to compare uterine remodeling during pregnancy. Similar changes to the cellular ultrastructure and molecular mechanisms allowing for implantation occur in both species with minimally invasive placentation (endotheliochorial). This supports the hypothesis that mechanisms allowing for successful pregnancy are conserved among mammals during the early stages of pregnancy.

Preconditioning with the indirect application of near infrared light as a potential neuroprotective intervention

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With life expectancy set to increase, the prevalence of age-related diseases, such as neurodegenerative disease is on the rise. Neurodegenerative diseases, such as Alzheimer’s disease (AD) and Parkinson’s disease (PD), manifest as neurons shrink and disappear, thereby compromising neuronal synapses and connections. Current treatments for neurodegenerative disease are only symptomatic; they do not prevent, slow, stop or reverse degeneration. A potential therapeutic intervention that may address this is near infrared light (nIR) therapy. Transcranial application of nIR has been shown to be neuroprotective in small animal models. However, in humans and larger animal models, it fails to penetrate through to deeper brain structures where it is needed to treat neurodegenerative diseases, like Parkinson’s. An alternate approach is the indirect application of nIR by targeting the limbs or dorsum, which has been shown to also be neuroprotective in preliminary studies. This project will utilise the MPTP model of Parkinsonism to investigate preconditioning with the indirect application of nIR, optimise its parameters and identify the underlying molecular mediators involved in neuroprotection.
Building Brains with Amino Acids: Understanding the mechanism of embryonic stem cell neural differentiation using L-Proline

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Mouse embryonic stem cells (ESCs) can be differentiated towards neurectoderm in the presence of exogenously added L-Proline. This amino acid acts as a novel growth factor stimulating differentiation to neural progenitors via embryologically relevant intermediate cell types. Initially, culturing ESCs with 400 µM L-Proline and 330 U/mL Leukaemia Inhibitory Factor (LIF) allows differentiation into a pluripotent population of early primitive ectoderm-like (EPL) cells. These cells are analogous to the in vivo primitive ectoderm in both gene expression and functional capacity. Further differentiation with L-Proline in the absence of LIF produces successive changes in gene expression consistent with the production of definitive ectoderm, then neurectoderm and, finally, mature neural cell types.

We aim to understand the L-Proline-mediated signalling activity underpinning ESC differentiation, with a focus on the ESC to EPL cells transition. Gene expression analysis using qPCR confirmed that ESCs converted to EPL cells. Protein analysis by western blot shows that serum-starved ESC and EPL cells were responsive to L-Proline. However, in standard culture medium, the basal activity of these pathways did not differ between ESC and EPL cells. Instead, basal pathway activity changes upon further differentiation to multipotent cell types. Inhibitors of the MAPK, AKT, mTORC and FGF pathways revealed that these pathways control specific aspects of cellular function, including as differentiation, proliferation, apoptosis and morphology changes.

L-Proline mediates the conversion of ESCs to EPL cells through activation of a number of signalling pathways. Blocking these pathways prevents this conversion. Studying these pathways will allow us to understand early embryogenesis and develop homogenous cell types for use in disease modelling.
Integration of multiple computational models of bioassays improve \textit{in vivo} rodent carcinogenicity prediction

