ABSTRACTS

NOVEMBER

2024

ASSCR MEETING

Stem Cell Research from Roots to Remedies

Adelaide, SA

Welcome to the 2024 Annual Meeting of the Australasian Society for Stem Cell Research (ASSCR). This year's theme reflects on the continuum of our field, from foundational science to life-changing therapies. With a remarkable roster of speakers from around the world, this gathering is designed to spark ideas, build connections, and inspire collaborations that will shape the next era of stem cell research.

CONNECT • COMMUNICATE • COLLABORATE

HOPX associated molecular programs control cardiomyocyte cell states underpinning cardiac structure and function

Ms Sumedha Negi[1], Dr Clayton Friedman[1,2], Dr Seth Cheetham[2], Nathan Palpant[1]
[1]Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia, [2]Institute for Stem Cell and Regenerative Medicine, University of Washington, Seattle, USA, [3]Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, Australia

Biography:
Sumedha is a PhD candidate at the Palpant Lab, University of Queensland, specializing in the intersection of stem cell disease modeling and statistical genetics. Her research is focused on characterizing variants related to dilated cardiomyopathy. Sumedha is driven by her passion for interdisciplinary research and translational outcomes.

Aims: Cardiac development requires temporal orchestration of the genome to enable stage specific changes in cell identity and function. These molecular changes underpin cell and organ-level physiology. HOPX (Homeodomain-only-protein-X) is a non DNA binding transcription factor known to be an early specifier of cardiomyocytes. However, the gene programs upstream and downstream of HOPX as well as physiological consequences of perturbating these programs remain unknown.

Methods: In this study we use CRISPR- mediated HOPX loss-of-function iPSC derived cardiomyocytes combined with multiomics such as transcriptomics (CAGE-seq) and genomwide footprinting (DamID-seq) to identify gene programs and chromatin architecture regulated by HOPX. We link HOPX-dependent loci in iPSC cardiomyocytes to complex cardiac traits through population genetics. We evaluate the physiological impact of HOPX loss-of-function in vitro through iPSC cardiomyocytes and organoids. We corroborate these findings *in vivo* using zebrafish.

Results: We show that HOPX suppresses cardiac progenitor gene programs and promotes cardiomyocyte maturation gene programs. Mapping HOPX-dependent loci to human complex trait genetic data, we show that HOPX-regulated enhancers and protein coding genes are primarily associated with variation in cardiac outflow tract traits and ventricular function traits, respectively. Given the centrality of HOPX in mediating cardiomyocyte cell identity, we evaluated mechanisms controlling transcription of HOPX. These data show that cell growth and mitogenic pathways compete for control of HOPX transcription and mitogenic pathways over-ride cell growth signals to control transcription of HOPX. Physiologically, we show that HOPX expression is required for maturation of cardiac electro-mechanical coupling and contractility. Lastly, in a zebrafish model of heart regeneration, we show that cardiomyocyte proliferation in the injury zone is dependent on suppression of HOPX.

Conclusion: Overall, this study provides a mechanistic link situating HOPX between competing upstream pathways where it acts as a molecular regulator of gene programs underpinning heart development and physiology.

Rising Star Award
Oral Presentation – Monday 11th November

Human heart valve tissue from pluripotent stem cells for treatment of heart valve diseases

Dr Holly Voges[1,2,3], Ms Jessica Durrant-Whyte[1,3], Mr Michael See[1,3], Dr Adam Piers[1,3], A/Prof Benjamin Parker[2], Ms Kaitlyn Bibby[1,3], Ms Ellen Keen[1,3], Dr Fernando Rossello[1,3], A/Prof Mirana Ramialison[1,3], Prof Enzo Porrello[1,3], Dr Alejandro Hidalgo[1,2,3]
[1]Murdoch Children's Research Institute, Melbourne, AUSTRALIA, [2]Department of Paediatrics, School of Medicine, Dentistry and Health Sciences, University of Melbourne, Melbourne, AUSTRALIA, [3]Novo Nordisk Foundation Center for Stem Cell Medicine, Murdoch Children's Research Institute, Melbourne, AUSTRALIA

Biography:
Dr Holly Voges is a Heart Foundation Postdoctoral Fellow and Senior Research Officer at the Murdoch Children's Research Institute. She obtained her PhD from the University of Queensland in 2019 focused on investigating innate regenerative potential and the role of cell-cell interactions in heart maturation. Her current research focuses on bioengineering heart tissue for treatment of heart disease. Her work has been recognised by multiple awards and distinctions including the Shirley E Freeman award from the Heart Foundation and publication prizes from the International Society of Heart Research (2023) and Stem Cells Australia (2017).

Heart valves are a critical internal structure that facilitate unidirectional blood flow which is important for maintaining cardiac output. Heart valve diseases are a rising cause of global cardiovascular mortality that has a projected disease burden expected to reach 4.5 million people in 2030. End stage heart valve disease is treated surgically and rely on biological prosthetics that have different composition and fibre organisation to native tissue.

AIM: Our aim is to generate a precision-made pluripotent stem cell-derived valve tissue to overcome the limitations and shortcomings of animal valve prosthetics.

METHOD: We have developed a directed differentiation protocol to derive heart valve interstitial cells that express key valve proteins including COL1A1, Vimentin and SOX9. Using tissue engineering approaches we generate self-organising large clinically relevant sized tissues called valve engineered tissue (VET) that exhibit high extracellular matrix deposition.

RESULT: Single cell RNA-sequencing revealed that VETs are comprised of valve interstitial cells and immune cells that mimic the cell identity and extracellular matrix of native tissue. Proteomic characterisation demonstrated that 98% of proteins detected in native valve are expressed in VETs, showing a similar global protein profile. In addition, we have determined the physical properties of engineered valve tissues using uniaxial stretch testing to test for clinical utility. Lastly, we have tested *in vivo* transplantation feasibility through subcutaneous implantation and found the absence of calcification.

CONCLUSIONS: Together, this study describes progress towards a stem cell-derived cellular therapy to treat patients with heart valve disease.

Oral Presentation – Electrifying Abstracts Tuesday 12th November

Our Moon's Mission: Brain organoids as a platform to advance AAV9 gene therapy to clinical trials: a hereditary spastic paraplegia 56 case study

Dr Hannah Leeson[1], Dr Connie Petroeschevsky[2], Prof Elizabeth Gillam[2], Prof David Coman[3], Prof Ernst Wolvetang[1]

[1]Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, St Lucia, Australia, [2]School of Chemistry & Molecular Biosciences, The University of Queensland, St Lucia, Australia, [3]School of Medicine, The University of Queensland, St Lucia, Australia

Biography:
Dr Hannah Leeson is an ECR specialising in using patient-derived stem cells and brain organoid models to investigate rare neurological conditions, and to develop and test therapeutics. She is currently implementing these organoid platforms for the pre-clinical testing of a novel gene therapy for Hereditary Spastic Paraplegia 56, and she leads a drug screening project to find appropriate anti-seizure medications for patients with drug resistant or intractable epilepsy. This work facilitates translation of scientific observation to clinical intervention, and she works closely with clinicians and families with the end goal of improved patient outcomes.

AIMS: Unreliable mouse models have been the downfall of many promising therapies for neurological disease. Here, for the first time we conduct safety and efficacy trials of an AAV9 gene therapy for hereditary spastic paraplegia 56 (SPG56) in patient-derived brain organoids, to more accurately recapitulate this neurological disorder and with the added objective of determining disease mechanisms.

METHODS: SPG56 is a rare neurodegenerative disorder characterised by early onset spasticity and axonal neuropathy. It is caused by mutations in CYP2U1, a CYP450 enzyme involved in long chain fatty acid metabolism. We generated iPSCs from two SPG56 patients and their heterozygous parents, and used these lines to generate neuronal and brain organoid cultures. We subsequently treated brain organoids with EF1α-CYP2U1-AAV9 and measured CYP2U1 enzymatic activity, mitochondrial function, and conducted transcriptomic, proteomic and metabolomic analysis. Mouse trials were performed in parallel to explore the potential of organoids as a pre-clinical platform.

RESULTS: We found that CYP2U1mut organoids and neuron cultures had impaired neuronal morphology (fragmented axons) and electrical function, reduced energy generation (altered Kreb cycle metabolites and down regulated fatty acid β-oxidation pathways), and impaired mitochondrial properties, including respiration. Treatment with AAV9 gene therapy increased CYP2U1 expression in target cell types, restored neuronal processes and significantly altered levels of Krebs cycle metabolites and gene expression make-up.

CONCLUSIONS: By comparing brain organoid data to data obtained from CYP2U1-AAV9 mouse trials conducted in parallel, we not only provide the data required to progress to clinical trials in a small number of patients, but are also validating the use of brain organoids in the pre-clinical gene therapy setting. The approved use of in vitro organoid systems in clinical trials reduces the barriers to gene therapy for many rare neurological diseases and is a key objective of Our Moon's Mission.

Oral Presentation – Electrifying Abstracts Tuesday 12th November

Re-assessment of clinically tested drugs using sporadic MND patient-derived motor neurons concords with clinical trial outcomes.

Elizabeth Qian[1], Katherine Lim[1], Bảo Trần-Lê[1], Bradley Turner[1], Thanuja Dharmadasa[1], **Christopher Bye**[1]
[1]Florey Institute of Neuroscience and Mental Health, Melbourne, AUSTRALIA

Biography:
Dr Bye leads the MND Drug Screening & Precision Medicine Program at the Florey Institute of Neuroscience. Focussing on the predominant sporadic form of MND/ALS, his team has generated one of the largest MND iPSC library in the world (> 100 lines), a world-leading model of sporadic MND recapitulating the hallmark degeneration of motor neurons, and the first phenotyped MND iPSC library combining comprehensive phenotypic, genetic and transcriptional profiling. His team are now using large-scale population level drug screening (across 15+ sporadic MND donors) to identify new therapeutics for people with MND.

