NSW StemCell NETWORK

15th NSW Stem Cell Network Workshop

Stem Cells and Developmental Biology

Wednesday, 31st August, 2011. Darlington Centre Conference Room, 174 City Road, Darlington

Dear Registrants,

The NSW Stem Cell Network has been in existence since 2002, and this is our 15th Workshop. There are ~500 members of the Network, almost of whom are professionals – scientists, clinicians, policy makers, lawyers, ethicists, politicians, commercial people, with some potential end users. Our aims have been (a) to network these professionals to encourage communication, cooperation and collaboration, and (b) to help educate the community. To the latter end, we are in-servicing clinicians at the Royal Australasian Colleges of Physicians and General Practitioners, "students" at the University of the 3rd Age, school students, and to some extent science teachers.

This is the 30th anniversary of the modern stem-cell era – when the first mouse embryonic stem cells were made – and it is just 13 years since Professor Jamie Thomson at Madison, Wisconsin, created the world's first human pluripotent stem-cell line. We have progressed a lot since then, especially with multipotent stem cells from adults, there being numerous clinical trials with this type of stem cell, including the mesenchymal stem cell derived from bone marrow or fat. Although pluripotent stem cells are capable of more feats than the multipotent cell, it was only in October last year that the first clinical trial with this type of stem cell, as a therapy for people with spinal cord paralysis, began. Further trials with these cells are planned soon, with loss of vision caused by macular damage next being targeted.

Stem cells may be also be used in the field of understanding how tissues and organs develop. At the workshop we will hear about their role in understanding the development of organs such as the eye, haemopoietic cells, and the heart.

The workshop features experts in the field of the developmental biology of stem cells, with internationally renowned Professor Richard Harvey from the Victor Chang Institute giving the key note address. We also have presentations from talented researchers in Sydney, and the Walter & Eliza Hall Institute in Melbourne.

We thank Dr Elizabeth Foley, Chief Executive Officer of Research Australia, for opening the Workshop, and hope you all enjoy the occasion, network and learn much on the day.

Bernie Tuch, Michael Morris & Sarah Mustafa

Program

9:30am	Registration opens / Light Refreshment
10:00am	Opening Address: **Dr Elizabeth Foley – CEO Research Australia**
Session 1	**A/Prof. Edna Hardeman** - Head, Neuromuscular and Regenerative Medicine Research Unit, University of New South Wales
10:10am	"Modelling eye development using pluripotent stem cells" **Dr. Michael O'Connor - University of Western Sydney**
10:35am	"Pluripotency in epiblasts and epiblasts derived stem cells" **Dr. Yoji Kojima – Children's Medical Research Institute.**
11:00am	Morning Tea / Networking
11:20am	"The role of MYST family of chromatin regulators in stem cells" **Dr. Tim Thomas - Walter and Eliza Hall Institute**
11:45pm	"Control of epithelial organization and differentiation of endoderm" **Dr. David Leobel – Children's Medical Research Institute.**
12:10pm	"An unexpected role for the copper transporter Ctr1 in embryonic stem cell differentiation and teratoma formation" **Dr. Stuart Fraser - University of Sydney**
12:35pm	Lunch / Networking
Session 2 **Chair:**	**Dr Margot Day** - Senior Lecturer, Bosch Institute
1:30pm	"Characterisation of Cardiac-resident MSC-like Stem Cells in the Mouse" **Prof. Richard Harvey – Victor Chang Cardiac Research Institute**
2:10pm	"Haematopoietic Stem Cell Development" **Dr. Samir Taoudi - Walter and Eliza Hall Institute**
2:35pm	"Amino acid-mediated development in embryonic stem cells and embryos" **Dr. Michael Morris - University of Sydney**
3:00pm	Refreshments / Networking

Opening Address : Elizabeth Foley Research Australia CEO

Elizabeth Foley is the new Chief Executive Officer of Research Australia, the national peak advocate for health and medical research. Ms Foley has enjoyed a very successful career in senior roles at blue chip organizations, most recently at AXA Asia Pacific. She has extensive experience and qualifications in marketing, communications, finance and governance. Elizabeth is passionate about the role of research in driving health improvement and the importance of Australian research to building a strong economy.