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\textit{In vitro} genotoxicity bioassays are cost-efficient methods of assessing potential carcinogens. However, many genotoxicity bioassays produce a substantial number of false positive results to non-carcinogens, risking the future of potential new therapeutic agents. Additionally, genotoxicity bioassays are inappropriate for detecting chemicals eliciting non-genotoxic carcinogenicity mechanisms such as tumor promotion and progression, necessitating the usage of \textit{in vivo} rodent carcinogenicity (IVRC) assays. While \textit{in vitro} and \textit{in vivo} bioassay batteries are currently utilised to address these issues, they are limited by cost, time and throughput. Consequently, \textit{in silico} models of bioassays, featuring equivalent predictive performance and expedient result output, are hypothesised to supplement current batteries. In this study, we developed computational QSAR models of novel bioassays, followed by statistical integration alongside the \textit{in vitro}-Ames bioassay and ToxTree SAR models to predict IVRC results ($n=822$). Bioassays were selected on data-abundance and \textit{in vitro} performance, resulting in the selection of the Ames ($n=6512$), Syrian Hamster Embryonic (SHE, $n=410$) and GreenScreen GADD45a-GFP ($n=601$) bioassays. QSAR model performance was assessed by accuracy (A) and ROC AUC from 10-fold cross-validation, resulting in 80.96%, 0.876 AUC; 82.56%, 0.893 AUC; 62.84%, 0.684 AUC for the Ames, SHE and GreenScreen models, respectively, followed by IVRC prediction results of 58.94%, 0.635 AUC, 65.8213%, 0.489 AUC and 60.63%, 0.562 AUC. Logistic regression was used to integrate IVRC prediction results from QSAR models, alongside \textit{in vitro}-Ames (A=66.35%) and SAR models (Ames [A=63.29%], genotoxicity [A=57.66%] and non-genotoxic carcinogenicity [A=15.57%]), producing an integrated model, which then predicted IVRC with 73.11% accuracy and 0.765 AUC. This study demonstrates an integrated approach with computational models is evidently more predictive of \textit{in vivo} rodent carcinogenicity than individual models alone and shows great merit in carcinogenicity prediction, leveraging the speed, scale and cost advantages of computational methodologies to enhance \textit{in vitro} bioassay performance.
Clusters of damaged neuronal terminals (dystrophic neurites) form around ruptured capillaries in Alzheimer’s disease.

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Growing evidence suggests that vascular pathology may be important in the pathogenesis of Alzheimer’s disease (AD). Cerebrovascular abnormalities are common with increasing age and have been associated with cognitive impairment in AD. Previously, we showed that all amyloid-containing plaques are found at sites of capillary damage. These plaques colocalise with haemorrhage markers and inflammatory cells. We now have substantial evidence that senile plaques are, in fact, microhaemorrhages of 100-200µm. Linking the neuritic degeneration to these microstrokes would provide a unifying hypothesis for the vascular aetiology of the hallmark AD lesions. This study was performed to correlate the location of neuritic plaques (clusters of hyperphosphorylated tau dystrophic neurites) to the microvascular network in post mortem brain tissue from AD and neurologically normal patients. Blocks of formalin fixed human brain tissue were obtained from the Australian Brain Bank Network. Cases were chosen with a range of neuritic pathology using the Braak and Braak staging criteria ranging from stages 0 to VI. Blocks of hippocampus, superior frontal and cingulate cortices were cryosectioned at 50-200µm and fluorescently labeled for tau (AT8) and collagen IV (for blood vessels). Entire neuritic plaques contained within the depth of the section were optically sectioned and examined for the presence of a microvessel. We find that neuritic plaques encircle capillaries in all stages of disease frequently appearing around collapsed vessels and near capillary branches.

This study provides evidence that the clinically significant neuritic lesions of AD form at sites of microhaemorrhage, which strengthens the notion, that vascular breakdown is the proximal cause of AD.
ABSTRACTS, BOSCH YOUNG INVESTIGATORS POSTER PRESENTATIONS

Cholesterol Regulates Syntaxin 6 Trafficking at trans-Golgi Network Endosomal Boundaries for cancer cell migration

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Inhibition of cholesterol export from late endosomes causes cellular cholesterol imbalance, including cholesterol depletion in the trans-Golgi network (TGN). Here, using Chinese hamster ovary (CHO) Niemann-Pick type C1 (NPC1) mutant cell lines and human NPC1 mutant fibroblasts, we show that altered cholesterol levels at the TGN/endosome boundaries trigger Syntaxin 6 (Stx6) accumulation into VAMP3, transferrin, and Rab11-positive recycling endosomes (REs). This increases Stx6/VAMP3 interaction and interferes with the recycling of αVβ3 and α5β1 integrins and cell migration, possibly in a Stx6-dependent manner. In NPC1 mutant cells, restoration of cholesterol levels in the TGN, but not inhibition of VAMP3, restores the steady-state localization of Stx6 in the TGN. Furthermore, elevation of RE cholesterol is associated with increased amounts of Stx6 in RE. We also characterized the location and activity of Focal Adhesion Kinase (FAK) and Src, key players in the regulation of focal adhesions at the plasma membrane, which are necessary for directional cellular migration. NPC1 mutant cell lines show fewer focal adhesions, while restoration of cholesterol level in the TGN through LDL incubation increases the number and size of focal adhesions. Furthermore, multi-scratch wound healing assays with EGFR overexpressing A431 cancer cells with intrinsic cholesterol imbalances similar to CHO NPC1 mutants, show strongly reduced activation of FAK and inhibited migration. This correlates with an increased phosphorylation of the inhibitory tyrosine residue 527 on Src, which is known to be regulated by FAK activation. Hence, the fine-tuning of cholesterol levels at the TGN-RE boundaries together with a subset of cholesterol-sensitive SNARE proteins may play a regulatory role in cell migration and invasion.
STAT1-independent, STAT2-dependent non-canonical type I interferon signaling in the host response against viral infection