Despite over 150 drugs reaching clinical trial for MND over the past 30 years, the prognosis for patients has not changed. Drugs developed using existing MND models have consistently failed to translate into effective treatments for patients, presenting a fundamental roadblock in the pursuit of new therapies. To develop an alternative drug discovery pathway for MND, we built an iPSC library from over 100 patients and successfully established a sporadic MND model and drug screening pipeline. In a first-of-its-kind screen, we re-assessed the effect of all drugs that have previously undergone evaluation in Phase 1-3 clinical trials for MND across the sporadic patient population. Strikingly, 95% of the drugs tested did not show efficacy on sporadic patient-derived motor neurons, results that are consistent with the clinical trial outcomes for the drugs. Combinatorial testing of the effective drugs from the screen identified a drug combination with 6.5x higher efficacy than the current standard of care in sporadic patient-derived motor neurons. As the first treatment identified and tested in a sporadic MND model, this promising therapeutic candidate offers fresh hope for people with sporadic MND.

Oral Presentation – Electrifying Abstracts Tuesday 12th November

Nucleosome eviction at TET/TDG-targeted enhancers occurs independently of the removal of the chromatin mark Oxidized-Methylcytosine.

Marion Turpin[1], Thierry Madigou[1], Rachael Acker[1,2], Maud Bizot[1], Katie Sawvell[1,3], Stephane Avner[1], Audrey Laurent[1], Gaelle Palierne[1], Christine Le Peron[1], Gilles Salbert[1]
[1]Saigenci, Adelaide, AUSTRALIA, [2]University of Pennsylvania, Philadelphia, US, [3]Cincinnati Children's Hospital Medical Center, Cincinnati, US

Biography:
I successfully defended my PhD thesis in the field of chromatin remodeling and enhancer activation dynamics last April in Brittany, France. In May, I moved to Adelaide and started my postdoctoral fellowship to study the effect of chromatin on transcription factor target gene shaping across the genome.

Genome-wide CpG methylation patterns reflect the competing action of DNA methyltransferases (DNMTs) and demethylation mechanisms, relying in part on 5-methylcytosine (5mC) oxidation by Ten-Eleven-Translocation (TET) enzymes. CpG methylation patterns can reflect cell-specific transcriptional programs and besides depending on DNMTs and TETs, these patterns can also be regulated locally by the engagement of transcription factors (TFs). This can be observed at small genomic regulatory regions called enhancers which undergo 5mC oxidation upon TF binding and recruitment of TETs. However, it remains unclear how 5mC oxidation functionally relates to enhancers, and what are the mechanisms underlying the successive oxidative steps. In this respect, priming is linked to the first step of 5mC oxidation into 5-hydroxymethylcytosine (5hmC), and further oxidation into 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC) correlate with enhancer activation. However, it is unknown whether removal of oxidized bases (oxi-mCs) is required for enhancer activation. Elimination of oxi-mCs in cycling cells can either occur through a passive mechanism of cell division, or the recognition of 5fC/5caC as DNA damages and their active removal by the T:G mismatch DNA glycosylase (TDG)/base excision repair (BER) machinery. Here, we identified enhancers undergoing a TET/TDG-dependent full demethylation process during differentiation of pluripotent embryonal carcinoma cells into neural progenitor-like cells (NPCs), through genome-wide mapping of 5caC in TDG null cells. The impact of oxi-mC persistence on the chromatin structure of these enhancers was analysed by MNase-seq and -qPCR assays. We identified hundreds of enhancers undergoing TDG-dependent oxi-mC removal during differentiation but observed that these regions undergo nucleosome eviction even without oxi-mC removal. Hence, we conclude that neural enhancer activation is linked to oxi-mC occurrence but does not require their removal by TDG.

Using mature, mitochondria rich hiPSC-cardiomyocytes to study cardiotoxicity

Dr Bin Bin Wu[1], Mr Jack Chen[1], Ms Chloe Ma[1], **Ellen Poon**[1]
[1]The Chinese University of Hong Kong, Hong Kong, Hong Kong

Biography:
Dr. Ellen POON received her BSc degree from the University of Sydney, where she graduated with First Class Honours and University Medal. She then received a Commonwealth Scholarship for her DPhil research in the University of Oxford and was also a recipient of the American Heart Association Postdoctoral Fellowship in the University of California, San Francisco where she first started her training in pluripotent stem cell technology. She later joined the Chinese University of Hong Kong as an Assistant Professor. Dr Poon's research focuses on the maturation of hPSC-cardiomyocytes and the application of these cells for cardiotoxicity studies.

Aims: Doxorubicin (DOX) is commonly used to treat cancer, but can damage the mitochondria and cause cardiotoxicity. Dexrazoxane is the only clinically-approved intervention for DOX-induced cardiotoxicity (DCT), but it can compromise the anti-cancer efficacy of DOX. New drug discovery is limited by the lack of suitable human models and the need to balance cardioprotection and cancer control. We aim to establish a clinically relevant model of DCT and identify novel treatment.

Methods: We previously showed CD36 to be a surface marker of maturation. Generic human pluripotent stem cell derived (hPSC)-cardiomyocytes (CMs) sorted for high CD36 (CD36hi CM) have more mitochondria and, unlike conventional hiPSC-CMs, respond to dexrazoxane-mediated protection. Using patient-derived hiPSC-CD36hi CMs and *in vivo* animal model, we studied the cardioprotective effects of ICG-001, which is a small molecule inhibitor of dynamin-related protein 1 (DRP1) and the Wnt/β-catenin signalling. The effects of ICG-001 on cancer cells were also evaluated.

Results: Patient-derived CD36hi hiPSC-CMs had more mitochondria relative to mixed and CD36lo CMs, and recapitulated the effects of dexrazoxane. Using this model, we demonstrated that ICG-001 could protect against cardiotoxicity induced by DOX and its analogue, daunorubicin, and its cardioprotective effects were comparable to that of dexrazoxane. Unlike its effects in CMs, ICG-001 reduced the viability of a panel of cancer cells. Mechanistic studies showed that ICG-001 protected CMs by inhibiting DRP1 protein while it suppressed cancer cells by inhibiting Wnt signalling. Lastly, ICG-001 restored cardiac function and ameliorated the pathological features of DCT in a *in vivo* mouse model of this disease .

Conclusion: In conclusion, patient-derived CD36hi CMs is critical for the identification of new treatment against DCT. We further showed that ICG-001 can protect the heart and suppress cancer, and is therefore potentially superior to traditional treatment with dexrazoxane.

Oral Presentation - Tuesday 12th November

Time-resolved multi-omics illustrates the impact of DNA replication stress on chromatin integrity and pluripotency loss

Dr Osvaldo Contreras[1,2], Chris Thekkedam[1], Dr. David Humphreys[1], Dr. Ling Zhong[3], Gabrielle Smith[1,4], Nicholas Murray[1,2], Dr. Emily Wong[1,4], Dr. Joanna Achinger-Kawecka[5], Dr. Richard P Harvey[1,4]

[1]Victor Chang Cardiac Research Institute and UNSW Sydney, Sydney, Australia, [2]School of Clinical Medicine, Faculty of Medicine and Health, UNSW Sydney, Sydney, Australia, [3]Bioanalytical Mass Spectrometry Facility, Mark Wainwright Analytical Centre, Wallace Wurth Building, UNSW Sydney, Sydney, Australia, [4]School of Biotechnology and Biomolecular Science, University of New South Wales, UNSW Sydney, Sydney, Australia, [5]Garvan Institute of Medical Research, Sydney, Australia

Biography:
I am a cell biologist and biomedical researcher studying tissue regeneration and homeostasis loss in humans, with a focus on the stromal and stem cell compartments. I work on human stem cell-based models to enhance our understanding of heart development and congenital and adult heart disease under the supervision of Prof. Richard Harvey (FRS) at the Victor Chang Cardiac Research Institute. I also hold a Conjoint Lecturer position at UNSW Sydney's Faculty of Medicine and Health. I have secured >600,000 USD in governmental fellowships and awards as an early-career researcher and established numerous national and international collaborations and networks.

BACKGROUND: By controlling the rate of cell division and cell fate, the cell cycle plays a crucial role in tissue development, organ morphogenesis, and disease progression. Thymidine analogues are widely used to study DNA replication and cell proliferation. However, despite their potential cytotoxicity, the underlying molecular mechanisms are not fully understood.

AIM: To investigate DNA replication stress in human iPS cells and its impact on the cell cycle and cell identity, with a focus on its relationship with chromatin dynamics.

METHOD: We used a comprehensive time-resolved multi-omics approach encompassing 3D cell imaging, RNA transcriptomics, nucleosome occupancy by MNase-seq, CUT&RUN, untargeted proteomics (LC-MS/MS), and phospho-proteomic analysis.

RESULTS: Our results show that the thymidine analogue EdU induces replication stress, triggering H2A.XS139 foci and ATM/ATR-mediated DNA damage response (DDR) and DNA repair, as well as Checkpoint Kinase 2 activation. EdU-induced replication stress and DDR are associated with time-dependent impairments in cell-cycle progression, mitotic timing, and increased nuclear size. Our integrative multi-omics also show that genotoxic stress in hiPSCs drives premature pluripotency exit and stochastic germ layer differentiation into ectodermal, mesodermal, and endodermal cell lineages. These transitions are associated with enhanced lineage gene expression, reduced H3K27me3, and decreased binding of the Polycomb Repressive Complexes to chromatin, impaired nucleosome assembly, and nucleolar ribogenesis. Importantly, our analysis identified hundreds of uncharacterized proteins associated with these processes.

CONCLUSIONS: By describing novel molecular mechanisms of cell cycle regulation and chromatin dynamics, our findings have broad implications for both developmental and adult defects where DNA replication is impacted by genetic or environmental factors. These insights potentially affect cell cycle regulation, organ growth, and lineage identity.

Oral Presentation - Tuesday 12th November

Controlled differentiation of induced pluripotent stem cells using a 3D bioprinted system mimicking the mechanical and topographical cues of the extracellular matrix

Dr Sara Romanazzo[1], Dr Thomas Molley[2], Prof. Kris Kilian[3]
[1]School of Chemistry, University of New South Wales, Sydney, Australia, [2]School of Bioengineering, University of California, San Diego, San Diego, United States of America, [3]School of Materials Science and Engineering, University of New South Wales, Sydney, Australia

Biography:
Dr. Sara Romanazzo is currently a senior postdoctoral researcher in the Kilian and Gooding groups, where she is mainly focusing on developing in vitro 3D tissue models for different applications, including stem cell plasticity in response to various physicochemical stimuli. Her passion is to apply innovative engineering techniques and novel materials as a tool to understand fundamental biological questions, and to use that knowledge for regenerative medicine and drug discovery applications.