Research Australia is a unique national alliance of over 170 member and donor organizations. It has built a sound reputation as a "whole of community" voice for health and medical research. Its capacity to contribute to debate, shape research policy and galvanize public opinion has positioned Research Australia to take on new challenges. This capacity, particularly in the face of economic uncertainty and growing competition both nationally and globally, reflects the strength of this organization's membership, values and mission.

Session Chairs

Session 1 Chair: Professor Edna Hardeman

Head, Neuromuscular and Regenerative Medicine Research Unit

School of Medical Sciences, University of New South Wales

Professor Hardeman is an internationally recognized authority on the genetics of muscle function in relation to disease, ageing and athletic performance. Her specific areas of expertise are diseases of skeletal muscle including muscular dystrophy, genetic indicators of athletic performance and gene therapy to treat muscle disease. Her laboratory has generated mouse models for human muscle diseases, discovered treatments that are being considered for human trials and is currently exploring novel methods of enhancing muscle stem cell transplantation. Professor Hardeman co-founded the conference 'Muscle Stem and Satellite Cells', a regular meeting of the US FASEB Society. She is a longstanding member of the European Neuromuscular Centre International Consortium on Nemaline Myopathy and is currently the President of the Australian New Zealand Society for Cell and Developmental Biology. Her research is funded by the NHMRC,

Session 2 Chair: Dr Margot Day - Senior Lecturer, Bosch Institute
(Bio and picture were not provided at the time of this printing)

Plenary Speaker: Prof. Richard Harvey - Victor Chang Cardiac Research Institute

Characterisation of Cardiac-resident MSC-like Stem Cells in the Mouse

Identification of multi-potent stem cells in the adult mammalian heart has promoted a revision of the dogma that the heart is a post-mitotic organ with limited regenerative reserve. Our laboratory has developed a quantitative framework for characterising colony-forming cells from the adult mouse heart. Such cells (cardiac colony-forming units-fibroblast; cCFU-F) are multipotent for a variety of mesodermal lineages in vitro and in vivo, and have a gene expression and surface receptor profile resembling bone marrow (BM) mesenchymal stem/progenitor cells (MSCs). We will present our recent data on the characterisation of cCFU-F in vivo and in vitro, their origins in development, and molecules regulating their cell cycle state and stemness.

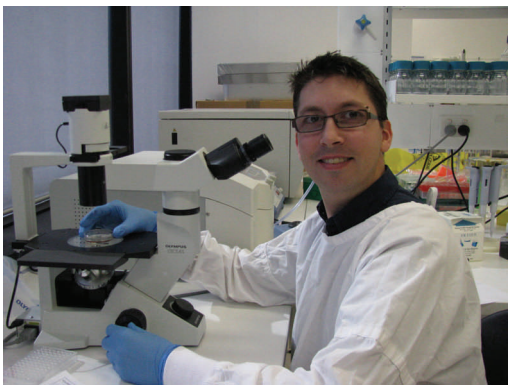
Professor Richard Harvey received his PhD in 1982 from the University of Adelaide, training in molecular biology. He undertook postdoctoral studies in embryology at Harvard University, then moved to the Walter and Eliza Hall Institute in Melbourne, establishing an independent group. In 1998, he relocated to the Victor Chang Cardiac Research Institute, where he is currently Co-Deputy Director and Head of the Developmental and Stem Cell Biology Division. He holds the endowed Sir Peter Finley Professorship of Heart Research at UNSW and an NHMRC Australia Fellowship, and is a member of EMBO and the Australian Academy of Science. His research has focused on the genetic basis of heart development and congenital heart disease, and more recently on the biology and origin of adult cardiac stem cells, and cardiac regeneration.

Speaker: Dr. Michael O'Connor - University of Western Sydney

Investigating Eye Development and Disease using Human Pluripotent Stem Cells.

