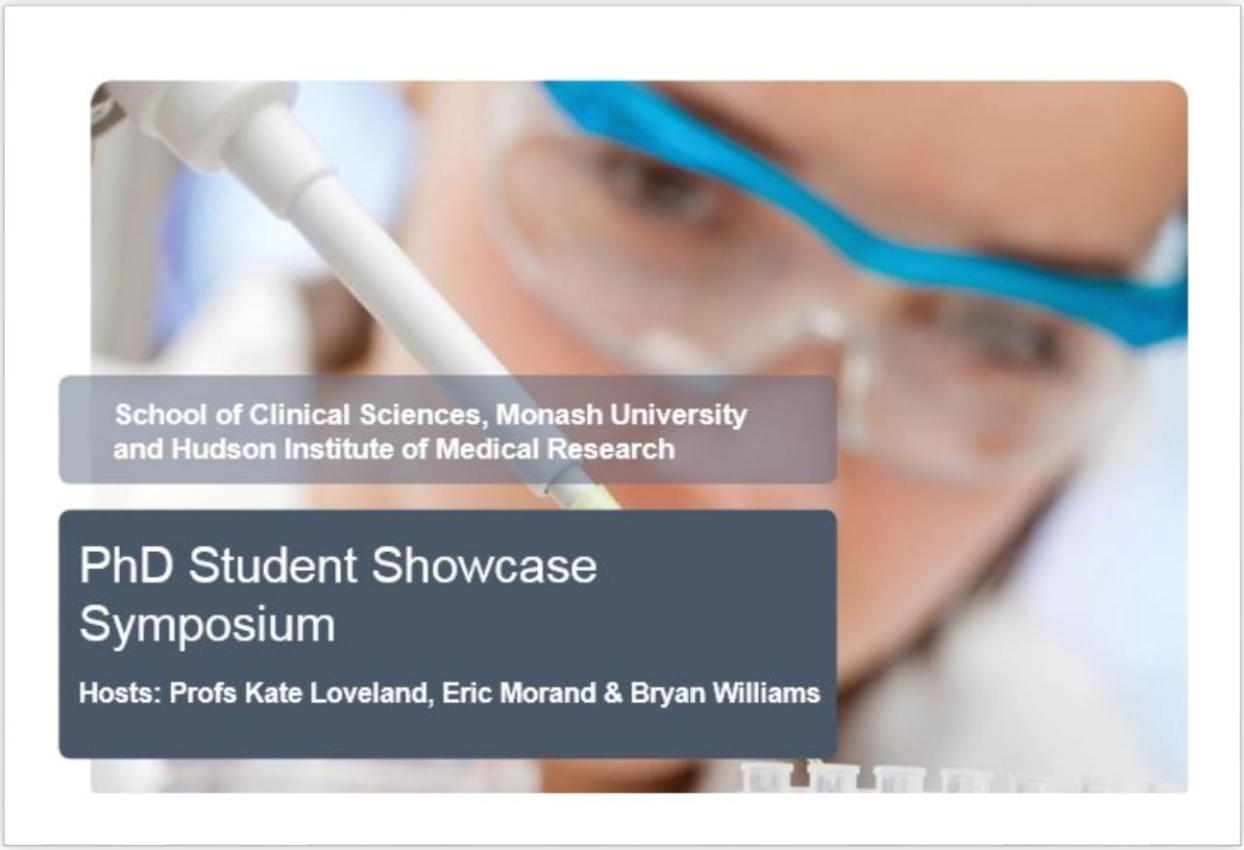


PROGRAM & ABSTRACTS

FRIDAY, 20TH NOVEMBER 2015

LECTURE THEATRE 1, MONASH MEDICAL CENTRE CLAYTON

MHTP
Monash Health
Translation Precinct

MonashHealth

HUDSON
INSTITUTE OF MEDICAL RESEARCH

MONASH
University

Table of Contents

PROGRAM AND SCHEDULE

Friday 20th November 2015. Lunch, from 12.15pm

12:40pm **Welcome**
Professors Kate Loveland, Eric Morand & Bryan Williams

SESSION 1 **ORAL PRESENTATIONS (12.50PM – 2.50PM)**
Chairs: Katharine Johnson, James Ong, Victoria Lyons

