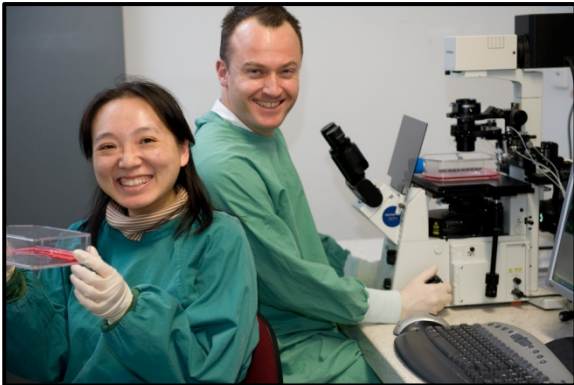


Research Centres and Projects

Centre for Reproduction & Development



Centre Director: Professor Justin St. John

Research at the Centre for Reproduction and Development focuses on understanding how human disease is propagated and transmitted using innovative reproductive and developmental biology approaches. Current work in the stem cell field involves understanding how amnion stem cells can be used for transplantation purposes and how somatic cells can be reprogrammed to behave as embryonic stem cells do. We also have a major interest in how mitochondrial DNA mutations accumulate and are transmitted.

Furthermore, our expertise in hormonal function and immune responses in the male reproductive system is also providing insights into areas such as cardiology and gastroenterology at Southern Health to help address problems such as inflammatory disorders and organ transplant rejection. Understanding the underlying mechanisms of disease will enable us to provide direction for the therapies of tomorrow. Projects on offer in the Centre explore testis development, testicular immunology and inflammation, potential therapeutic applications of human amnion stem cells, differentiation and re-programming of human embryonic stem cells, bovine cloning, nuclear transfer and the transmission of mitochondrial DNA in animal models and embryonic stem cells.

Understanding how mitochondrial DNA is propagated through stem cell models of mitochondrial DNA disease

Project Leader: Prof. Jus St. John
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Project Description:

Mitochondrial DNA (mtDNA) encodes key genes associated with the generation of the majority of cellular ATP through oxidative phosphorylation. However, single point mutations to mtDNA result in the loss of ATP output leading to cellular impairment and the onset of severely debilitating neurodegenerative and muscular disorders. In this project, we will take skin cells from patients with a mtDNA mutation and induced these to become stem cell-like, a process known as induced pluripotency. Once fully characterised, we will differentiate these cells into neuronal precursor cells and at various points of subsequent differentiation determine how and when the mutation affects neuronal function. We will also analyse mtDNA replication events to determine how mutant mtDNA molecules are selected during the process of differentiation.

Along with learning how to generate induced pluripotent stem cell models of disease you will learn stem cell culture techniques, real time PCR and RT-PCR, Western blotting, immunocytochemistry and confocal microscopy, and siRNA and expression vectors to downregulate and to overexpress key genes associated with mtDNA replication. You will also use DNA methylation assays to determine how the expression of key mtDNA replication factors is regulated and 2-D agarose gels to analyse modes of mtDNA replication.

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The generation of heteroplasmic models of mitochondrial DNA disease

Project Leader: Prof. Jus St. John
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Project Description:

The mitochondrial genome (mtDNA) encodes some of the proteins that constitute the electron transfer chain (ETC), which generates the vast majority of cellular ATP through the process of oxidative phosphorylation. Mutations and deletions to mtDNA can be either severely debilitating or even lethal. These outcomes are dependent on the ratio of mutant to wild type molecules (heteroplasmy) present in the affected cells. There is currently a shortage of models to understand how mutant molecules are selected for, segregate and transmitted from one generation to the next. The aim of this project is to generate models of mtDNA disease in order to track the fate of mutant molecules in a time dependent manner. You will learn basic embryology techniques along with microinjection skills in order to make the heteroplasmic models. You will then use real time PCR to analyse the degree of heteroplasmy and how this changes over time; and RT-PCR, Western blotting, immunocytochemistry and confocal microscopy and immunohistochemistry to determine how the heteroplasmy affects the expression of the genes of the electron transfer chain. You will also perform metabolic assays to determine the effects on tissue and organ function.