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Type I interferons (IFN-Is) have a crucial role in both host defense against viral infections and modulation of innate and adaptive immune responses. However, there are an increasing number of reports that IFN-I can be detrimental in various pathological conditions. We have previously found that following systemic infection with lymphocytic choriomeningitis virus (LCMV), STAT1 KO but not WT, IFNAR KO, STAT1/IFNAR DKO, STAT2 KO or STAT1/STAT2 DKO mice develop lethal IFN-I driven, STAT2-dependent, CD4+ T-cell-mediated disease characterized by a ‘cytokine storm’ and severe immune pathology in peripheral organs. This study aimed to determine the underlying molecular mechanisms of this lethal disease. Following infection, significantly higher levels of LCMV nucleoprotein mRNA were observed in peripheral organs of all the KO genotypes of mice compared with the WT counterparts. However, IFN-b levels were significantly higher in the serum of LCMV-infected STAT1 KO mice compared with WT, STAT1/IFNAR DKO and STAT1/STAT2 DKO mice. The cytokine levels were elevated to an even greater degree in LCMV-infected IFNAR KO mice. IFN-g levels were similar between all genotypes of mice on day 7 post-infection. Moreover, STAT1 phosphorylation was observed in peripheral organs of LCMV-infected WT and IFNAR KO mice, while STAT2 phosphorylation was found in STAT1 KO mice, but not in WT, IFNAR KO or STAT1/IFNAR DKO mice. LCMV-infected STAT1 KO mice also showed greater STAT3 phosphorylation than WT and STAT1/IFNAR DKO mice. These studies showed that STAT1-independent, STAT2-dependent non-canonical IFN-I signaling during LCMV infection: 1) was insufficient to limit viral replication and spread, 2) positively regulated the production of IFN-b, and 3) resulted in activation of downstream signaling molecules, STAT2 and STAT3.
Additive (alcohol) Drug on Neural Stem Cells: Identification of Growth Regulatory Metabolic Pathways through Proteome Analysis

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Background: Neural stem cells (NSCs) attract biologists and clinicians alike because of their potential in the development of treatments and the understanding of disease. Various chemicals, such as alcohol and antipsychotics agents, and mental disorders, such as schizophrenia, have been shown to influence neurogenesis through changes in the activities of NSCs, leading to alterations of brain plasticity and cognitive functions. The molecular mechanisms underlying the above factors on NSC activity in particular growth and metabolism, are largely unknown. Methods: NSCs were treated with 0, 25, 50 and 100 mM of ethanol (Eoth) for 96 h. Cell growth and protein expression (only 0 and 50 mM) were studied by immunohistochemical and proteomics techniques respectively. Proteins were identified with the MASCOT search engine (http://www.matrixscience.com/) and validated expression through western blotting. Results: NSC growth was significantly inhibited by 50 mM of Eoth. Proteome analysis was performed 0 and 50 mM of Eoth treated cells. Total 30 protein spots were altered by ethanol relative to control. Interestingly, few nuclear proteins such as nucleophosmin (B-23), heterogeneous nuclear ribonucleoprotein and dead end homology-1 are detected that have involved in chromatin modification and microRNA biogenesis process. Identified proteins have been classified in cytoskeletal, metabolism, signal transduction, oxidative stress and apoptosis classes. Conclusion: The inhibition of the growth of NSC might be due to activation of oxidative stress and apoptosis metabolic pathways.
A role for EphA7 in the formation of the ipsilateral retinal projection