Cataract and macular degeneration combined account for two-thirds of Australians with either low vision or blindness. Due to the increasing age of Australia's population, the total number of people affected by these two diseases is expected to almost double over the coming decade. A better understanding of how these diseases develop is therefore needed to develop new treatments that can reduce both the number of people affected by, and the costs associated with, these two diseases. Human pluripotent stem cells offer a unique opportunity to generate large numbers of human lens and retinal cells in vitro. Investigation of the differentiation processes involved in generating these ocular cell types will increase our understanding of the molecular events involved in normal lens and retinal development, as well as the initiation and progression of cataract and macular degeneration. Our laboratory uses step-wise protocols that generate lens and retinal cell types by mimicking aspects of normal embryonic development in vitro. While somewhat successful, these differentiation methods also produce other, unwanted cell types. A major aspect of our research therefore is aimed at better defining the signalling and gene expression networks required at each stage of lens and retinal differentiation, from maintenance of the undifferentiated pluripotent stem cells through to production of mature lens and retinal cell types.

Dr. Michael O'Connor is a Research Lecturer in the School of Medicine at the University of Western Sydney. He obtained his PhD from the University of Sydney in 2005, creating an animal-based culture system that can regenerate functional ocular lenses in vitro. Upon completing his PhD Michael undertook postdoctoral studies with Prof. Connie Eaves in Vancouver, Canada, where he identified new genes that are required to maintain human pluripotent stem cells. During this time he was also closely involved in the commercial development of the feeder-independent pluripotent stem cell media, mTeSR®1 and TeSR™2. Michael's current research uses human pluripotent cells to learn more about normal and pathological eye development.



Speaker: Dr Michael Morris - University of Sydney

Selected Amino Acids Stimulate ES-Cell Differentiation and Pre-Implantation Embryo Development

ES cells: The phenotypic status of ES cells is controlled in part by signalling pathways which translate inputs mediated by extracellular molecules. Here we show that LIF, the ligand responsible for ES-cell self-renewal, together with L-proline promote neurogenesis of mouse ES cells via a series of embryologically relevant cell types including epiblast-like pluripotent primitive ectoderm, germ-layer-like multipotent definitive ectoderm and neurectoderm (the first cells of the developing neural system). L-proline is first taken up into ES cells via a specific transporter. Inhibitor studies and kinome array analysis show that it then (i) changes the activity of signalling pathways already stimulated by LIF and (ii) activates additional signalling pathways.

These results indicate that through the addition of small, nontoxic activators and inhibitors of signalling pathways, the differentiation of pluripotent cells might be controlled sufficiently well to allow homogeneous production of specific cell types suitable for use in animal models of human disease.

Preimplantation embryos: Selected amino acids, including L-proline, also stimulate development of cultured pre-implantation mouse embryos. The mechanism does not appear to be a simple metabolic effect nor is it an osmolyte effect. Instead, it appears to be autocrine-like, promoting the development of embryos when they are cultured at low density but not when they are cultured at high density. As with the differentiation-inducing effects of amino acids in ES cells, these amino acids first require uptake into the blastomeres of the embryo via specific amino-acid transporters.

Dr Michael Morris is a cell biologist and biophysicist who has worked in the field of adult and embryonic stem cells since 1997. He holds the position of Sesqui Senior Lecturer in Embryonic Stem Cells at the University of Sydney, and has previously run groups for the Cell Therapy Program of BresaGen Ltd and for the commercially oriented Australian Stem Cell Centre. He has held postdoctoral fellowships at Harvard Medical School and the Children's Hospital, Boston and previously held an ARC Postdoctoral Fellowship. Currently, his research is focused on the signaling and gene circuitry driving neural differentiation of embryonic stem cells and pre-implantation embryo development. His lab is located at the Centre for Developmental and Regenerative Medicine, Kolling Institute, Royal North Shore Hospital.

He is a also a member of the Bosch Institute, University of Sydney, and the executive committee of the New South Wales Stem Cell Network.