Understanding the regulation of mitochondrial DNA copy number in undifferentiated and differentiating embryonic stem cells

Project Leader: Prof. Jus St. John
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Project Description:

Mitochondria are organelles within a cell that are responsible for generating the vast majority of cellular energy, ATP. This process, known as oxidative phosphorylation, takes place in the electron transfer chain and is the only cellular apparatus dependent on proteins produced from both the chromosomal and mitochondrial genes (mtDNA). mtDNA is replicated by nuclear-encoded factors necessitating a high degree of cooperative interaction between the nucleus and the mitochondria. This is especially so as different cell types possess different numbers of mtDNA copies to meet their specific requirements for energy. Little is known about the mechanisms regulating mtDNA copy number and replication during development and cellular differentiation. This project aims to define the key mtDNA replication events during differentiation by using undifferentiated and differentiating embryonic stem cells. Along with learning stem cell culture techniques, you will use real time PCR, Western blotting, immunocytochemistry and confocal microscopy, and siRNA and expression vectors to downregulate and to overexpress key genes associated with mtDNA replication. You will also use DNA methylation assays to determine how the expression of key mtDNA replication factors is regulated and 2-D agarose gels to analyse modes of mtDNA replication.

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The regulation of mitochondrial DNA copy number in undifferentiated and differentiating human amnion epithelial stem cells

Project Leaders: Prof. Jus St. John & Dr. Ursula Manuelpillai
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Project Description:

Adult stem cells are harvested from specific tissues and can differentiate into cell lineages associated with that tissue. They also have the potential to transdifferentiate into other lineages. For example, adipocytes can give rise to pancreatic B-islet cells; mammary gland stem cells can populate the prostate with prostate-like cells; and amnion epithelial cells have the potential to give rise to specialised cells, such as hepatocytes and neurons. Although these transdifferentiated cells express genes associated with and morphologically resemble the new lineage, we do not know whether they are capable of generating sufficient cellular energy to meet the cell's specialised functions.

Nearly all eukaryotic cells possess mitochondria, which generate the vast majority of cellular energy, ATP, through a process known as oxidative phosphorylation (OXPHOS). OXPHOS takes place in the electron transfer chain, which is dependent on proteins generated from both the chromosomal and mitochondrial genes (mtDNA). During differentiation, cells acquire the appropriate numbers of mtDNA to match their requirements for OXPHOS in order that they can perform their specialised functions. However, little is known about the mechanisms regulating mtDNA copy number and replication as adult stem cells differentiate into mature cells. Furthermore, we need to determine whether those adult stem cells with the capability to transdifferentiate have the potential to acquire the appropriate copies of mtDNA to match cellular function. This project will determine how mtDNA is replicated as adult stem cells differentiate into

mature cells and whether their ability to transdifferentiate is coupled to appropriate regulation of mtDNA copy number. Along with learning stem cell culture techniques, you will use real time PCR, Western blotting, immunocytochemistry and confocal microscopy, and siRNA and expression vectors to downregulate and to overexpress key genes associated with mtDNA replication and cellular differentiation. You will also use DNA methylation assays to determine how the expression of key mtDNA replication factors is regulated and 2-D agarose gels to analyse modes of mtDNA replication

Centre for Reproduction & Development

Immunogenicity of transdifferentiated human amnion epithelial stem cells

Project Leader: Dr. Ursula Manuelpillai
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Project Description:

Human amnion epithelial cells (hAEC) line the amnion membrane attached to the placenta. We have shown that hAEC isolated from term delivered placentae have properties of pluripotent and multipotent stem cells and transdifferentiate into several lineages including hepatocytes, type II alveolar epithelial and pancreatic cells. Transplantation of these cells could offer immense benefits to patients with chronic liver and lung diseases and type I diabetes. As a pre-requisite to cell transplantation, we have been investigating potential immune responses that could be elicited by the transdifferentiated hAEC in human recipients. Recent data from our lab shows that the expression of HLA antigens and co-stimulatory molecules that could induce immune responses differs significantly among different cell lineages derived from hAEC. The mechanisms leading to this differential expression is unknown.

This study will determine the immunogenicity of pancreatic, hepatic and type II alveolar epithelial cells using *in vitro* assays and following transplantation into mice. The role of pro-inflammatory cytokines including interferon gamma in inducing / suppressing HLA antigens, co-stimulatory molecules and other allo-antigen recognition molecules will also be investigated. The study will involve human amnion stem cell isolation, transdifferentiation of hAEC and their characterisation, allogeneicity assays, FACS, xenotransplantation and molecular techniques.