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EphA7 is expressed in a number of places in the brain development. Recently, the expression of EphA7 was shown to decrease in knockout (KO) mice which lack the gene encoding the trans-membrane protein tenerurin-m3 (Ten-m3). This molecule has been shown to play an important role in the formation of ipsilateral retinal ganglion cell (RGC) projections to brain centres. This indicates that EphA7 might have functional associations with Ten-m3, and could have important roles in regulating optic nerve development. The preliminary results of the anterograde tracing showed a decrease in the size of the ipsilateral retinal projections to the dorsal lateral geniculate nucleus (dLGN) in EphA7 KO mice (n=6). In addition, an additional cluster of the RGCs were also observed in the ipsilateral region of the dLGN and the superior colliculus (SC) in EphA7 KO mice (n=2). The results suggested EphA7 has an association with the ipsilateral retinal projection pathway.

What’s in a NuRD?

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The Nucluesome Remodeling and Deacetylase (NuRD) complex is a co-regulator complex found in all higher organisms and has essential roles in development. Possessing nucleosome remodelling and deacetylase activities, it has been shown to be involved in both the repression and activation of genes, as well as DNA repair. Thus, it is unsurprising that the NuRD complex has strong links to cancer biology.

Although it is known that the NuRD complex is made up of ~10 protein subunits, namely CHD3/4, HDAC1/2, MTA1/2/3, RBBP4/7, p66α/β and MBD2/3, information on its exact subunit stoichiometry, structure and biochemical functional is sorely lacking. Given both its biological significance and its widespread distribution throughout the animal and plant kingdoms, knowledge of NuRD structure and function will provide broad insight into mechanisms of eukaryotic gene regulation.

To this end, we have undertaken a program of research to probe the structure of the NuRD complex and substantial progress has been made using single particle electron microscopy (EM), mass spectrometry (MS)-based quantitation and crosslinking mass spectrometry. Our goal is to combine the MS data with EM-derived structural envelopes to derive the first glimpses of the structure of a mammalian chromatin remodelling complex.

Currently, we have a preliminary EM-derived envelope and have identified 30+ inter-molecular NuRD crosslinks and many more intra-crosslinks. In addition, our MS-based quantification has begun to give us an insight into the stoichiometry of the complex. Progress towards our goal will be described in this presentation.
Replication stress induces the prolonged mitotic arrest telomere-dependent DNA damage response

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Telomeres suppress DNA damage response (DDR) activation by forming a protective structure that sequesters the chromosome termini. Changes in telomere structure expose chromosome ends as substrates that activate either an ATM- or ATR-dependent DDR. Activation of a telomere DDR is identified by co-localization of telomeres with DDR markers in “telomere dysfunction induced foci (TIF)”. Unlike broken genomic DNA, an ATM-dependent telomere DDR does not activate the G2/M checkpoint. Instead ATM-dependent TIF are passed through mitosis to the following G1-phase. In addition, Aurora B controls a mechanism whereby telomere structure is altered in cells experiencing prolonged mitotic arrest, resulting in ATM-dependent TIF. Metaphase-TIF can therefore result from passage of DDR-positive telomeres into mitosis from the previous G2, and the accrual of DDR-positive telomeres due to prolonged mitotic arrest.

It is known some cancer cell lines exhibit spontaneous metaphase-TIF, though it remains unknown if these TIF are ATM or ATR-dependent or if they result from prolonged mitotic arrest. We found that ATM inhibition suppresses the majority of spontaneous metaphase-TIF suggesting an ATM-dependent origin. Surprisingly, in some cancer cell lines ATR inhibition caused an increase in ATM-dependent metaphase-TIF and separated sister chromatids. Live-cell imagining revealed that cancer cells lines exhibiting spontaneous metaphase-TIF often also exhibit spontaneous prolonged mitotic arrest. This mitotic arrest was exacerbated through treatment with ATM and ATR inhibitors. Due to the role for ATR in repairing replication stress, and the connection between replication and cohesion, we hypothesized endogenous replication stress was driving mitotic arrest. We verified this hypothesis by identifying that the induction of replication stress by treatment with hydroxyurea or aphidicolin also induced mitotic arrest due to spindle assembly checkpoint activation. We will report on the expansion of these preliminary findings and the relevance to telomere and cell cycle biology at the meeting.
Glucose stress induced-lysosomal P-glycoprotein formation sensitizes multi-drug resistant tumours to novel anti-cancer agents