AIM: Induced pluripotent stem cells (iPSC) have the potential to differentiate into any cell type, making them a promising tool for tissue engineering and regenerative medicine. The efficiency and specificity of iPSC differentiation can be improved by integrating mechanical and topographical cues from the extracellular microenvironment. The extracellular matrix surrounding cells provides physical and biochemical signals that can influence cell behaviour.

During embryonic development, the formation of different germ layers is tightly regulated by complex signalling pathways and spatial cues, influenced by the position and orientation of neighbouring cells and tissues. Similarly, in-vitro iPSC differentiation can be enhanced by controlling the spatial organization of iPSCs within the extracellular microenvironment. The emergence of 3D bioprinting techniques opened the door to new opportunities for recreating in-vitro the natural 3D microenvironment surrounding cells.

METHODS: In this study, human iPSC were deposited in a controlled spatial and temporal manner within a jammed microgel suspension composed of gelatin-methacrylate gels with different microgel sizes and porosity, previously developed in our group.

RESULTS: The iPSC printing in the microgel suspension demonstrated the successful deposition of high-density iPSC aggregates, without affecting their viability, pluripotency and differentiation potential. Surprisingly, the physical properties of the microgel material were able to tune germ layer specification without the need for any biochemical factors.

CONCLUSIONS: In conclusion, our study demonstrates that the physical properties of the microgels alone were sufficient to direct germ layer specification, eliminating the need for additional biochemical factors. This highlights the critical role of the extracellular matrix's mechanical and topographical cues in stem cell differentiation and opens new avenues for creating more effective and precise regenerative therapies. In particular, the potential of combining iPSC technology with advanced bioprinting techniques to engineer tissues and organs, paves the way for breakthroughs in personalized medicine and the treatment of various diseases.

Examining the neurogenic potential of enteric glia for the treatment of achalasia

Ms Linxuan Jiang[1], A/Prof Jing Zhao[1], Ms Gunes S Yildiz[1], Mr David Lai[1], Dr Lincon Stamp[1], Dr Marlene M Hao[1]
[1]Department of Anatomy and Physiology, University of Melbourne, Melbourne, Australia

Biography:
Graduated with a Bachelor of Medical Science (Honours class I) degree in Physiology at The University of Sydney in July 2021. PhD candidate at The University of Melbourne, eager to participate a role in health sciences.

Aims: Oesophageal achalasia is a debilitating disorder characterised by the inability of the lower oesophageal sphincter to relax, its symptoms include dysphagia (difficulty swallowing) and bolus impaction (food getting stuck) in the oesophagus, regurgitation (food refluxing back up), unintended weight loss, chest pain, and coughing. Achalasia involves the loss of nitrergic inhibitory motor neurons from the enteric nervous system, specifically those expressing neuronal nitric oxide synthase (nNOS). Current treatments manage symptoms but not the underlying cause. This study aimed to isolate adult mouse enteric glia and evaluate their neurogenic potential following transplantation into the oesophagus of an nNOS-knockout mouse model of achalasia.

Methods: Enteric glia were isolated from Sox10creERT2;R26R-YFP or R26R-GCaMP3 mice one week after tamoxifen induction. Following in vitro culture and expansion, these enteric glia-derived "gliospheres" were transplanted into the external muscular layer of the nNOS-KO oesophagus at the level of the lower oesophageal sphincter. Immunohistochemical analysis was performed to assess glial cell survival, integration and neuronal differentiation.

Results: After 14 days of in vitro expansion, enteric glia had proliferated and formed gliospheres. Following -transplantation into the nNOS-KO oesophagus, we found that Sox10-YFP/GCaMP expressing cells survived, with a subset differentiating into HuC/D-expressing enteric neurons. Subpopulations of these glia-derived neurons expressed markers of inhibitory (nNOS) and excitatory (ChAT- and Calbindin) enteric neurons. These findings indicate that enteric glia can survive and differentiate into neurons following transplantation.

Conclusion: This study demonstrates the potential of enteric glia as a novel stem cell source for generating neurons and restoring gut motility in the upper gastrointestinal tract. Further investigation will focus on assessing the restoration of oesophageal motility using manometry studies.

Oral Presentation – Wednesday 13th November

A new immunocompetent brain organoid model to study demyelinating diseases

Ms Soumya Garawadamath[1], PhD Huiwen Zheng[1], PhD Selin Pars[1], A/Prof Jessica Mar[1], Prof Ernst Wolvetang[1], **Dr Giovanni Pietrogrande**[1]
[1]Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, Brisbane, Australia

Biography:
Dr. Giovanni Pietrogrande is a senior postdoctoral researcher at the Australian Institute for Bioengineering and Nanotechnology. During his PhD, he researched microglia activation's impact on brain processes, leading to a treatment for preventing neuronal death post-ischemia. Now, he focuses on translating his findings from mice to humans and understanding neuroinflammation mechanisms and their role in neurodegeneration. Using CRISPR-Cas9, he modifies induced pluripotent stem cells to create brain and spinal cord organoids for modeling neurological diseases. He collaborates with biotech companies on genetic engineering and organoid-based compound screening and provides consultancy services, advancing stem cell research.

AIM: We aim to harness the potential of immunocompetent brain organoids to study neurodegeneration, particularly in the context of demyelination and multiple sclerosis. Our goal is to uncover critical mechanisms underlying neurodegeneration and facilitate the development of innovative treatments.

METHOD: We developed a novel protocol to create organoids that intrinsically generate various neuronal types, myelinating oligodendrocytes, microglia, and astrocytes (MOLBOs), effectively representing a complete model of the human brain. We treated these organoids with lysolecithin to induce demyelination. This approach allows us to examine the relationship between demyelination, inflammatory responses of microglia, and alterations in neuronal activity.

RESULT: Our method provides valuable insights into the dynamics between demyelination, inflammation, and neuronal activity within the brain organoids. By treating the organoids with lysolecithin, we induce demyelination and can observe the subsequent inflammatory responses of microglia and changes in neuronal activity. This enables us to assess the role of microglia in neurodegenerative processes in real-time and their impact on neuronal function.

CONCLUSIONS: This comprehensive brain organoid model opens new avenues for understanding neurodegenerative diseases and identifying potential therapeutic targets. By adopting this advanced model, we aim to develop treatments to combat neurodegenerative conditions and promote remyelination.

Oral Presentation – Wednesday 13th November

A cloaked human stem cell-derived neural graft evades immune detection in humanised mice

Dr Chiara Pavan, Dr Kathryn Davidson, Dr Natalie Payne, Dr Stefano Frausin, Dr Cameron P Hunt, Dr Niamh M Moriarty, Dr Kwaku Dad Abu-Bonsrah Abu-Bonsrah, Miguel Ángel Berrocal-Rubio, Zahra Elahi, Le Wang, William Clow, Andrew Quattrocchi, Dr Eric Jong, Prof Marc Pellegrini, Prof Christine A Wells, Prof Lachlan H Thompson, Prof Andras Nagy, Prof Clare L Parish

[1]Florey Institute of Neuroscience and Mental Health, Melbourne, AUSTRALIA

Biography:

Chiara completed her PhD candidate at The Florey Institute of Neuroscience, the University of Melbourne. Her doctoral research focused on the use of human stem cells to enhance our understanding of Parkinson's disease and for the advancement of a cell-based therapy to treat neurological conditions. Chiara graduated from a Bachelor of Biotechnology at the University of Milan, where she investigated NFAT signaling in immunity. She then undertook a Master's degree in Immunology at the University of Copenhagen, studying glymphatic system impairment in meningitis and multiple sclerosis. With her research, she hopes to advance stem cell therapy in the clinical setting.

The ability of new cells to anatomically and functionally integrate into host tissue after transplantation has been the ambition of regenerative medicine since the discovery of human pluripotent stem cells (hPSCs). For Parkinson's disease (PD) patients this is becoming a close reality, with several ongoing clinical trials. While our ability to generate the necessary replacement neuron is well understood, there remains the complications associated with rejection and need for complex immunosuppressants regimes. For the foreseeable future, the ability to generate autologous donor cells from a patient's own hPSCs remains an unrealistic prospect due to high costs, time and exhaustive regulatory demands. An alternative approach is to generate a Universal PSC, capable of evading immune rejection by the otherwise functioning immune system of the recipient. Here we adopted a hPSC line (termed H1-FS-8IM), engineered to overexpress 8 immunomodulatory transgenes, to demonstrate the ability to evade immune rejection in an in vitro neural-based co-culture system and following transplantation. In vitro we confirmed the ability of the 'universal' PSC-derived neurons to evade rejection by T-lymphocytes, natural killer cells, macrophages and detection by dendritic cells. In a humanised mouse model, allogenic neural grafts derived from the cloaked H1-FS-8IM hPSCs similarly evaded rejection, while neural grafts generated from the control hPSC line evoked activation of human immune cells, resulted in elevated peripheral inflammatory cytokines and enlarged spleen and lymph nodes. Finally, in an immune-compromised rat PD model, we confirmed the ability of the cloaked H1-FS-8IM neural grafts to reverse motor deficits. By additional incorporation of the thymidine kinase-suicide gene into the H1-FS-8IM line, with established ability to eliminate unwanted proliferative cells within the grafts, we demonstrate the feasibility of generating a population-wide applicable, safe, universal, off-the-shelf therapeutic cell product suitable for the treatment of diseases for which cell-based therapies are a viable option, including PD.