Speaker: Dr Tim Thomas - Walter and Eliza Hall Institute of Medical Research

The Role of the MYST Family of Chromatin Regulators in Stem Cells

The regulation of gene expression requires co-activator complexes. Co-activators are large multi-protein complexes and typically contain at least one enzyme subunit that has chromatin modifying activity, such as histone acetyltransferase activity. Since normal development is dependent on precise regulation of time/space patterns of gene expression it is not surprising that co-activators are intimately associated with the regulation of embryonic development. The MYST family of histone acetyltransferases, with five members the largest family in humans, has a diverse range of functions in embryonic development and, perhaps not surprisingly, is required in adult stem cells.

Monocytic leukaemia zinc finger protein (Moz, Myst3, Kat6a) is closely related to Querkopf (Qkf, Myst4, Kat6b), a co-activator we have shown previously to have function in neural development. By studying mouse strains carrying mutations in *Moz* and *Qkf* we have shown that these genes have essential functions in adult stem cell populations; Moz is essential for the development of definitive hematopoietic stem cells whereas Qkf is essential for the maintenance of adult neural stem cells. The function of Qkf in adult neural stem cells shows strong parallels to the function of Moz in haematopoietic stem cells. These results show that, surprisingly, these closely related co-activators have highly specific, non-redundant, functions in discreet stem cell populations.

More recently we have studied the function of the MYST family histone acetyltransferase, HBO1 (MYST2, KAT7) during mouse embryonic development. HBO1 is thought to be involved in regulating DNA replication, however our studies have shown that HBO1 is entirely dispensable for cell proliferation. In contrast, we show that HBO1 is an essential activator for a number of key regulatory genes controlling lineage specification in the post-gastrulation embryo. Our in vitro studies using stem cells suggest that HBO1 is essential for activation of new patterns of gene expression as cells differentiate.

Dr Tim Thomas completed his Ph.D at Melbourne University. After post-doctoral positions in the Centre for Early Human Development at Monash Medical Centre and at the Max-Planck-Institute of Biophysical Chemistry in Göttingen, Germany, Tim established his laboratory at the Walter and Eliza Hall Institute of Medical Research in Melbourne in 2000. Tim is researching the transcriptional regulation of undifferentiated cell populations with particular emphasis on the regulation of chromatin structure both during embryonic development and in adult stem cell populations. Tim has published articles in *Development, Genes & Development and Developmental*

Speaker: Dr Yoji Kojima - Children's Medical Research Institute

Pluripotency in Epiblasts and Epiblast Derived Stem Cells

Mouse epiblast stem cells (EpiSCs) are pluripotent stem cells derived from post-implantation embryos. EpiSCs differ from mouse embryonic stem cells (ESCs), which are derived from pre-implantation embryos, in several respects, but share many characteristics with human ESCs including colony morphology, pluripotency marker genes expression, cytokine requirement for self renewal and low contribution rate for chimera formation.

EpiSCs lines studied to date have been derived from early post-implantation stage embryos (around 5.5 to 5.75 days post coitum), prior to gastrulation. The signal requirements to differentiate EpiSCs into certain germ layer lineages are known to be distinct from those of mouse ESCs reflecting the difference between the character of the cells of the developmental stages they are derived from. This brought an hypothesis that the stem cells derived from later stages of deveelopment might maintain the characteristics of their origin.

Recently, we have shown that EpiSCs lines can be isolated from the epiblast of mouse embryos developed well into gastrulation (around 6.5 to 7.5 days post coitum). These lines also share the features of EpiSCs with similar morphology, marker genes expression of pluripotency, and the ability to form teratoma with three germ layer derivatives.

In my talk I will compare the characteristics of EpiSCs derived from gastrula-stage embryo with those

Dr Yoji Kojima is a research fellow in the Embryology Unit at Children's Medical Research Institute (CMRI) headed by Professor Patrick Tam. From his working experience as a clinical cardiologist, Dr Kojima recognizes the need to develop alternative paradigm to promote tissue repair and regeneration to treat cardiac diseases, and to gain further understanding of cardiac development to control the differentiation of the stem cells more precisely for clinical use, which underpinned his PhD study at Kyoto University, Japan. Dr Kojima joined embryology unit at CMRI after he obtained his PhD, and he is now focusing in earlier stages of developmental process, and explores the key mechanisms that lead the transition of the pluripotent state of the epiblast into three germ layer lineages, using the mouse epiblast derived stem cells (EpiSCs) as the experimental model. In particular, Dr Kojima investigates how the epiblast cells lose their pluripotent state and the cell fates are committed.