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Recently, it has been identified that lysosomal membrane bound P-glycoprotein (Pgp) has an active role in increasing drug accumulation within the organelle. Interestingly, lysosomal sequestration of the novel agent and Pgp substrate, di-2-pyridylketone-4,4-dimethyl-3-thiosemicarbazone (Dp44mT), is able to induce lysosomal membrane permeabilization (LMP) and enhance cytotoxicity selectively to MDR cells. This study investigated how cellular stresses present within the tumour environment, namely glucose variation, influenced the production of lysosomes and consequently multi-drug resistant (MDR) cell sensitivity to thiosemicarbazones compared with Doxorubicin (DOX). Glucose concentrations were modulated from complete glucose starvation to hyperglycaemic conditions in human cervical carcinoma cells. Early endosomes and lysosomes were assessed by western blotting and immunofluorescence. LMP was assessed by Acridine Orange staining. The effect on Pgp-mediated drug resistance to the thiosemicarbazones, Dp44mT and DpC, were examined by MTT assays. We demonstrate that changes in glucose availability confers Pgp-mediated drug sensitivity against the thiosemicarbazones Dp44mT and DpC. Low and high glucose induced endosomal and lysosomal formation with functional membrane bound Pgp. Notably, this led to greater drug resistance to DOX while increasing LMP and drug sensitivity to Dp44mT and DpC. The observed effects were found to be Pgp mediated as it was removed by the Pgp inhibitor, Ela. These studies highlight the rapid MDR response of tumours to glucose levels. It was demonstrated that as tumour cells become glucose-deprived or exposed to high glucose levels, early endosomes and lysosomes are formed containing functional organelle membrane bound Pgp. Lysosomal membrane Pgp serves to locally protect the cells against intracellular insult from chemotherapeutics.
ABSTRACTS, BOSCH YOUNG INVESTIGATORS POSTER PRESENTATIONS

Neuroprotective pre-conditioning with saffron: dose and mechanisms

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Neurodegenerative diseases continue to rise in prevalence but yet lack effective treatment to slow or stop their progression. Though the mechanisms behind these diseases are yet to be fully elucidated, protective strategies are increasingly being discovered in laboratory models, offering potential therapeutic options for human conditions. One example is the spice saffron, one of the most potent antioxidants known to science. Clinical data suggest dietary saffron can improve visual function in patients with age-related macular degeneration. These observations have recently been extended to models of brain disease.

Using a neurotoxin (MPTP) mouse model of Parkinson’s disease, this study aims to determine an effective pre-conditioning dose of dietary saffron for inducing neuroprotection and identify mechanisms involved. Ten week-old Balb/c mice were given saffron in their drinking water (0.01% w/v) for 2, 5 or 10 days (n=10/group). Following this pre-conditioning regime, mice were injected with 50mg/kg MPTP over two days. Throughout the experimental period, motor function was monitored using an open field test. Brain tissue was collected 7 days after MPTP injections and processed for immunohistochemistry.

Untreated mice injected with MPTP showed significant loss of dopaminergic cells in the substantia nigra pars compacta (SNc) relative to saline-injected control mice (~20% reduction, p=0.0112). Saffron pre-conditioning for 5 days significantly mitigated this neuronal loss (p=0.001). Correspondingly, MPTP increased striatal expression of FOS, a marker of abnormal neuronal firing, while saffron pre-conditioning mitigated this effect (p<0.05). Open-field testing showed a pattern of locomotive deficit corresponding to the level of dopaminergic cell loss across treatment groups.

This study indicates that saffron pre-conditioning protects against MPTP-induced parkinsonian neuropathology, with 5 days of pre-conditioning the most effective of the doses trialled. I will next explore the mechanisms underlying neuroprotection at this dose by assessing the brain transcriptome by microarray. It is hoped that this study will lay the foundation for the development of saffron as a potential therapeutic in human conditions.
Synthetic Omega-3 Epoxyfatty Acid As Antiproliferative and Pro-apoptotic Agent in Multiple Human Cancer Cells

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Rationale: The cytochrome P450-mediated 17,18-epoxide of omega-3 eicosapentaenoic acid (EPA) is anti-tumourigenic. To exploit this property we synthesised a novel analogue (C20-epoxide) with greater stability than 17,18-epoxy-EPA and greater efficacy against MDA-MB-231 breast cancer cells. However, the effects of C20-epoxide in other tumour-derived cell lines, reflecting cancers in other tissues, are unclear.