12:50pm **Dr Jonathan Dick**
Complement is an Important Mediator of Anti-Myeloperoxidase Autoimmunity and Glomerulonephritis

1:10pm **Ruth Tatnell**
NSSI: A developmental model of attachment and emotion regulation

1:30pm **Dr Jingang Li**
Cord blood cell therapy to reduce preterm brain injury

1:50pm **Natalie Bitto**
Properties and transport of DNA carried by bacterial outer membrane vesicles

2:10pm **Dr Diane Apostolopoulos**
The Cost of Glucocorticoids in Systemic Lupus Erythematosus – an Australian Perspective

2:30pm **Dr Moya Vandeleur**
Sleep quality in children with cystic fibrosis: associations with mood

2.50pm **BREAK**

SESSION 2 **ORAL PRESENTATIONS (3.00PM – 4.20PM)**
Chairs: Dean Popovski, Charlotte Nejad

3.00pm **Maureen Humphrey**
Long term effects of medically prescribed diets on growth, body composition, and nutritional markers in children with Inborn Errors of Metabolism (IEM)

3:20pm **Dr Om Narayan**
Novel insights into Central Aortic Blood pressure, its generation, relationship to cardiovascular outcomes and interaction with coronary blood flow

3:40pm **Dr Luke Larmour**
A patient derived xenograft model and cervical cancer

4:00pm **Lexie Prokopuk**
Programming the oocyte epigenome: establishing a foundation for the next generation

4:20pm **Afternoon Tea in Foyer**
Judges convene to decide winners

4:40pm **PRIZE AWARDS**

- Special award to Professor Rosemary Horne in recognition of long service to Hudson PhD program
- Prizes for each speaker - $50 Coles-Myer gift card
- Student Choice Award - $200 Coles-Myer gift card
- 2nd Prize, Best Presentation - $300 Conference Travel Award
- 1st Prize, Best Presentation - $600 Conference Travel Award

SPEAKERS

DR JONATHAN DICK

Jonathan studied Medicine at the University of Oxford and University College London before training in internal and renal medicine in London. He joined the Centre for Inflammatory Diseases at Monash University at the end of 2013 and is currently studying for a PhD under the supervision of Professors Stephen Holdsworth and Richard Kitching.

RUTH TATNELL

Ruth completed her Bachelor of Science and Master of Science (Psychology) at the University of Otago, and is in the final year of her PhD with the Centre for Developmental Psychiatry and Psychology. Her research interests include: attachment theory, development of emotion regulation and self-injury.

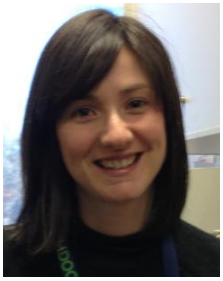
DR JINGANG LI

Jingang is a third year PhD student, who has been working as a neonatologist and pediatrician for over 10 years in Japan. Now he is investigating the utility of umbilical cord blood stem cells to reduce brain injury in preterm infants, in the Neurodevelopment and neuroprotection group, Hudson Institute of Medical Research.

NATALIE BITTO

Natalie completed her undergraduate studies at Monash University and Honours with Dr. Hans Netter in the Department of Microbiology. After working at CSIRO as a research assistant in the protein production group, Natalie began her PhD with Associate Professor Richard Ferrero at the Centre for Innate Immunity and Infectious Diseases, Hudson Institute. Her project is focussed on characterising the properties and innate immune effects of DNA associated with bacterial outer membrane vesicles.