Speaker: Dr Stuart Fraser - University of Sydney

An Unexpected Role for the Copper Transporter Ctr1 in Embryonic Stem Cell Differentiation and Teratoma Formation.

Copper is a trace element essential for cell metabolism and function. Copper transporter-1, Ctr1 (encoded by *Slc31a1*) is the predominant cell surface transporter of copper into the cell. Recently, we have shown that *Xenopus* mesoderm development requires FGF signaling via Ctr1. Null mutation of *Ctr1* results in early embryonic lethality and defective mesoderm development in the mouse. We have examined the role of *Ctr1* in development using *in vitro* differentiation of wild type (WT), *Ctr1(+/-)* and *Ctr1(-/-)* embryonic stem (ES) cells. *Ctr1(-/-)* embryoid bodies (EBs) differentiate poorly *in vitro*. Differentiating *Ctr1(-/-)* ES cells down-regulate E-cadherin expression but fail to up-regulate surface expression of the mesoderm marker Flk1 and exhibit poor mesodermal development. Gene expression analysis revealed that *Ctr1(-/-)* EBs maintain transcription of stem cell markers such as *Oct3/4*, *Nanog* and *Rex1*, even 15 days after removal from LIF. We evaluated the impact of *Ctr1* gene dosage on teratoma formation. The single resectable teratoma derived from numerous injections of *Ctr1(-/-)* ES exhibited poorly differentiated histology in comparison to the variety of cell types present in the WT ES-derived teratomas. *Ctr1(+/-)* ES cells can differentiate successfully *in vitro* but failed to form teratomas. The loss of a single allele of *Ctr1* is therefore sufficient to ablate all teratoma activity while still allowing the ES to differentiate normally *in vitro*. Surprisingly, ES cells of all three genotypes were able to contribute to the formation of embryos in vivo, following injection into blastocysts. These data demonstrate that *Ctr1* is a crucial regulator of ES cell differentiation and teratoma formation.

Dr Stuart Fraser completed his B.Sc (Honours) in Immunology at Monash University in 1993 and a Ph.D in Biochemisty from the University of Hong Kong in 1998. He then spent 4 years in the Department of Molecular Genetics at Kyoto University as a post-doctoral fellow with Professor Shin-Ichi Nishikawa. It was during this time that he developed his interest in mesoderm and haematopoietic development using the mouse embryo and embryonic stem cells as model systems. Following two years in the University of Mainz, Germany developing transgenic reporter model mouse, Dr. Fraser moved the Mount Sinai School of Medicine as Assistant Professor. Here, he investigated the development of the first haematopoietic lineages to appear in the developing mouse embryo at the cellular and molecular levels. Dr. Fraser joined the Discipline of Physiology as a Lecturer in 2010.