Objective: To analyse the anti-cancer effects of C20-epoxide in human cell lines representative of cervical (Hela), liver (HepG2), lung (A549), prostate (DU145), placental (BeWo) and breast (MDA-MB-468) tumours.

Methods and Results: After treatment with C20-epoxide, cell proliferation was estimated by mitochondrial MTT reduction, cell viability by ATP production, and apoptosis by caspase-3/7 activity. C20-epoxide decreased MTT reduction in all cells in a concentration-dependent fashion (by 30±4%-76±15% from control at 40 µM, 48 hours of treatment). C20-epoxide impaired ATP production by 49±8% (A549), 23±6% (DU145), 30±8% (Hela), 11±5% (MD-MBA-468), 40±33% (BeWo) relative to control (40 µM, 48 hours). Similarly, caspase-3/7 activity was increased by 26±4%, 49±1%, 49±8%, 30±7%, 82±59% over control in A549, DU145, Hela, MDA-MB-468 and BeWo cells, respectively. Cell cycle kinetics were analysed in propidium iodide-stained cells. Treatment with C20-epoxide (40 µM, 48 hours) decreased the proportion of all cells in S-phase, with the exception of BeWo. Western immunoblotting for cyclin D1 was undertaken because this protein regulates the G1-S-phase transition, and was down regulated in MDA-MB-231 cells by C20-epoxide. A decrease in cyclin D1 expression at 40 µM in A549, DU145 and Hela cancer cell lines occurred after 48 hours of C20-epoxide treatment.

Conclusion: The novel C20-epoxide decreased the viability of multiple cancer cell lines by inhibiting cell cycle progression, likely due to cyclin D1 down-regulation, and increased apoptosis.
ABSTRACTS, BOSCH YOUNG INVESTIGATORS POSTER PRESENTATIONS

Functional exhaustion of the immune response protects IRF9-deficient mice from lethal lymphocytic choriomeningitis

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The interferon regulatory factor 9 (IRF9) is a key component of type I interferon signalling and critical for the host anti-viral response.

While wild-type mice die following intracranial infection with lymphocytic choriomeningitis virus (LCMV), IRF9-deficient mice recover from the acute disease but develop a persistent infection. This is accompanied by viral spread to peripheral organs and chronic encephalitis. During chronic viral infection, virus-specific CD8+ T-cells become functionally exhausted. This status refers to a state of T-cell dysfunction characterised by poor effector function and sustained expression of inhibitory receptors. In particular the increased expression of the co-inhibitory receptor, programmed death-1 (PD-1), is considered to be a signature of functionally exhausted cells. Here we studied whether functional exhaustion of the T-cell response contributes to survival and virus persistence in IRF9-deficient mice. We found that CD8+ T-cells from LCMV-infected IRF9-deficient mice showed upregulation of PD-1, indicative of functional exhaustion. In contrast to IRF9-deficient mice, mice lacking both IRF9 and B7-H1, the primary ligand for PD-1, developed a lethal wasting disease following LCMV infection. Surprisingly, expression of key proinflammatory cytokines and T-cell infiltrates in the CNS and peripheral organs were more pronounced in LCMV-infected IRF9-deficient mice than in the double-deficient mice. Further, neutralisation of B7-H1 with antibodies during the subacute and chronic stages of LCMV infection in IRF9-deficient mice increased cytokine serum levels and decreased viral RNA levels. Importantly, neutralisation of B7-H1 had no impact on the clinical symptoms or body weight of the treated mice compared to untreated mice. These findings suggest that B7-H1 dependent T-cell exhaustion is crucial for the survival of LCMV-infected IRF9-deficient mice. Furthermore neutralisation of B7-H1 results in a ‘controlled’ reactivation of the immune response.
A third splice variant of β-tropomyosin is differentially expressed in representative cancer cell lines.