DR DIANE APOSTOLOPOULOS



Diane completed her medical degree at the University of Melbourne in 2006, and was accepted as a Fellow of the Royal Australian College of Physicians in 2015 as a General Physician and Rheumatologist. She is currently employed as a Consultant Rheumatologist and Clinical Fellow at Monash Health. Diane is in her second year of PhD (part-time) through the Monash University Centre for Inflammatory Diseases and the Rheumatology Unit at Monash Health.

DR MOYA VANDELEUR

Moya is a respiratory and sleep paediatrician and 3rd year PhD candidate (part-time) studying the effects of sleep disturbance in children with cystic fibrosis. This research is funded by a NHMRC postgraduate scholarship and she has received previous funding from the RACP ResMed Foundation/ Sleep Health Foundation Research Entry Scholarship and The Royal Children's Cystic Fibrosis Research Trust.

MAUREEN HUMPHREY

Maureen is a Senior Dietitian in the Department of Metabolic Medicine at the Royal Children's Hospital in Melbourne. Her clinical workload involves working with infants, children and adolescents diagnosed with an Inborn Error of Metabolism requiring lifelong medically prescribed and highly modified diets. Maureen has a particular interest in examining protein and energy relationships in protein modified diets and this has resulted in her PhD examining long term effects of protein modified diets on growth and body composition in children with IEM.

DR OM NARAYAN

Om is an Interventional Cardiology Fellow at MonashHeart. His principal research interest is in coronary physiology and in particular the interrelationships between coronary blood flow, proximal aortic function and left ventricular function. In addition to competencies in coronary wave intensity analysis, aortic pressure wave morphological analysis and advanced echocardiographic analysis, Dr. Narayan is level A certified in Cardiac CT. His PhD research studies are currently funded by an NHMRC Postgraduate Scholarship.

DR LUKE LARMOUR

Luke is a trainee of the Royal Australian and New Zealand College of Obstetricians and Gynaecologists. He is currently in the third year of his PhD research undertaken at the Ritchie Centre, under the supervision of A/Prof Caroline Gargett and A/Prof Tom Jobling, investigating the development of cervical cancer form precancerous disease. His professional and research interest is gynaecological cancer and women's health.

LEXIE PROKOPUK

Lexie is a 2nd year PhD student in the Centre of Genetic Diseases at the Hudson Institute of Medical Research. Her research is focussed on the establishment of epigenetic modifications during fetal germline development, and how changes to epigenetic information in a parent can affect outcomes in offspring. This year Lexie was selected as the winner of the MIMR-PHI PhD Travel Fellowship that supported her attendance to the 48th Annual Meeting for the Society for the Study of Reproduction (SSR) in Puerto Rico, where her abstract was accepted for an oral presentation.

ABSTRACTS

Complement is an Important Mediator of Anti- Myeloperoxidase Autoimmunity and Glomerulonephritis – Dr Jonathan Dick

J.S.C. DICK[1], P.Y.GAN[1], S.L FORD[1], M. ALIKHAN, A.R. KITCHING[1], S.R HOLDSWORTH[1],
[1] *Centre for Inflammatory Diseases*

Introduction: The anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitides are autoimmune diseases that cause rapidly progressive glomerulonephritis resulting in renal failure. The common auto-antigens in this disease are neutrophil myeloperoxidase (MPO) and proteinase-3 (PR3). Complement has recently been discovered play an important role in the activation of neutrophils by autoantibodies: a key step in the disease pathogenesis. The effect of complement on anti-myeloperoxidase autoimmunity has not previously been investigated.

Methods: MPO autoimmunity was induced by injecting C57BL/6 (WT) or C5aR-/- mice with MPO in Freund's adjuvant. In separate experiments WT mice were injected with $1x10^6$ WT or C5aR-/- MPO-pulsed bone marrow derived dendritic cells (DCs). Anti-MPO glomerulonephritis was triggered by a sub-nephritic dose of anti-GBM globulin.