Speaker: Dr David Leobel - Children's Medical Research Institute

Control of epithelial organization and differentiation of endoderm

The definitive or gut endoderm forms at gastrulation and contributes to the epithelial linings of the lungs, stomach, oesophagus and trachea, as well as organs including the liver, pancreas, thyroid and thymus. In a microarray study designed to detect genes that are preferentially expressed in the endoderm in early somite stage mouse embryos, we identified *Rhou*, which encodes a Cdc42-related atypical Rho GTPase that influences actin organization in cultured cells. *Rhou* is expressed in the columnar endoderm epithelium lining the lateral and ventral wall of the anterior intestinal portal in early-somite to early-organogenesis stage mouse embryos. During foregut development, *Rhou* is down-regulated in regions where the epithelium becomes multilayered prior to the budding of organ primordia. Embryonic stem (ES) cell lines in which *Rhou* was stably knocked down were tested for their ability to differentiation as embryoid bodies (EBs) in the presence of Activin A. *Rhou*-deficient cells in the EBs displayed abnormal F-actin localization and loss of apical ZO-1 staining at tight junctions. This was accompanied by impaired differentiation of endoderm derivatives and reduced expression of c-Jun/AP-1 target genes, consistent with a role for Rhou in regulating JNK activity. In embryos generated from *Rhou* knockdown embryonic stem cells, the embryonic foregut displayed an abnormally flattened shape. The epithelial architecture of the endoderm was disrupted, the cells were depleted of microvilli and the F-actin content of their apical domain was reduced. Down-regulation of *Rhou* in individual endoderm cells resulted in an impaired ability of these cells to occupy the apical territory of the epithelium. Our findings highlight epithelial morphogenesis as a required intermediate step in the differentiation of endoderm progenitors.

Dr David Loebel is the Kimberley-Clark Research Fellow in the Embryology Unit at the Children's Medical Research Institute (CMRI) in Westmead. His research interests encompass the development and differentiation of the gut endoderm, the genetic networks underlying limb and craniofacial development and transcriptomics of embryo-derived pluripotent cells. His research uses a combination of mouse embryo and in vitro models. Dr Loebel completed his PhD at Macquarie University, working on DNA methylation and X-chromosome inactivation in marsupials before moving to the UK in 1997 to work at the Marie Curie Research Institute, studying the proteins that replicate DNA in fly embryos. Pursuing an interest in early mammalian development, he returned to Australia and joined Professor Patrick Tam's laboratory at the CMRI in 2000.

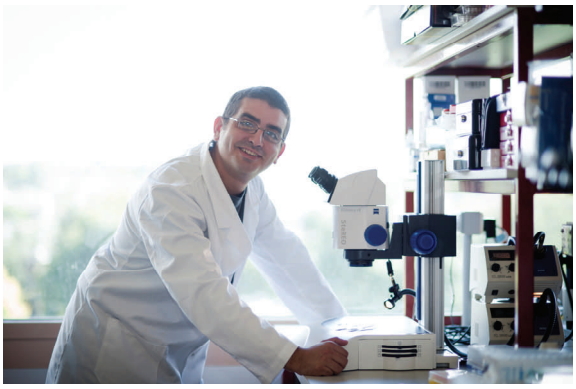
Speaker: Dr Samir Taoudi - Walter and Eliza Hall Institute of Medical Research

Haematopoietic Stem Cell Development

Hematopoietic development occurs sequentially: First, primitive nucleated erythroid cells are generated in the yolk sac (YS); next, hematopoietic progenitors are formed in both the YS and embryo body, after which hematopoietic stem cells (HSCs) emerge within the aorta-gonad-mesonephros (AGM) region. From this point onwards, definitive hematopoiesis (self-sustaining lifelong hematopoiesis) is driven by the HSCs.

Many regulators of hematopoiesis have been described, the absence of which results in failure of hematopoietic specification or inadequate formation of multipotential progenitors. However, it is not possible to resolve whether these factors specifically regulate HSC development or if they are required for general hematopoietic function. At the core of this issue is uncertainty about whether the mechanisms controlling hematopoietic specification, HSC formation, and HSC function are common or if distinct regulatory mechanisms are successively employed. During this talk, we will provide evidence that ERG is at the center of a distinct regulatory program that is not required for hematopoietic specification or differentiation but is critical for HSC maintenance during embryonic development.

Dr Samir Taoudi undertook his PhD studies with Professor Alexander Medvinsky in the United Kingdom at the MRC Centre for Regenerative Medicine (University of Edinburgh) where he studied the cellular basis of haematopoietic stem cell (HSC) emergence during embryogenesis. In 2008, Dr Taoudi was recruited to the Hilton laboratory at the Walter and Eliza Hall Institute where he investigated the role of the transcription factor ERG in control of HSC maintenance. In 2011, Dr Taoudi accepted a faculty position at the Walter & Eliza Hall Institute where he has established a laboratory which focuses on the regulatory mechanisms of haematopoietic development.



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