Rising Star Award
Oral Presentation – Wednesday 13th November

Alzheimer's disease induced neurons bearing PSEN1 mutations exhibit reduced excitability

Dr Simon Maksour[1], Dr Rocio Finol-Urdaneta[1], Dr Amy Hulme[1], Dr Mauricio Castro Cabral-da-Silva[1], Helena Targa Dias Anastacio[1], Dr Rachelle Balez[1], Dr Tracey Berg[1], Calista Turner[1], Dr Sonia Sanz Muñoz[1], Dr Martin Engel[1], Dr Predrag Kalajdzic[2], A/Prof Leszek Lisowski[2], Prof Kuldip Sidhu[3], Prof Perminder Sachdev[3], Prof Mirella Dottori[1], Prof Lezanne Ooi[1]
[1]University of Wollongong, Wollongong, AUSTRALIA, [2]Children's Medical Research Institute, Westmead, Australia, [3]University of New South Wales, Sydney, Australia

Biography:
Dr Simon Maksour is an Associate Research fellow in the Neurodevelopment and Neurodegeneration lab at the University of Wollongong. Dr Maksour has extensive experience utilising stem cell models to better understand and develop novel therapeutics for neurodegerative diseases, in particular Alzheimer's disease.

Background: Alzheimer's disease (AD) is a devastating neurodegenerative condition that affects memory and cognition, characterized by neuronal loss and currently lacking a cure. Mutations in PSEN1 (Presenilin 1) are among the most common causes of early-onset familial AD (fAD). While changes in neuronal excitability are believed to be early indicators of AD progression, the link between PSEN1 mutations and neuronal excitability remains to be fully elucidated.

Aim: Therefore, this study aimed to investigate early intrinsic excitability changes in neurons generated from fAD patients with PSEN1 mutations.

Method: This study used induced pluripotent stem cell (iPSC)-derived NGN2 induced neurons (iNs) from fAD patients with PSEN1 mutations S290C or A246E, alongside CRISPR-corrected isogenic cell lines, to investigate early changes in excitability.

Results: Electrophysiological profiling revealed reduced excitability in both PSEN1 mutant iNs compared to their isogenic controls. Neurons bearing S290C and A246E mutations exhibited divergent passive membrane properties compared to isogenic controls, suggesting distinct effects of PSEN1 mutations on neuronal excitability. Additionally, both PSEN1 backgrounds exhibited higher current density of voltage-gated potassium (Kv) channels relative to their isogenic iNs, while displaying comparable voltage-gated sodium (Nav) channel current density.

Conclusion: This suggests that the Nav/Kv imbalance contributes to impaired neuronal firing in fAD iNs. Deciphering these early cellular and molecular changes in AD is crucial for understanding the disease pathogenesis.

RAPID FIRE POSTERS

P1

Understanding Circadian Disturbances in Neurodegenerative Diseases using Hypothalamic Organoids

Dr Aswathi Gopalakrishnan[1], Dr Cecilia Gomez Inclan[1], Prof. Stefan Thor[1]
[1]School of Biomedical Sciences, The University of Queensland, St Lucia, Brisbane, AUSTRALIA,

Biography:
Dr. Aswathi Gopalakrishnan is a dedicated bioengineer specializing in human stem cell-based disease models. She is working under the guidance of Prof. Stefan Thor. Her research focuses on developing human hypothalamic organoid models to understand sleep and circadian disturbances in neurodegenerative disorders. Aswathi is experienced in developing stem cell-derived tissue-engineered constructs. Aswathi's academic journey is decorated with notable achievements, including the AIBN HDR International Travel Award (2022) and the University of Queensland Career Development Award (2022). Her contributions to stem cell culture platforms have been published in reputable journals with over 150 citations, underscoring her commitment to advancing biomedical sciences.

AIM: As the population ages, neurodegenerative disorders are becoming increasingly prevalent, characterized by a complex interplay of motor and non-motor symptoms. In the early stages of these diseases, non-motor symptoms, such as sleep disorders, anxiety, and depressive episodes, predominate. These symptoms are closely linked to disruptions in circadian rhythms—the nearly 24-hour cycles controlled by the hypothalamus.
Evidence suggests a reciprocal relationship between circadian rhythm disruptions and neurodegenerative disorders. Irregular circadian rhythms may predispose individuals to neurodegenerative conditions and exacerbate disease progression, indicating a harmful feedback loop at the molecular level. Understanding this relationship is crucial for early diagnosis and management of these diseases.
By coupling a human induced pluripotent stem cell (hiPSCs)-derived hypothalamic organoid (HyOrgs) protocol with a programmable expression of mutant proteins associated with neurodegenerative diseases, such as Parkinson's disease (PD), this project focuses on understanding the role of neurotoxicity in driving circadian disturbances in neurodegenerative disorders.
METHODS: Using lentiviral vectors, hiPSC lines were transduced with plasmids encoding PD-associated alpha-synuclein proteins, both wild-type and mutant A53T variants. A reliable protocol for generating HyOrgs was established in control hiPSCs and characterized using rt-PCR and immunofluorescence and scRNA-sequencing.Circadian entrainment of HyOrgs was performed using dexamethasone.
RESULTS: Inducible expression of alpha-synuclein was achieved through the tet-ON system activated by doxycycline treatment. Generation of HyOrgs was evidenced by rt-PCR and immunofluorescence analysis confirming the presence of major hypothalamic markers, especially the sleep marker HCRT. scRNA-seq data further confirmed the cellular heterogeneity within the HyOrgs, encompassing expression profiles characteristic of the posterior hypothalamus, alongside crucial marker genes implicated in PD, including Parkin, PINK1, SNCA, and GBA1. Circadian entrainment revealed rhythmic oscillations of key clock genes.
CONCLUSIONS: This methodology lays the foundation for generating oscillating HyOrgs expressing mutant neurodegenerative proteins, offering a platform to investigate their involvement in circadian disturbances associated with neurodegenerative disorders.

Rapid Fire Posters

P1 Understanding circadian disturbances in neurodegenerative diseases using hypothalamic organoids
Aswathi Gopalakrishnan, *Cecilia Gomez Inclan, Stefan Thor*

P2 NR2F2/COUP-TFII haploinsufficiency may lead to 46,XX ovotesticular DSD through the impaired regulation of bipotential gonad factors
Lucas Ferreira, *Svenja Pachernegg, Antonia Zech, Mauricio Cabral-da-Silva, Gorjana Robevska, Jocelyn van den Bergen, Elizabeth Ng, David Elliott, Magnus Dias-da-Silva, Andrew Sinclair, Katie Ayers*

P3 AATD reshapes infection responses in human stem-cell models
Sahel Amoozadeh, *Declan Turner, Katelyn Patatsos, Tanya Labonne, Ed Stanley, Rhiannon Werder*

P4 Pathogenic E. coli infections: Studying interactions using human intestinal organoids
Eva Chan, *Cristina Giogha, Garrett Ng, Ruo Wang, Paul Hertzog, Elizabeth Hartland*

P5 Deconstructing the impacts of CLN3 disease on the blood-brain barrier
Adelene Chiam, *Sueanne Chear, Natalie E King, Jana Talbot, Elizabeth Read, Emma Wilkinson, Richard Wilson, Brad A Sutherland, Anthony L Cook*

P6 Characterisation of the purinergic P2X7 receptor in iPSC-derived astrocytes and microglia-like cells from ALS & AD donors
Andre McKenzie, *Eryn Werry, Michael Kassiou*

Poster Presentations

P8 Investigating the crosstalk between inflammation and regeneration in inflammatory bowel disease
Khanh Ha Do, *Diana Micati, Lucy Porter, Andrew Pattison, Anne Fletcher, Edward Giles, Helen Abud*

P9 Scaling up cell production of iPSC-derived haematopoietic stem cells: challenges, advantages, and a gateway to resource sharing
Mauricio e Castro Cabral-da-Silva, *Jacky Li, Hasindu Edirisinghe, Gulcan Sarila, Chantelle Inguanti, Ed Stanley, Andrew Elefanty, Elizabeth Ng*

P10 From human placenta to vascular organoids: investigating endothelial plasticity and SOX9's role in endothelial progenitor cells
Haiming Li, *Laura Sormani, Seen Ling Sim, Mitchell Mostina, Edwige Roy, Abbas Shaffie, Kiarash Khosrotehrani*

P11 Embryonically derived endothelial-macrophage progenitors promote reparative neovascularisation in skeletal muscle after ischaemia
Sanuri Liyanage, *Anna Williamson, Dang Tran, Khalia Primer, Thalia Salagaras, Vashe Chandrakanthan, Joanne Tan, Alex Pinto, Claudine Bonder, Christina Bursill, Shiwani Sharma, Peter Psaltis*

P12 Heparan sulfate proteoglycan: the pivotal regulators for neurogenesis and pathogenesis in the fight against Alzheimer's Disease
Duy Nguyen, *Lyn Griffith, Rachel Okolicsanyi, Larisa Haupt*

P13 Unveiling the neural potential of human mesenchymal stem cells in 3D differentiation models
Sofia Petersen, *Tayla Elliott, Rachel Okolicsanyi, Larisa Haupt*

P14 New insights into links between the microtubule cytoskeleton and pluripotent cell identity
Oliver Anderson, *Jessica Greaney, Israa Hameed, Asma Aberkane, Sue Mei Lim, Jose M Polo, Jennifer Zenker*

P15 Harnessing the plasticity of enteric glia for cell and gene therapy for Hirschsprung Disease
David Lai, *Jing Zhao, Gunes Yildiz, Annette Bergner, Marlene Hao, Lincon Stamp*

P16 Inflammatory cytokines trigger the activation of the kynurenine pathway of tryptophan metabolism: Effects on embryonic mouse neural stem cell proliferation, health and NAD state
Michael Lovelace, *Ben Summers, Kazuo Suzuki, Bruce Brew*

P17 Spatially charting the developing fat-tailed dunnart
Monika Mohenska*, Kellie Wise, Elly D Walters, Ning Liu, Michael J Roach, Owen JL Rackham, Anja S Knaupp, Fernando J Rossello, Luciano G Martelotto, Oliver Griffith, Jose M Polo*

P18 A 2D in-vitro model of early-stage placental development
German Atzin Mora Roldan*, Alexa Mae Gill, Karoline Swiatczak, Jose Polo*

P19 Investigation of transcription factor Tead1 in hydrocephalus and neural development
Alexandra Pelenyi*, Michael Piper*