Results: Humoral immune response at 10 days measured by anti-MPO IgG ELISA was significantly reduced in C5aR-/- mice (0.64±0.05 vs 0.20±.0.02 OD450nm $p<0.001$) T cell Th1 response to MPO was significantly attenuated in C5aR-/- mice as measured by IFN-γ ELISPOT (26±6 vs 55±11 cells $p=0.03$).The proportion of CD4+CD25+Foxp3+ T regulatory cells were significantly increased in C5aR-/- mice (13.5±0.4 vs 11.8±0.2% $p<0.001$). To determine whether C5aR expression on DCs accounted for the effect on T-cells anti-MPO autoimmunity was induced by injection of MPO-pulsed C5aR-/- or WT DCs. Anti-MPO glomerulonephritis was then induced resulting in significantly reduced renal injury (segmental glomerular necrosis 9.9±2 vs 30.9±5% $p=0.005$) in the group receiving C5aR-/-DCs.

Conclusion: Complement acting through the C5aR plays a crucial role in modulating both humoral and cellular anti-MPO autoimmunity in mice. This suggests an additional mechanism by with C5aR inhibition in ANCA associated vasculitis may be an effective treatment strategy.

NSSI: A developmental model of attachment and emotion regulation - Ruth Tatnell

Non-suicidal self-injury (NSSI), the deliberate destruction of body tissue without suicidal intent, is a behaviour affecting up to 20% of adolescents and young adults, costing over $14million in monthly hospital expenses. Primarily used for emotion regulation, little is known about what differentiates those who self-injure for affect regulation from those who use more productive coping techniques. This research proposes and examines a developmental model of NSSI, based in infant attachment theory as a basis for emotional development. Using cross-sectional, longitudinal and experimental methodology, aspects of the relationship between early attachment, emotion regulation development and NSSI is examined in adolescents and emerging adults. The aim of this research is to assist in the early identification of at risk children to enable prevention strategies, as well as potential areas for later intervention.

Cord blood cell therapy to reduce preterm brain injury – Dr Jingang Li

Li J, Yawno T, Sutherland A, Loose J, Nitsos I, Bischof R, Wong F, Jenkin G, Miller SL

The Ritchie Centre, Hudson Institute of Medical Research

Aim: This study examined the neuroprotective effects and mechanisms of action of allogeneic umbilical cord blood (UCB) cells in a fetal sheep model of hypoxic-ischemic (HI) preterm brain injury. Methods: Umbilical cord occlusion (UCO) or sham was performed for 25 minutes to fetal sheep at 100 d gestational age (0.7 gestation). 50 million UCB cells were administered intravenously to the fetus at 12 h or 5 d after UCO. Fetal brains were obtained at 10 d after UCO for analysis.

Results: UCO reduced the number of oligodendrocytes (olig2+) and myelinated axon density (CNPase+) in white matter, while UCB cells administered at 12 h, restored white matter development. UCO animals showed a significant increase in TUNEL- and Ki67-positive cells in the white matter, while UCB cells, administered at 12 h, prevented these effects. The number of activated microglia (Iba1+) was also increased in the white matter in UCO fetuses, whereas UCB cell administration at 12 h provided an anti-inflammatory effect. However, UCB cell administration at 5 d failed to show these, potentially, therapeutic effects. Additionally, UCB cells at 12 hours induced a systemic increase in interleukin-10 ($p < 0.05$), and reduction in oxidative stress (malondialdehyde, $p < 0.05$) following HI.

Conclusion: Early UCB cell administration at 12 h after hypoxia-ischemia reduces white matter injury through its anti-inflammatory, anti-apoptotic, and antioxidant effects.