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β-Tropomyosin (TPM2) encodes either a high molecular or a low weight actin-binding protein involved in the stabilisation of actin microfilaments in the cell cytoskeleton. Previously our group identified the presence of a third splice variant of TPM2 within prostatic cancer. This third splice variant produces a truncated, low molecular weight form of the protein that we hypothesise contributes to the oncogenic transformation of cells by destabilising the cytoskeletal organisation. To determine if this splice variant is a unique feature of prostate cancer, or is common among all cancers, we have screened a range of representative cell lines including prostate (PC3, LNCaP and DU145), breast (MDA-MB-468, HCC1806, MCF-7), uterine (Ishikawa), cervical (HeLa), colorectal (HT29), pancreatic (MIA-PaCa), brain (SKN-MC) and lung (A549) for the presence of this particular splice variant using RT-PCR. Of those cell lines assayed, Ishikawa, MDA-MB-468, MCF-7, PC3, DU145 and LNCaP cell lines produce the splice variant. Preliminary western blot analysis of splice factors; SC35, ASF/SF2 and hnRNPA1 suggests that they control the relative proportions of the normal versus the splice variant transcripts in different cancer cell lines. Further study will be undertaken to explore their underlying effect upon transcript splicing. Additionally, we aim to demonstrate the importance of this splice variant in oncogenesis given that it appears to be present in some, but not all cancer cell types.
DPI and pMDI of simvastatin formulations for Pulmonary Diseases

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Simvastatin (SV) is an oral anti-cholesterol prodrug used to treat cardiovascular diseases and hypercholesterolemia via complete inhibition of the HMG-CoA reductase. There is increasing evidence to show that SV has anti-inflammatory and muco-inhibitory properties unrelated to its lipid lowering activity, making it potentially useful in the management of pulmonary diseases that have an inflammatory component. These findings support the current investigation on the pulmonary delivery of SV as a pressure metered dose inhaler (pMDI) and dry powder inhaler (DPI) for direct lung delivery. This study examines and compares the physico-chemical properties that could influence the absorption of both formulations in a bronchial epithelial in vitro cell model. It was found that both SV formulations, pMDI and DPI, have the ability to penetrate into the respiratory epithelium at differing rates. Formulations of inhaled SV have the potential to open up new exciting anti-inflammatory therapeutic opportunities for the treatment of pulmonary diseases.

STAT1 suppresses endogenous retroviral gene expression in the murine CNS

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Endogenous retroviral elements occupy approximately eight percent of the human and murine genomes and their activation has been linked with disease and cancer. Although the role of the transcription factor signal transducers and activators of transcription 1 (STAT1) in the host response against exogenous viruses is well established, whether STAT1 has a role in the regulation of endogenous retroviruses is unknown and was the focus of this study. Microarray analysis of total RNA from murine mixed glial cells revealed a strong induction of the expression of the retroviral gene, melanoma antigen (Mela), in STAT1-deficient cells. Follow-up studies revealed Mela mRNA was significantly increased in STAT1-deficient primary murine microglia compared with WT counterparts and was not detectable in astrocytes. To localise Mela mRNA in vivo, dual-label in situ hybridisation and immunohistochemistry was performed. This showed colocalisation of Mela mRNA in microglia and astrocytes in a punctate pattern, which was associated with astrogliosis, throughout the CNS of STAT1 KO mice but not in the WT CNS. To further elucidate the mechanism of regulation of Mela, its expression was analysed in mice deficient in other interferon signalling pathway components. STAT1 and to a lesser extent interferon-α/b receptor deficiency but not interferon-γ receptor, STAT2 or interferon regulatory factor 9 (IRF9) deficiency caused induction of Mela expression in the brain. These findings show that homeostatic STAT1-dependent non-canonical type I interferon signalling is involved in suppressing the re-emergence of Mela mRNA. Hence, in the healthy CNS, STAT1 may have an important role in restricting the expression of endogenous retroviral elements.
CNS-infiltrating T cells, from the draining cervical lymph node reservoir, are not essential for viral control in the non-adapted Dengue virus-2 encephalitis mouse model.

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Aims: Dengue (DENV) has been increasingly associated with neurological complications, including dengue encephalitis. However, the pathogenic mechanisms underlying the complications remain unclear.

Methods: These were therefore investigated in C57BL/6 mice, intracranially infected via the postglenoid foramen non-adapted DENV-2. Trafficking of leukocytes was examined by histological, flow cytometric and mRNA analysis of the brain, cervical lymph nodes (CLN), spleen, and bone marrow, at various timepoints post infection (p.i.).