P20 Investigating the effect of culture time on inflammatory phenotype retention in stem-cell derived pediatric intestinal organoids
*Lucy Porter**,** Diana Micati, **Khanh Ha Do**, Andrew Pattison, Eva Chan, Wing Hei Chan, Stuart Archer, Edward Giles, Helen Abud*

P21 Derivation of cynomolgus monkey induced trophoblast stem cells via nuclear reprogramming
Elly Walters*, Jia Tan, Naiara Bediaga, Sue Mei Lim, Monika Mohenska, Guizhi Sun, Yu Bo Yang Sun, Joseph Chen, Qiaoyu Chen, Xiaodong Liu, Jose Polo*

P22 Applying stomics to pluripotent stem cell derived organoids
Maria Rosaria Nucera*, Natalie Charitakis, Ryan Leung, Anna Leichter, Emma Scully, Ritika Saxena, Alexander Maytum, Michelle Scurr, Gulcan Sarila, Rebecca Sutton, Lynn Rowley, Natasha Tuano, Holly Voges, F Ahmad, Denis Bienroth, Hieu Nim, Micheal See, X Sun, Ka Leung Li, B Yang, Vallari Sawant, Merzena Walkiewicz, David Eisenstat, Andrew Elefanty, David Elliot, Alejandro Hidalgo-Gonzales, Marie Faux, Melissa Little, S Lamande, Richard Millis, E Ng Enzo Porrello, Fernando Rossello, Ed Stanley, J Vanslambrouck, R Werder, S Velasco, Mirana Ramialison*

P23 RUNX1 expression tracks human haematopoiesis from haemogenic endothelium to haematopoietic stem cell
Ritika Saxena*, Alexander Maytum, Tanya Labonne, Gulcan Sarila, Chantelle Inguanti, Hasindu Edirisinghe, Ed Stanley, Elizabeth Ng, Andrew Elefanty*

P24 Blood vessel networks generated from human stem cells
Connor Sherwood*, Tony Pang*

P25 Harnessing germline stem cells as tools for marsupial conservation
Gerard Tarulli*, Dorthea Hansen, Christina Alsterberg, Patrick Tatt, Nicola Kolaitis, Sara Ord, Stephen Frankenberg, Andrew Pask*

P26 Developing a platform of quality-controlled, validated stem cell models for researchers within a multi-user facility
Johana Tello Velasquez*, Robyn Yeh, Renee M Whan, Alison Ferguson*

P27 A dual-reporter human pluripotent stem cell line for live-cell imaging of human immune responses
Zhengqi Cheng, ***Christine Wells***

P28 Macrophages influence epithelial responses to respiratory viral infections in human stem cell models of the lung
Hannah Baric, Katelyn Patatsos, Declan Turner, Sahel Amoozadeh, Kathleen Strumila, Tanya Labonne, Ed Stanley, ***Rhiannon Werder***

P29 Accessing cord blood units for research: expanded consent options and pathways towards cellular therapies
Keren Abberton*, Junann Wish-Wilson, Tricia McDonald, Ngaire Elwood*

P30 Transient reduction of electrophysiological activity enhances energy reserves for cellular repair and protecting human midbrain neurons
Robert Adams*, Bridget Milky, Paris Mazzachi, Amal Abdisharid Ali, Glenda Halliday, Glenn King, Tim Sargeant, Cedric Bardy*

P31 Gene editing for usher syndrome type 2A: Unravelling photoreceptor degeneration mechanisms and exploring therapeutic potential using retinal organoids
Deborah Aubin*, Hani J Kim, Pengyi Yang, Mark Graham, Leszek Lisowski Anai Gonzalez-Cordero*

P32 Vascular phenotype switching in induced pluripotent stem cell-derived models of spontaneous coronary artery dissection
***Monique Bax**, Keerat Junday, Muhammad Alsherbiny, Stephanie Hesselson, Emily Hurley, David Muller, Ingrid Tarr, Lucy McGrath-Cadell, Siiri E Iismaa, Eleni Giannoulatou, Robert M Graham*

P33 Modelling Rubinstein-Taybi Epigenetic Neurodevelopmental Disorder to Test and Develop New Therapeutic Strategies
***Rudrarup Bhattacharjee**, Suzan de Boer, Dulce Medina Garcia, German A Mora-Roldan, Joseph Chen, Sue Mei Lim, Stefan White, Jose M Polo*

P34 Bioengineering functional skeletal muscle derived from pluripotent stem cells
***Kaitlyn Bibby**, Rebecca McElroy, Richard Mills*

P35 An automated protocol for generating microglia-containing cortical organoids
***Jordan Elli Clarke**, Angela Connelly, Maciej Daniszewski, Yumiko Hirokawa, Alice Pébay, Murray Cairns, Maria Di Biase*

P36 Generation of iPSC-derived adipocytes to investigate the role of epicardial adipose tissue in atrial fibrillation
***Lauren Cook**, Jordan Thorpe, Matthew D Perry, Adam P Hill*

P37 Betacellulin is a central target for Schizophrenia
***Agustin Cota-Coronado**, Rachel Hill, Andrew Gibbons, Joseph Rosenbluh, Suresh Sundram*

P38 Making mature muscle: Investigating metabolic and developmental pathways for improving the maturation of bioengineered muscle
***Callum Dark**, Kaitlyn Bibby, Natasha Tuano, Rebecca McElroy, Tabitha Cree, Richard Mills*

P39 Optimisation of a whole genome CRISPR-Cas9 screen in PD-iPSC derived dopamine neurons
***Isabelle de Luzy**, Wenna Chen, Alexander Henderson, Marco Rosso, Sanjoy Mehta, Ting Zhou, Nathalie Saurat, Gabrielle Ciceri, Lorenz Studer*

P40 Understanding the cellular environment of the heart to advance the development of cardiac therapies
***Sebastian Bass-Stringer**, Yinghan She, Hayley Pointer, Adam Piers, Kevin Watt, James Hudson, David Elliott, Enzo Porrello*

P41 Stem cell modelling of mitochondrial disease-linked cardiomyopathy
***Ann Frazier**, Yau Chung Low, Cameron McKnight, David Stroud, David Elliott, Diana Stojanovski, David Thorburn*

P42 Preclinical iPSC drug screen and machine learning predict neuroprotective agents for a form of childhood dementia
***Zarina Greenberg**, Ella McDonald, Alejandra Noreña Puerta, Manam Inushi De Silva, Cade Christensen, Robert Adams, Jenne Tran, Paris Mazzachi, Sebastian Loskarn, Siti Mubarokah, Megan Maack, Kris Elvidge, Mark Hutchinson, Kim Hemsley, Lisa Melton, Nicholas Smith, Cedric Bardy*

P43 Creating a micro-exon splicing map of early human brain development with genetically engineered pluripotent stem cell derived brain organoids
***Pallavi Gupta**, Hannah Leeson, Ishaan Gupta, Ernst Wolvetang*

P44 Optimizing the *in vitro* neuron microenvironment to mitigate phototoxicity in live-cell imaging
***Cassandra Hoffmann**, Simon Maksour, Jordan Clarke, Maciej Daniszewski, Alice Pébay, Paul Gleeson, Mirella Dottori, Ellie Cho, Andrew Zalesky, Maria Di Biase*

P45 Using patient-derived stem cell models to study childhood onset Facioscapulohumeral muscular dystrophy (FSHD)
***Peter Houweling**, Vanessa Crossman, Kathrin Mattes, Natasha Tuano, Rebecca McElroy, Chantal Coles, Katy de Valle, Peter Jones, Richard Mills, Ian Woodcock*

P46 What makes us breathe? Uncovering the neural rhythm generators for involuntary breathing using human pluripotent stem cells
***Kevin Law**, Clare Parish, Lachlan Thompson*

P47 The PSEN1 H163R mutation in an iPSC-retinal model of Alzheimer's disease causes detectible disease-associated features involving ß-amyloid pathways.
Grace Lidgerwood, *Damián Hernández, Jenna Hall, Anne Senabouth, Mehdi Mirzaei, Allison van de Meene, Celeste Karch, Alison Goate, Joseph Powell, Alice Pébay*

P48 Developing a model of incomplete penetrance in arrhythmogenic cardiomyopathy using patient-derived induced pluripotent stem cells
Serena Li, *Matisse Fox, Samantha B Ross, Mira Holliday, Richard D Bagnall, Seakcheng Lim, Christopher Semsarian*

P49 Reduced connexin-43 expression, slow conduction, and repolarisation dispersion in a model of hypertrophic cardiomyopathy.
Seakcheng Lim, *Melissa Mangala, Mira Holliday, Henrietta Cserne Szappanos, Samantha B. Ross, Serena Li, Jordan Thorp, Whitney Liang, Ginell N. Ranpura, Jamie I. Vandenberg, Christopher Semsarian, Adam P. Hill, Livia C. Hool*

P50 Exploring cell senescence in iAstrocytes from C9orf72 ALS patients
Alexandra Maximova, *Michael Sullivan, Eryn Werry, Michael Kassiou*

P51 Hyperactive synaptic circuits in neurons derived from children with a form of dementia
Paris Mazzachi, *Ella McDonald, Zarina Greenberg, Alejandra Noreña Puearta, Jenne Tran, Manam Inushi De Silva, Cade Christensen, Robert Adams, Sebastian Loskarn, Helen Beard, Michael Zabolocki, Meera Elmasri, Megan Maack, Kris Elvidge, Mark R. Hutchinson, Cara O'Neill, Kim Hemsley, Lisa Melton, Nicholas Smith, Cedric Bardy*

P52 Identifying new therapies for Facioscapulohumeral muscular dystrophy using a phenotypic screening approach
Rebecca McElroy,*Natasha Tuano, Kaitlyn Bibby, Timothy Johanssen, Henry Beetham, Alejandro Hidalgo-Gonzalez, Peter Houling, Kevin Watt, Sara Howden, Richard Mills*