Properties and transport of DNA carried by bacterial outer membrane vesicles – Natalie Bitto

Natalie Bitto[a], Adam Costin[b], Camden Lo[b], Ross Chapman[a], Jodee Gould[c], Jasmine Choi[a], Tanya D'Cruze[d], Eric Reynolds[d], Stuart Dashper[d], Lynne Turnbull[e], Cynthia B. Whitchurch[e], Katryn J. Stacey[f] and Richard L. Ferrero[a]

[a] Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, Victoria 3168, Australia;
[b] Monash Micro Imaging, Monash University, Clayton, Victoria 3168, Australia;
[c] Monash Health Translation Precinct Medical Genomics Facility, Clayton, Victoria 3168, Australia;
[d] Oral Health CRC, Melbourne Dental School, Bio21 Institute, The University of Melbourne, Melbourne, Parkville, Victoria 3052, Australia;
[e] The ithree institute, University of Technology Sydney, Ultimo, NSW 2007, Australia; and
[f] University of Queensland, Brisbane 4000, Australia

The release of nano-sized membrane vesicles is a conserved phenomenon amongst Gram-negative bacteria. These bi-layered structures, known as outer membrane vesicles (OMVs), are adept at entering eukaryotic cells, facilitating delivery of bacterial factors into host cells. As well as a range of bacterial proteins and glycolipids, OMVs have been shown to contain DNA. Despite its putative importance in both bacterial and immune functions, very little is known about the nature of OMV-associated DNA and its interactions with host cells.

This study investigates the mechanisms by which OMV-associated DNA may play a role in host-pathogen interaction. To this end, we characterised the amount, form and sequence of OMV associated DNA, demonstrated its uptake into eukaryotic cells and determined the destination of OMV-derived DNA once within the cell. To characterise the amount, form and sequence of OMV-associated DNA, we used PicoGreen fluorescent quantitation, Ion Torrent semiconductor sequencing and electron microscopy to show that OMVs are associated with bacterial genomic DNA. This genomic DNA is predominantly bound to the OMV surface, however a small portion is protected within the vesicle lumen. Sequence analysis identified a number of genes that were enriched in the internal and external OMV-derived DNA. Using immunofluorescence, we have shown for the first time the uptake of OMV-associated DNA into eukaryotic cells. Furthermore, we have separated the nuclear and cytosolic fractions of eukaryotic cells treated with OMVs and have shown that OMV-derived DNA can be detected in the nuclear fraction of treated cells.

Collectively, our findings show that OMVs are associated with bacterial DNA which is carried as cargo into eukaryotic cells and localises in the nucleus or perinuclear space. The outcomes of this study will give insight into host-pathogen interaction of Gram-negative bacteria, as well as shedding light on the mechanism of action of OMV-based vaccines, such as the current meningococcal vaccine.

The Cost of Glucocorticoids in Systemic Lupus Erythematosus – an Australian Perspective – Dr Diane Apostolopoulos

Systemic lupus erythematosus (SLE) is a chronic multisystem autoimmune disease in which tissue inflammation results in the accrual of irreversible damage to multiple organs including, the kidneys, skin, joints, lungs and brains. The aetiology of SLE is incompletely understood and this has restrained the discovery of curative medicines for this disease. Patients with SLE, the majority of whom are young women, face chronic illness, loss of health, reduced work participation, and a very significant risk of premature death.

Because of a lack of significant advances in therapy, the majority of patients still receive acute and/or chronic treatment with glucocorticoids, a drug class unchanged since the 1950s. Severe, predictable, dose-dependent metabolic adverse effects accompany glucocorticoid use, and the continued use of glucocorticoids despite their toxicity reflects a failure to discover tractable alternatives. Studies including those from our own centre at Monash University indicate that up to 70% of SLE patients take chronic glucocorticoid therapy.

Despite the universally accepted clinical dictum that glucocorticoids are harmful in the treatment of SLE, data on the costs of glucocorticoid use in SLE, both health-related and economic, is lacking.