Results: While virus was controlled by d3 p.i in the brain, DENV-specific IgG was detectable only at d9 p.i. Although no infected animals showed particular signs of pathophysiology or mortality, there were significantly more infiltrating effector CD8+T (GrB+CD62L\textsuperscript{low}CCR7\textsuperscript{low}IL7R-\alpha\textsuperscript{int}\textsuperscript{low}), CD69+GrB+NK cells, conventional DC (MHC-II\textsuperscript{hi}CD11c\textsuperscript{hi}CD8-CD4+/+CD11b+Ly6C\textsuperscript{hi}) and inflammatory CD45\textsuperscript{hi}CD11b+Ly6C\textsuperscript{hi} macrophages (iM\Phi) in the brains of infected mice than those of the mock-infected group with different kinetics. NK cells and CD8+T cells peaked at d6 and d9 p.i., respectively, correlating with increased intracellular granzyme B and perforin mRNA expression, while leukocyte recruitment correlated generally with increased CCL5, CCL2, CCL3, CXCL10, CXCR3, TNF and IFN-\gamma mRNA expression observed at d6 p.i. Intriguingly, intracerebral accumulation of T cells, but not NK cells and DC, was significantly impaired after cervical lymphadenectomy. However, the reduction of T cells did not alter clinical outcomes or brain viral burden. Additionally, the shedding of CD62L, which steadily increased from d3 to d9 and significantly declined at d13 p.i., was also detected on CLN T lymphocytes.

Discussion: The significant reduction of intracerebral infiltrating T cells and the kinetic of the shedding of CD62L on T cells in CLN clearly demonstrate an important role of the draining CLN as a reservoir for these infiltrating CD8+T cells. However, CNS-infiltrating CD8+T cells are not essential for controlling infection. Presumably, microglia, Ly6Chi iM\Phi and activated NK cells synergistically play an important role in restricting DENV-2 infection in resistant mice.
Paradoxical effect of endocannabinoids on visually-evoked responses of mouse retinal ganglion cells

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Vision starts in the retina, a complex yet structurally organised extension of the brain. This remarkable piece of nervous tissue not only allows for the conversion of light signals into neuronal impulses, but demonstrates higher order processing, previously thought only to occur in the brain. Adaptation to mean illumination and contrast adaptation are just two processes that take place in the retina. For this to occur, several mechanisms that modulate retinal signal transmission operate. The endocannabinoid (eCB) system has been characterised as a modulator of central nervous system synapses, regulating transmitter release. Recently, the eCB system has been detected in the retina, with studies indicating functional changes after application of cannabinoid receptor agonists and antagonists. Whether or not eCBs are tonically released in the retina, however, and their physiological function, are unknown. We aimed to establish whether a tonic role for eCBs exists in the retina, and if so, how it modulates retinal function and consequently visual processing. Light-evoked responses and voltage-gated Na\textsuperscript{+} currents were studied using single cell patch-clamping of retinal ganglion cells (RGCs) of C57/BL6J mice in control conditions, and in the presence of URB597, the fatty acid amide hydrolase (eCB degradatory enzyme) inhibitor. Addition of URB597 confirmed a tonic presence of eCB modulation: the amplitude of light-evoked responses of RGCs measured in current-clamp mode was reduced, as expected for an increase in eCB concentration, however a paradoxical increase in RGC excitability occurred. A shift in RGC excitability could be explained by changes in Na\textsuperscript{+} currents observed in voltage-clamp mode. Significant modulation of light-evoked response strength, contrast sensitivity, short-term plasticity, and sensory acuity were observed. These results provide evidence for a tonic role of eCBs in the retina. Implications for treatment of conditions such glaucoma with cannabinoids may involve altered visual processing.
BOSCH YOUNG INVESTIGATOR EVENTS

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A three-day retreat on the beautiful beach of Kioloa gives BYI’s a chance to develop skills for their career and create lasting memories

Welcome Harbour Cruise
A welcome cruise in fancy dress where honours students can meet one another and the other BYI’s in a relaxed, fun setting

Seminar Series
Catch up with your fellow BYI’s over some drinks and snacks, and learn what exciting data is being generated by our talented BYI’s.

Trivia Night and Games Night
This year we have already hosted an exciting night of board games and pizza, and look forward to our up-coming trivia night
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