P53 Phosphoproteomic analysis of the signalling networks controlling human cardiomyocyte proliferation
Yulia Mitina, *Yi Sing Gee, Hannah Huckstep, Rosie Hyslop, Hayley Pointer, Benjamin Parker, Sean Humphrey, Jonathan Baell, Paul Stupple, Kevin Watt, David Elliott, Enzo Porrello*

P54 Transcriptome analysis of ADHD dopaminergic neuron derived from iPSC: Evidence of signalling disruption and maturation delay
Atefeh Namipashaki, *Mark Bellgrove, Ziarih Hawi*

P55 Using human stem cell-derived macrophages to examine the effects of platinum dissolution from cochlear implants
Bryony Nayagam, *Steven Iem, Jackie Ogier, Ulises Aregueta Robles, Dhyey Shah, Marnie Maddock, Mirella Dottori, Laura Poole-Warren*

P56 Single cell transcriptomics elucidates intra-cellular signalling networks involved in donor-host interactions following transplantation of hPSC-derived photoreceptor cells to restore visual function
Michelle Sophie O'Hara-Wright, *Hani Kim, Pengyi Yang, Anai Gonzalez-Cordero*

P57 Modelling the influence of the autonomic nervous system in atrial fibrillation using induced pluripotent stem cell models
Sutapa Saha, *Azadeh Zahabi, Jordan Thorpe, Shiang Lim, Adam Hill*

P58 Innovative stem cell-based disease modeling: pioneering discoveries and future directions in RP11, ADOA and PMS disease
Priyanka Sain, *Caitlyn Richworth, Wissam Chiha, Sudhanshu Gupta, Resmi Menon, Maria Hoyos Carmona, Suzy Juraja, Richard Francis, Bridget Flis, Laura Florez, Anna Mills, Clarissa McDonagh, Danie Champain, Dean De Alvis, Abbie Francis, Tenielle George, Janya Grainok, Jessica Nichols, Carla Jackson (née Mellough)*

P59 Modelling SETBP1 Haploinsufficiency Disorder using CRISPR gene editing and neural differentiation of iPSCs
Nicole Shaw, *Kevin Chen, Kathryn Farley, Mitchell Hedges, Gareth Baynam, Timo Lassmann, Vanessa Fear*

P60 TRAPPC4 disorder: neuronal models of disease for therapeutic validation studies
Nicole Van Bergen*, Riley Hall, Vallari Sawant, Jinchao Gu, Tim Sikora, Ben Rollo, Silvia Velasco, Jinkuk Kim, John Christodoulou*

P61 Bioengineering complex, functional kidney tissue with spatially organised proximal nephrons
Jessica Vanslambrouck*, Ker Sin Tan, Sophia Mah, Kathleen Dominic, Melissa Little*

P62 Characterization of a novel human Amyotrophic lateral sclerosis (ALS) three- dimensional (3D) Neurovascular Unit (NVU) *in vitro* cell model
Stephani Viljoen*, Juliana C. S. Chaves, Ian Peall, Anthony R White, Lotta Oikari*

P63 Investigating the cell biology of childhood dementia using high-content imaging of organelles and macromolecules
Emma Wilkinson*, Elizabeth Read, Sueanne Chear, Adelene Chaim, Alex Hewitt, Jana Talbot, Anthony L Cook*

P64 Multicellular stem cell-derived human heart valve organoids to model Rheumatic Heart Disease
Serene Yeow*, Jessica Durrant-Whyte, Adam Piers, Hannah Frost, Andrew Steer, Enzo Porrello, Holly Voges*

P65 Investigating the Role of Hypoxia-Immune Tumour Microenvironment in Colorectal Cancer Using Patient-derived Organoids
Ruobing Zhang*, Wing-Hei Chan, Rebekah Engel, Zhengqi Cheng, Andrew D. Pattison, Thierry Jardé, Paul J. McMurrick, Lochlan Fennell, Christine A. Wells, Helen E. Abud*

P66 Don't call me "iPSC1". Addressing identity and provenance in Australian Stem Cell Research
Suzanne Butcher*, Noel Faux, Stuart Lee, Mengqi Hu, Daniel Russo-Batterham, Priyanka Nair-Turkich, Masoud Kamali, Thao Nguyen, Christine Wells*

P67 How FAIR are hPSCs?
Mengqi Hu*, Christine. A Wells*

P68 The research-teaching nexus
Julia Young*, Sonja McKeown*

P69 Targeting colon cancer stem cells as new therapeutic approach in the treatment of colorectal cancer
Reem ALHulais*, Stephen Ralph*

P70 identifying genomic drivers of *in vitro* human mesenchymal stem cells proliferation via targeted transcriptome sequencing
Nghia Nguyen*, Sofia Petersen, Duy Nguyen, Martina Gyimesi, Rachel Okolicsanyi, Larisa Haupt*

RAPID FIRE POSTERS

P2

NR2F2/COUP-TFII haploinsufficiency may lead to 46,XX ovotesticular DSD through the impaired regulation of bipotential gonad factors

Lucas Ferreira[1,2], Svenja Pachernegg[2,3], Antonia Zech[2,3,4], Mauricio Cabral-da-Silva[2,3,4], Gorjana Robevska[2], Jocelyn van den Bergen[2], Elizabeth Ng[2,3,4], David Elliott[2,3,4], Magnus Dias-da-Silva[1], Andrew Sinclair[2,3], Katie Ayers[2,3]

[1]Laboratory of Molecular and Translational Endocrinology (LEMT), Endocrinology Division, Department of Medicine, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, Brazil, [2]The Murdoch Children's Research Institute, Melbourne, Australia, [3]Department of Paediatrics, The University of Melbourne, Melbourne, Australia, [4]Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), Murdoch Children's Research Institute, Melbourne, Australia

Biography:

Dr Ferreira holds a PhD degree in molecular biology at the Universidade Federal de São Paulo (Brazil). His research is focused on studying genetic aspects of differences of sex development (DSD) and developing protocols to differentiate human stem cells into cells resembling fetal ovarian and testicular tissue to understand how genetic variants impact gonad differentiation.

The bipotential gonads develop and adopt either a testicular or an ovarian cell fate between 5th-6th weeks of human gestation. Disruption to this process leads to differences of sex development (DSD). Heterozygous loss-of-function genetic variants in NR2F2, encoding COUP-TFII, represent a novel cause of SRY-negative 46,XX ovotesticular (OT) DSD, in which children with female sex chromosomes are born with both ovarian and testicular tissues. By ablating NR2F2 expression in the ovarian-like human cell line COV434, we have recently shown that COUP-TFII regulates the maintenance of the bipotential gonad state (Ferreira et al., 2024). However, more work is required to understand why haploinsufficiency of COUP-TFII causes sex reversal. To address this, we have obtained a hESC reporter cell line (NKX2.5EGFP/+-COUP-TFIImCherry/+) and have created heterozygous and homozygous NR2F2 knockout (KO) iPSC lines (Ferreira et al., 2024). We differentiated reporter ESCs into embryoid bodies (EB) and then into ovarian-like cells using an adapted protocol from Lan et al. (2013). Upon EB attachment, we identified mCherry fluorescence mainly in areas of higher cell density at day 9. The upregulation of NR2F2/COUP-TFII expression was observed along with bipotential gonad markers GATA4, WT1, NR0B1, and STAR. We then differentiated wild-type (WT) and both NR2F2 KO iPSCs into granulosa-like cells. In cells differentiated from the homozygous KO line we observed a downregulation of LHX9 mRNA and protein. LHX9 is a key player in the early development of the bipotential gonad. Interestingly, compared to WT, the differentiated heterozygous NR2F2-KO line expressed higher levels of FOXL2 and AMH, markers of ovarian pre-granulosa and fetal testicular Sertoli cells, respectively. Our findings highlight a novel interaction between COUP-TFII and LHX9 in the early gonad, and corroborate the hypothesis that NR2F2/COUP-TFII haploinsufficiency leads to 46,XX OT-DSD through the dysregulation of bipotential gonad factors instead of the downregulation of pro-ovarian genes.

RAPID FIRE POSTERS

P3

AATD reshapes infection responses in human stem-cell models

Mrs Sahel Amoozadeh[1,2], Dr Declan Turner[1], Ms Katelyn Patatsos[1], Ms Tanya Labonne[1], Prof Ed Stanley[1,2], Dr Rhiannon Werder[1,2]
[1]Stem Cell Medicine, Murdoch Children's Research Institute, Melbourne, Australia, [2]Department of Paediatrics, University of Melbourne, Melbourne, Australia

Biography:
Sahel Amoozadeh is a 2nd year international PhD student studying Stem Cell Biology at the University of Melbourne, based at the Murdoch Children's Research Institute. Sahel completed a Master's in Medical Biotechnology at Iran University of Medical Sciences in Tehran, Iran, and holds a Bachelor's degree in Biotechnology. Their current project focuses on modeling Alpha-1 Antitrypsin Deficiency disease using induced pluripotent stem cells. By developing a robust lung alveolar model, Sahel aims to investigate the cellular and molecular mechanisms underlying this genetic disease.

Aims: Alpha-1 Antitrypsin Deficiency (AATD) stems from genetic mutations in the SERPINA1 gene. AATD patients face early-onset chronic obstructive pulmonary disease, characterised by rapid lung function decline and frequent exacerbations, commonly triggered by respiratory viral infections. In AATD patients with the PiZZ genotype, alpha-1 antitrypsin (AAT) protein misfolds and accumulates within the endoplasmic reticulum. We hypothesise that AATD mutations will interfere with appropriate responses to viral infections. The aim of our study is to explore the pathogenic cellular and molecular mechanisms underlying infections in AATD.

Methods: AAT in the lung is predominantly expressed by type 2 alveolar epithelial cells and alveolar macrophages. To overcome limitations in accessing primary sources of these cells, we used induced pluripotent stem cells (iPSCs) from an AATD donor (PiZZ genotype) and isogenic control (PiMM genotype). iPSCs underwent directed differentiation to type 2 alveolar epithelial cells (iAT2s) or macrophages (iMacs) using established protocols.