Immunosuppressive properties of primary and transdifferentiated human amnion epithelial stem cells

Project Leaders: Dr. Ursula Manuelpillai & Dr. James Chan (Dept. of Medicine)
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Project Description:

The expansion of T-cells induced by auto-antigens can lead to the onset of auto-immune diseases including multiple sclerosis and type I diabetes. Factors that could suppress T-cell proliferation are being widely tested to combat these diseases. Undifferentiated, bone marrow derived mesenchymal stem cells have been shown to suppress T-cell proliferation and their effects on auto-immune diseases are being currently evaluated. We have shown that human amnion epithelial cells (hAEC) isolated from term delivered placenta display features of pluripotent and multipotent stem cells and have transdifferentiated the hAEC into several lineages. More recently we showed that pancreatic cells derived from hAEC inhibit T-cell proliferation *in vitro*. In the case of type I diabetes, the transplantation of functional, insulin secreting pancreatic beta-islet cells that could also suppress T-cell proliferation would be a major advancement. hAEC are extremely plentiful and easily obtained from placental tissue and therefore offer a better alternative to bone marrow derived stem cells. In this study we will evaluate the immunosuppressive effects of primary hAEC and pancreatic cells derived from these cells on different human T-cell populations, the cellular mechanisms and secreted factors that are involved in the suppression of T-cell proliferation.

Techniques employed will include human amnion stem cell isolation, transdifferentiation of hAEC and their characterization, isolation of human T-cell sub-populations, T-cell proliferation assays, FACS, Luminex / multikine protein assays and gene knock down assays.

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Human amnion epithelial stem cells for the treatment of liver cirrhosis

Project Leaders: Dr. Ursula Manuelpillai & A/Prof. William Sievert (Dept. of Medicine)
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Project Description:

Persistent inflammation arising from a wide variety of causes including alcohol, drugs and viruses lead to liver fibrosis and ultimately to cirrhosis with extensive collagen deposition and widespread loss of hepatocytes that perform the vital functions in the liver. These diseases pose major global health burdens with over 450 million people having viral and alcohol related liver fibrosis and cirrhosis. Stem cell based therapies aimed at reducing hepatic tissue inflammation and fibrosis are currently being tested. Recently, we transplanted undifferentiated, primary human amnion epithelial cells (hAEC) into immunocompetent mice with liver fibrosis and showed that hAEC engraft, escape host immune detection and importantly reduce hepatic tissue inflammation and collagen deposition. In previous studies we have shown that hAEC isolated amnion membranes of term delivered placenta possess stem cell like characteristics. Strategies aimed at achieving optimal hAEC engraftment in the liver, the efficacy and safety of hAEC transplantation will be tested in mice with extensive liver fibrosis that is more representative of cirrhosis. The interactions between hAEC and the hepatic cell populations will be investigated using *in vivo* and *in vitro* approaches to elucidate mechanisms leading to hepatocyte regeneration, reduced inflammation and fibrosis with a view to developing targeted therapies. Techniques employed will include hAEC isolation and characterization, labelling and tracking of hAEC, xenotransplantation into diseased mice, immunohistochemistry, FACS, in-direct and direct co-cultures, molecular techniques and ELISA.

Cell reprogramming

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Project Description:

Embryonic stem cells (ESCs) can potentially generate specific cell types for regenerative medicine. A major problem limiting the clinical use of ESCs is the potential for tissues derived from these cells to be rejected by receiving patients. The most attractive solution to this problem comprises transplanting tissues derived from ESCs genetically matched to each patient.

Somatic cell nuclear transfer (SCNT), where an adult somatic cell is returned to an embryonic state (a process called reprogramming) following transplantation to an enucleated oocyte, can be used to provide such cells, however, ethical and practical limitations associated with both oocyte donation and human SCNT raise serious concerns about the suitability of this method. An alternative approach to reprogramming cells involves forced expression of a few key stem cell genes in somatic cells, also known as induced pluripotent stem cells (iPSCs). The following projects examine the potential to improve reprogramming of somatic cells and generation of functional cells.