Sleep quality in children with cystic fibrosis: associations with mood – Dr Moya Vandeleur

Moya Vandeleur 1,2,3,4, Gillian M Nixon 1,2,3, David S Armstrong 1, Philip J Robinson 4, Rosemary SC Horne 1,2.
1. Department of Paediatrics, Monash University, Melbourne
2. The Ritchie Centre, Hudson Institute of Medical Research
3. Melbourne Children's Sleep Centre, Monash Children's Hospital
4. Department of Respiratory Medicine, Royal Children's Hospital

Introduction: In adults with cystic fibrosis (CF) sleep disturbance is common and associated with depressed mood however there is a paucity of international data regarding children.

Aim: To determine sleep patterns and quality in children with clinically stable CF and healthy controls and to examine the relationship to mood.

Methods: Children with CF, free from pulmonary exacerbation and age matched healthy control children (age range 7-18y) were recruited. Each completed 2 weeks of sleep diary together with sleep questionnaires [OSA-18, Paediatric Daytime Sleepiness Scale (PDSS), Sleep Disturbance Scale for Children (SDSC)]. Overnight SpO_2 was measured using pulse oximetry. Questionnaires were used to assess mood [Children's Depression Inventory (CDI), Beck Depression Inventory-Youth (BDI-Y)]. Data were compared between groups with one way ANOVA with Student Newman Keuls posthoc analysis if normally distributed or Kruskal-Wallis one way ANOVA on Ranks with Dunns posthoc analysis if not.

Results: 46 CF (24M/22F) and 39 control (19M/20F) children well-matched for age completed the study. Mean (±SD) FEV_1 in the CF group; 80±19% predicted. CF and control subjects reported no significant differences in sleep duration or frequency of night waking. Children with CF had significantly lower mean SpO_2 than controls (96.9±1.7% vs 98.4±0.8%, $p<0.001$). Children with CF had higher total scores than controls for the OSA-18 (median 35 vs 24, $p<0.05$), PDSS (mean14.3±4.5 vs10.2±4.4, $p<0.001$) and SDSC (median 45.5 vs 35.0, $p<0.05$). Young children (7-12y) with CF

(n=28) exhibited higher mood scores on the CDI (reflecting lower mood) than controls (n=24) (mean 45.8±7.3 vs 41.3±5.1, p=0.01). In the CF group, there was no correlation between mood scores and any of the sleep questionnaire scores or FEV1.

Conclusion: Children with clinically stable CF report significantly more sleep problems than healthy children despite similar durations of sleep. Young children with CF have lowered mood compared to controls however it is not directly associated with poor subjective sleep quality. This relationship needs to be examined in children with more severe CF lung disease.

Long term effect of medically prescribed diets on growth, body composition, and nutritional markers in children with Inborn Errors of Metabolism (IEM) – Maureen Humphrey

An Inborn error of metabolism (IEM) is an inherited disorder of body chemistry, due to a genetic mutation that affects the production of a specific enzyme, transporter or channel, which is responsible for the metabolic process. In many IEM dietary therapy is the mainstay of treatment in order to reduce the intake of the offending substrate that cannot be efficiently metabolised, and to help supply essential nutrients for normal function. These diets are often extremely restrictive with a natural protein intake significantly altered or lower than healthy children and a high fat and carbohydrate (CHO) load to provide energy which may increase longer term nutritional risk.

A novel way to describe and prescribe diets in this group may be the use of a Protein to Energy ratio (P:E) as a single index to describe dietary quality and to ensure that not only adequacy, but also correct balance of these intakes is ensured. In order to determine if this concept may have some validity for use in patients in whom protein and energy intake is so carefully controlled, we aimed to elucidate the long term nutritional status of our patients and document in detail their dietary intake particularly in terms of protein (quality and quantity) and energy intake and P:E ratios.