Results: PiZZ and PiMM iAT2s at an air-liquid interface were infected with respiratory syncytial virus (RSV), a common viral trigger, and infection responses were profiled for 5 days. Compared to PiMM iAT2s, PiZZs had elevated cell death and decreased trans-epithelial electrical resistance. Strikingly, both viral transcription and shedding was significantly altered in AATD iAT2s. Expression of antiviral interferons, interferon-stimulated genes, cytokines and chemokines were significantly different following infection of AATD iAT2s. To explore how iMac function is affected by AATD mutations, we focused on their ability to phagocytose fluorescently-tagged bacteria, as this is an essential component of alveolar macrophage defence. PiZZ iMacs had a significant impairment in phagocytosis compared with PiMM iMacs.

Conclusions: Our findings suggest that AATD mutations rewire iAT2 and iMac infection responses. In future experiments, we will compare these findings to responses to other viral or bacterial respiratory infections, with the goal to uncovering new avenues for innovative AATD therapeutics.

RAPID FIRE POSTERS

P4

Pathogenic E. coli infections: Studying Interactions Using Human Intestinal Organoids

Dr Eva Chan[1,2], Cristina Giogha[1,2], Garrett Ng[1,2], Ruo Wang[1,2], Paul Hertzog[1,2], Elizabeth Hartland[1,2]

[1]Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, Australia, [2]Department of Molecular and Translational Science, Monash University, Clayton, Australia

The gastrointestinal tract is lined by a single layer of epithelial cells that forms a barrier to the external environment. This barrier is exposed to several environmental stressors, and on occasion, encounter gastrointestinal pathogens, such as enteropathogenic Escherichia coli (EPEC). This bacterium can disrupt the epithelial lining leading to symptoms such as diarrhoea, fever, vomiting, and in extreme cases death. This is possible due to the bacterial effector proteins that are injected into the host cell, resulting in disruptions to host cell cytoskeleton and cell signalling. Such findings have been possible due to the use of immortalised cell lines in the laboratory, however, immortalised cell lines are not representative of the cellular heterogeneity exhibited in the human gastrointestinal tract.

AIM: To establish an organoid co-culture model to allow efficient infection of human intestinal epithelial cells with EPEC, to study epithelial response.

METHODS: Human intestinal organoid cultures grown as 2-dimensional cultures (monolayers) have been established to allow access to the apical cell surface by EPEC for infection. Immunofluorescent staining and imaging, Western blotting, and cytometric bead array, were performed following infection.

RESULTS: Immunofluorescent staining and imaging confirmed infection, through the visualisation of EPEC attaching to the epithelial surface, leading to reorganisation of host ACTIN. The degradation of host proteins by EPEC effector proteins was detected by Western blotting and measurement of cytokines secreted by organoid derived monolayers, through cytometric bead array, further confirmed epithelial response to infection.

CONCLUSION: By establishing a bacteria-organoid co-culture system with human intestinal organoid derived monolayers, this will facilitate the study of host cell response and epithelial cell function following infection.

RAPID FIRE POSTERS

P5

Deconstructing the impacts of CLN3 disease on the blood-brain barrier

Ms Adelene Chiam[1], Dr Sueanne Chear[1], Ms Natalie E King[2], Dr Jana Talbot[1], Ms Elizabeth Read[1], Dr Emma Wilkinson[1], Dr Richard Wilson[3], Associate Professor Brad A Sutherland[2], Associate Professor Anthony L Cook[1]

[1]Wicking Dementia Research and Education Centre, University of Tasmania, Hobart, AUSTRALIA, [2]Tasmanian School of Medicine, College of Health and Medicine, University of Tasmania, Hobart, AUSTRALIA, [3]Central Science Laboratory, College of Science and Engineering, University of Tasmania, Hobart, AUSTRALIA

Biography:

Cook Lab, Stem Cell Models Group, Wicking Dementia Research and Education Centre, College of Health and Medicine, University of Tasmania
B. Laboratory Medicine, University of Tasmania (2018 - 2020)
B. Medical Research (Hons), University of Tasmania (2021)
Doctor of Philosophy (Medical Studies), University of Tasmania (2022 - present)

AIM: CLN3 disease is a lysosomal storage disorder that causes childhood dementia. Previous literature suggests that the blood-brain barrier (BBB) is compromised in CLN3 disease. However, there are limited cell-based models further exploring this. Here, we investigated the effects of pathological CLN3 genetic variants on the function of BBB cells (astrocytes, pericytes, endothelial cells).

METHOD: Fibroblasts with a compound heterozygous mutation in the CLN3 gene (1kb-deletion / E295K) were reprogrammed into induced pluripotent stem cells (iPSCs). Using CRISPR/Cas9 editing, an isogenic pair of cell lines (i.e. CLN3 and Corrected) was generated. iPSCs were differentiated into astrocytes, pericytes, and endothelial cells. Marker expression was verified through immunofluorescence. Functional activity for each cell type was evaluated. In astrocytes, glutamate uptake was examined by measuring extracellular glutamate using a luminescence assay. Pericytes were treated with platelet derived growth factor-BB (PDGF-BB) and proliferation was determined based on EdU incorporation. In endothelial cells, barrier permeability was assessed by culturing endothelial monolayers on inserts and quantifying apical-to-basolateral transport of FITC-dextran.

RESULT: Successful differentiation of iPSCs into BBB cells was verified by positive expression of multiple canonical markers per cell type. Corrected astrocytes demonstrated higher glutamate uptake compared to CLN3 astrocytes when treated with exogenous glutamate. Furthermore, the Corrected endothelial barrier was less permeable to FITC-dextran compared to CLN3. Interestingly, the proliferative capacity of CLN3 pericytes was higher than that of Corrected pericytes at baseline, and when treated with PDGF-BB (with or without imatinib, a PDGFRβ inhibitor).

CONCLUSIONS: The functional activity of CLN3 and Corrected iPSC-derived astrocytes, pericytes and endothelial cells is similar to that expected *in vivo*. Significant differences between CLN3 and Corrected cells, particularly astrocytes and endothelial cells, suggest that CLN3 disease may result in BBB impairment. Future directions include generating additional CLN3 models, high-content imaging, co-cultures, and proteomic analysis of BBB cells.

RAPID FIRE POSTERS

P6

Characterisation of the purinergic P2X7 receptor in iPSC-derived astrocytes and microglia-like cells from ALS & AD donors

Andre McKenzie[1], Dr Eryn Werry[2,3], Professor Michael Kassiou[3]
[1]School of Medical Science, University of Sydney, Darlington, Australia, [2]Central Clinical School, University of Sydney, Darlington, Australia, [3]School of Chemistry, University of Sydney, Darlington, Australia

Biography:
Andre Mckenzie is a final-year PhD Candidate at the University of Sydney, exploring the role of the purinergic system in iPSC derived glial cells in Alzheimers disease and amyotrophic lateral sclerosis. He received a bachelor's degree with honours from the University of Sydney focusing on P2X7 receptor neuroinflammation in Alzheimer's disease. He then worked as a research assistant within the Drug Discovery Lab under Professor Michael Kassiou with an interest in finding treatments and detection methods for CNS diseases.

Amyotrophic lateral sclerosis (ALS) and Alzheimer's disease (AD) are progressive neurodegenerative diseases characterized by upper and lower motor neuron loss, and brain atrophy, respectively. The pathomechanisms of both diseases are still poorly understood with current hypotheses involving genetic mutations, excitotoxicity, toxic proteins and neuroinflammation. In the absence of disease-altering clinically approved therapeutics, there is an ever-increasing need to identify new targets to develop drugs that delay disease onset and/or progression. The purinergic P2X7 receptor (P2X7R) has been implicated widely across both diseases, providing a potential therapeutic strategy. However, there are no clinically approved P2X7R antagonists. Utilisation of iPSC-derived CNS cells would allow for therapeutic testing in disease-state and healthy donors helping to identify translatable and successful therapeutics.

Aims: This study aims to characterise the P2X7R in iPSC-derived astrocytes (iAstrocytes) and microglia-like cells (iMLCs) sourced from ALS & AD patients, by exploring expression and functionality of the P2X7R.

Methods: iAstrocytes & iMLCs were developed from fibroblast lines donated by ALS, AD patients and age matched healthy controls (n=3 per disease state). To explore P2X7R expression we performed western blotting and a fluorescent dye-uptake assay to explore functionality.

Results: P2X7R expression was present on all ALS & AD iAstrocytes, with no significant difference in expression compared to healthy controls ($p>0.05$). ALS & AD iMLCs showed a significant increase in P2X7R mediated fluorescence delta change (60 min – 0 min) following stimulation with the P2X7R agonist BzATP ($p<0.05$). This result was not observed in the iMLC control group, nor in any iAstrocytes($p>0.05$).

Conclusion: The data confirms that the P2X7R receptor is functionally over-active in ALS & AD iMCLs, compared to healthy controls, and is not functionally active in iAstrocytes. This provides evidence for the use of iMCLs as a model for exploring P2X7R-directed drug design.

POSTER PRESENTATIONS

P8

Investigating The Crosstalk Between Inflammation and Regeneration in Inflammatory Bowel Disease

Ms Khanh Ha Do[1,2], Dr Diana Micati[1,2], Ms Lucy Porter[1,2], Dr Andrew Pattison[1,2,3], Associate Professor Anne Fletcher[4], Dr Edward Giles[5,6,7], Professor Helen Abud[1,2]
[1]Department of Anatomy and Developmental Biology, Monash University, Clayton, 3800, Australia, [2]Development and Stem Cells Program, Monash Biomedicine Discovery Institute, , Clayton, 3800, Australia, [3]Monash Bioinformatics Platform, Clayton, 3800, Australia, [4]Department of Biochemistry and Molecular Biology, Monash Biomedicine Discovery Institute, Monash University, Clayton, 3800, Australia, [5]Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, 3168, Australia, [6]Department of Molecular and Translational Sciences, Monash University, Clayton, 3800, Australia, [7]Department of Paediatrics, Monash University, Clayton, 3800, Australia

Biography:
I am a first-year Ph.D. candidate in Abud Lab at Monash Biomedicine and Discovery Institute with a passion for passionate stem cell and disease modelling. My project focuses on investigating the relationship of inflammation and intestinal regeneration. I also completed my undergraduate studies (BBiomedSc and Hons) at Monash University. My Honours project was also completed in Abud Lab at Monash BDI, and focused on modelling inflammation in IBD-patient derived organoids.