1. Generation of iPSCs without genetic modification.
2. Derivation of ESCs from non-obese diabetes (NOD) mice, as a model for diabetes research.
3. Differentiation of ESCs and iPSCs to functional β -cells and hepatocytes.
4. Improving reprogramming efficiency of somatic cells by altering expression of transcription factors.

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Understanding the novel phenotype of testicular macrophages

Project Leaders: A/Prof. Mark Hedger & Dr. Wendy Winnall
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Project Description:

Mouse testicular macrophages have several unique functional properties associated with testicular immune privilege, but remain poorly characterised. We have recently undertaken a microarray and real-time PCR analysis to compare these cells from rats and mice to macrophages from other tissues. This project will involve a broad range of computing, molecular and cellular techniques: the use of GeneSpring software to analyse genomic data, cell culture to isolate and purify macrophages from mice, real-time PCR, protein expression analysis and assays for specific cell functions. This project is a collaboration with the Centre for Innate Immunity and Infectious Disease.

Investigating the role of Sertoli cells and Toll-like Receptors in testicular inflammation

Project Leaders: A/Prof. Mark Hedger & Dr. Wendy Winnall
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Project Description:

Inflammation and infection in the male tract compromise androgen production and male fertility. Sertoli cells are epithelial cells in the testis, which regulate spermatogenesis, but these cells are capable of responding to inflammatory stimuli and help protect the testis from damage due to infection. This project examines Sertoli cell responses to bacterial and viral molecules, and the emerging role of the Toll-like receptors and their signalling

pathways and products in testicular disease and in normal function. These studies involve both in vivo and in vitro approaches, employing a range of morphological, immunological and molecular techniques.

Activin and follistatin in inflammatory diseases and immunity

Project Leaders: A/Prof. Mark Hedger & Dr. Wendy Winnall
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Project Description:

Activin is a growth factor and cytokine produced by many cells in the body. We have made an exciting discovery that activin is released very rapidly as an 'alarm' cytokine in response to inflammatory signals. Studies have shown that follistatin, which is the binding protein for activin, has potential therapeutic applications for controlling inflammatory diseases, such as idiopathic lung fibrosis, septicaemia and testicular autoimmunity. Work is currently focussed on how this system operates, and whether that knowledge could be used to develop new screening methods for patients or new classes of medical treatments.

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Fetal germ cell differentiation and testis cancer

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Male fetal germ cells are the precursors of spermatogonial stem cells that differentiate to form sperm. Failure of fetal germ cell differentiation can lead to testis cancer, the most common malignancy in young men. Primordial germ cells are specified from the pluripotent epiblast and share features with pluripotent stem cells, including the expression of key genes that regulate developmental potency. Undefined signalling pathways induce the primordial germ cells to form the male germ cell lineage, a process that involves their entry into mitotic arrest and suppression of the core regulators of pluripotency, *Oct4*, *Sox2* and *Nanog*. Disruption of male germ cell differentiation can result in maintained pluripotency, failed mitotic arrest and germ cell tumour formation. Despite this, our understanding of male germ cell development remains limited. Our work is focused on identifying the signalling pathways controlling male germ cell differentiation, their entry into mitotic arrest and the suppression of pluripotency. By combining analyses of germ cell differentiation in the mouse and candidate germ cell tumour genes in human testis cancer patients we aim to determine how germ cell differentiation is disrupted during the reacquisition of pluripotency and the formation of testis cancer.

Project Description:

This project aims to examine the molecular processes controlling male germ cell differentiation in the mouse model. We have previously used expression micro-array profiling and bioinformatic analyses to identify genes associated with fetal male germ cell development. To isolate pure populations of germ cells we use a germ cell specific transgenic mouse model combined with fluorescent activated cell sorting. This model also allows direct visualisation of germ cells in organ culture experiments. This project will involve the use of *ex-vivo* organ culture technologies combined with

specific small molecule chemical inhibitors to disrupt germ cell development. Analysis of samples generated in these experiments will involve the use of established technologies including flow cytometry and immunostaining. Furthermore, in order to test the pluripotent potential of the germ cells manipulated in the organ culture system this project aims to use these germ cells to establish pluripotent cell cultures. This work is expected to yield substantial insight into the molecular mechanisms controlling normal germ cell development and to identify genetic pathways that are misregulated in germ cell tumours.