Novel insights into Central Aortic Blood pressure, its generation, relationship to cardiovascular outcomes and interaction with coronary blood flow – Dr Om Narayan

Measurement of the central aortic blood pressure (CBP) waveform has emerged as potentially superior to traditional brachial blood pressure estimation in the prediction of cardiovascular risk. This has led to a proliferation of devices and techniques with the promise of rapid, non-invasive and accurate central blood pressure estimation despite a lack of robust data demonstrating the prognostic utility of the central aortic pressure waveform in predicting cardiovascular risk. Additionally, the role of central aortic blood pressure and myocardial microvascular function in directing coronary blood flow was poorly defined. As part of this thesis, a systematic, quantitative meta-analysis was performed to clarify the role of measurement factors in the non-invasive estimation of the central aortic pressure waveform. This analysis demonstrated substantial variability between devices and a tendency for a consistent under or over-estimation of the central blood pressure. Secondly the prognostic role of the CBP waveform in addition to traditional cardiovascular risk factors was also evaluated in the 853 patient strong ANBP2 cohort. Reservoir-waveform analysis was applied to the CBP waveforms in this patient cohort and a marker of arterial compliance was found to independently predict death, stroke and MI. Finally, we applied coronary wave intensity analysis to investigate the role of early diastolic suction generated by the myocardial microvasculature (in conjunction with CBP) in stenosed coronary arteries. We identified a significant impairment of early diastolic coronary suction in the setting of severe coronary artery stenosis with rectification of this abnormality following PCI. These findings provide novel insights into the CBP waveform, its measurement, prognostic utility and role in coronary blood flow.

A patient derived xenograft model and cervical cancer – Dr Luke Larmour

Background and Aims: Limited animal models exist for the study of cervical carcinoma, and none exist for examining the progression of cervical dysplasia to carcinoma. Our aim was to develop a patient derived xenograft (PDX) model of cervical dysplasia and carcinoma using the sub-renal capsule.

Methods: Biopsy tissue from either high-grade cervical dysplasia (n=4) or carcinoma (n=14) was transplanted into adult female NOD/SCID IL-2R mice and harvested after 6 months. Portions of harvested tissue was then retransplanted beneath the kidney capsule of new recipient mice. Both primary biopsies and harvested graft tissue were immunostained for p16INK4a, HPV, HPV-16 and -18 E6, and cytokeratin-17 using standard immunohistochemical techniques.

Results: The engraftment rate of primary cancer samples (n=14) was 71.4%. The time to generate tumours was similar for up to four subsequent serial retransplantions. Three of four dysplasia samples formed cystic structures lined by epithelium, staining positive for p16INK4a, HPV, and cytokeratin 17 in 2 of the samples.

Conclusions: The sub-renal capsule is an excellent site for a PDX model for cervical cancer, regardless of degree of copy number variation. This is potentially the first time that cervical dysplasia has been grown in a PDX model, although further characterization is required. Our model enhances the study of cervical cancer development and progression.

Programming the oocyte epigenome: establishing a foundation for the next generation – Lexie Prokopuk

Lexie PROKOPUK, Jessica M STRINGER and Patrick S WESTERN
Centre for Genetic Diseases, Hudson Institute of Medical Research, Clayton, Victoria, Australia.

Epigenetic modifications involve chemical alterations of the DNA and associated histones that package the chromatin within the nucleus and alter the accessibility of genes to transcriptional machinery. As epigenetic modifications are heritable through cell divisions they collectively regulate ongoing lineage identity and cell function.

Significantly, epigenetic information is reset in developing germ cells, which form oocytes and sperm and pass an individual's genetic and epigenetic information to the offspring. This new epigenetic information regulates development in the next generation.

Polycomb repressive complex 2 (PRC2) mediates trimethylation of lysine 27 in histone 3 (H3K27me3), which is an epigenetic modification that is critical for gene silencing and is important for regulating developmental gene expression.

The roles of PRC2 in regulating germline epigenetic information remain unknown. We have used immunofluorescence to determine the temporal and spatial profile of PRC2 components and H3K27me3 during epigenetic reprogramming and development of the female germline. Our data indicates that PRC2 regulates the epigenome in maturing oocytes, which is likely to be important for health and development in offspring.