Aims: Inflammatory bowel disease (IBD) is marked by unpredictable periods of intestinal inflammation and remission, influenced by genetic, environmental, and immunological factors. Due to the cyclic nature of injury and regeneration in IBD and the limited efficacy of immunomodulatory treatments, there is a need for alternative therapeutic approaches that promote mucosal healing. However, the impact of inflammation on epithelial regeneration is poorly understood. Organoids, which preserve the three-dimensional structure and cellular heterogeneity of the intestinal epithelium in vitro, offer a promising system for studying intestinal regeneration. However, organoids still fail to fully replicate the *in vivo* cellular composition, spatial organization, and microenvironment. Thus, this project aims to identify molecules that disrupt epithelial regeneration and to use these signals to develop a comprehensive model and timeline of regeneration in IBD using patient-derived organoids.

Methods: We utilized publicly available datasets to identify the expression of proinflammatory factors in the IBD environment and analyse their relationship to the foetal-reprogramming process in the epithelium, thus inferring the relationship between inflammation and intestinal regeneration. The identified molecules are used to create a regeneration model using patient-derived organoids.

Results: Transcriptomic analysis revealed a significant enrichment of the foetal-reprogramming transcriptional signature in the intestine during the injury phase in mice. Among the upregulated proinflammatory cytokine signals, interferon-gamma (IFN-γ) signalling showed a strong correlation with the enrichment of this signature within epithelial cell populations. Treatment of organoids with IFN-γ following injury successfully induced and sustained this regenerative signature.

Conclusion: Identifying the association of IFN-γ with intestinal regeneration provides crucial insights into its role in influencing epithelial regenerative processes. This understanding may

pave the way for developing novel therapeutic interventions for IBD, enhancing mucosal healing and improving patient outcomes.

POSTER PRESENTATIONS

P9

Scaling Up Cell Production of iPSC-derived Haematopoietic Stem Cells: Challenges, Advantages, and a Gateway to Resource Sharing

Mauricio e Castro Cabral-da-Silva[1], Jacky Li[1], Hasindu Edirisinghe[1], Gulcan Sarila[1], Chantelle Inguanti[1], Ed Stanley[1], Andrew Elefanty[1], Elizabeth Ng[1]
[1]Murdoch Children's Research Institute - MCRI, Melbourne, Australia

Biography:
I am a stem cell biologist interested in protocol optimisation, process development, developmental biology and disease modelling.

Aims: Our laboratory aims to generate haematopoietic stem cells (iHSCs) from patient-derived induced pluripotent stem cells (iPSCs) as a potential autologous therapy for replenishing bone marrow progenitors in patients with blood cell diseases.

Methods: We developed a systematic method for generating transplantable HSCs from human iPSCs (iHSCs). This method was recently published in Nature Biotechnology. We conducted proof-of-concept experiments to model the manufacture of iHSCs at ‘patient scale’ using a flask system and analyzed the phenotypic, transcriptional, and functional characteristics of the iHSCs produced.

Results: Our method represents a significant advance in iPSC differentiation technology, enabling the scalable manufacturing of personalized CD34+ iHSCs. Currently, iHSCs are differentiated at ‘laboratory scale’ (yielding millions of cells) and functionally evaluated by engraftment in an immunocompromised mouse model. We demonstrated the feasibility of scaling up cell production to ‘patient scale’ (hundreds of millions of cells) while maintaining consistency in the iHSCs produced.

Conclusion: The development of a scalable manufacturing workflow for iHSCs brings potential cell-based therapies for blood cell diseases closer to realization. Ongoing assessment is essential to confirm the consistency of iHSCs produced at ‘patient scale’ for future clinical translation.

POSTER PRESENTATIONS

P10

From Human Placenta to Vascular Organoids: Investigating Endothelial Plasticity and SOX9's Role in Endothelial Progenitor Cells

Mr Haiming Li[1], Dr Laura Sormani[1], Dr Seen Ling Sim[1], Mr Mitchell Mostina[1], Dr Edwige Roy[1], Dr Abbas Shaffie[1,2], Professor Kiarash Khosrotehrani[1]
[1]Frazer Institute. The University of Queensland, Brisbane, Australia, [2]Metro North Hospital and Health Service, Queensland Health, Brisbane, Australia

Biography:
Haiming Li is a PhD candidate of Frazer Institute in the University of Queensland. He received his bachelor degree from South University of Science and Technology in 2020 and his master degree from University of Queensland in 2022. His areas of interests include the stem cells, in particular, the endothelial progenitor cells and endothelial to mesenchymal transition.

Aims: This study aims to explore the heterogeneity of endothelial cells, understand the role of SOX9 in human endothelial progenitors, and evaluate vascular organoids (VOs) as a novel source for isolating endothelial colony-forming cells (ECFCs).

Methods: We utilized single-cell RNA sequencing (scRNA-seq) on human term placenta to assess cellular heterogeneity and state transitions. Key transcription factors as well as SOX9, were identified and analyzed based on their expression levels during different stages. Functional assays were performed to understand the roles of these transcription factors. VOs were generated from human induced pluripotent stem cells (hiPSCs), and ECFCs were isolated. Comparative analyses were conducted on gene expression profiles and functional characteristics of ECFCs derived from both VOs and placenta.

Results: Thirteen peaks/clusters were identified and progenitor peaks were found to develop into endothelial or mesenchymal fates. Four transcription factors (Lhx6, Hes4, Nr2f2 and Hoxd9) were selected from scRNA-seq datasets, which contribute to the endothelial/epithelial to mesenchymal transition in previous literatures. SOX9 drives the mesenchymal transition in ECFCs. Overexpression of SOX9 in ECFCs leads to the following mesenchymal characteristic, including more mesenchymal morphology, upregulation of mesenchymal genes, decrease of tube formation ability and self-renewal ability, and increase of proliferation rate. We successfully generated the VOs which forming the vascular structures. The CD45-CD34+CD31low population isolated from VOs remained the endothelial properties.

Conclusion: Our findings highlight the heterogeneity of endothelial cells and the pivotal role of SOX9 in their function. Vascular organoids represent a viable alternative to human placenta for isolating ECFCs, offering a promising tool for vascular disease research and therapeutic applications.

POSTER PRESENTATIONS

P11

Embryonically derived Endothelial-Macrophage Progenitors promote reparative neovascularisation in skeletal muscle after ischaemia

Dr Sanuri Liyanage[1,2], Dr Anna Williamson[1,2], Miss Dang Tran[1,2], Dr Khalia Primer[1,2], Miss Thalia Salagaras[1], Dr Vashe Chandrakanthan[1], Dr Joanne Tan[1], Dr Alex Pinto[3], Professor Claudine Bonder[4], A/Prof Christina Bursill[1,2], Dr Shiwani Sharma[1,5], A/Prof Peter Psaltis[1,2,6]
[1]Vascular Research Centre, SAHMRI, Adelaide, AUSTRALIA, [2]Adelaide Medical School, The University of Adelaide, Adelaide, AUSTRALIA, [3]Baker Heart and Diabetes Institute, Melbourne, AUSTRALIA, [4]Centre for Cancer Biology, University of South Australia and SA Pathology, Adelaide, Australia, [5]Flinders Medical Centre, Flinders University, Bedford Park, Australia, [6]Department of Cardiology, Royal Adelaide Hospital, Central Adelaide Local Health Network, Adelaide, Australia

Biography:
Sanuri Liyanage is a Postdoctoral Research Fellow at the Vascular Research Center at SAHMRI. She completed her PhD in Medicine from the University of Adelaide in 2023. Her main focus of research involves the discovery and characterisation of a novel bipotent progenitor in postnatal tissues such as the adventitia of the aorta, skin and skeletal muscle.

Background and Aim: Macrophages and endothelial cells share an intimate relationship in regulating inflammation and neovascularisation in tissues following ischaemic insult. We previously discovered bipotent, embryonically derived endothelial-macrophage (EndoMac) progenitor cells in mouse aorta. Here, we studied these cells in steady-state and ischaemic skeletal muscle.

Methods and Results: Digests of mouse gastrocnemius and quadriceps formed macrophage colony-forming units in methylcellulose, that self-renewed in secondary culture from single cells. These colonies contained homogeneous Lin-CD45+CD11b-F4/80-Sca-1+c-Kit+CX3CR1+ progenitors, that were also identified in fresh muscle digests. Flt3-Cre lineage-mapping revealed that these muscle progenitors were independent from Flt3+ bone marrow haematopoiesis, indicating they are tissue-resident. They were more abundant in neonatal mice and declined with ageing, suggesting prenatal seeding. This was confirmed by fate-mapping using Csf1rMer-iCre-Mer and Cx3cr1CreER-YFP mice which showed their origins from an E8.5 CSF1R+ and E9.5 CX3CR1+ embryonic source. Differentiation studies demonstrated that muscle progenitors were vasculogenic and bipotent for macrophages and endothelial cells. Furthermore, they expanded rapidly following acute hindlimb ischaemia and promoted neovascularisation, perfusion recovery and myocyte repair. Exposure to high glucose in vitro and streptozotocin-induced diabetes *in vivo* attenuated their self-renewal, vasculogenic and differentiation properties, accompanied by mitochondrial dysfunction. While transplantation of progenitors from non-diabetic donor mice led to robust engraftment and perfusion recovery in ischaemic hindlimbs of diabetic recipient mice, these salutary properties were markedly diminished in progenitors from diabetic donors.

Conclusion: EndoMac progenitors mediate local inflammation and neovascularisation to promote perfusion recovery in ischaemic muscle. Their inhibition by high glucose may contribute to the impaired angiogenesis that leads to poor outcomes from ischaemia in diabetes.

POSTER PRESENTATIONS

P12

Heparan Sulfate Proteoglycan: The Pivotal Regulators for Neurogenesis and Pathogenesis in the Fight Against Alzheimer's Disease

Mr Duy Nguyen[1], Distinguished Professor Lyn Griffiths[1], Dr Rachel Okolicsanyi[1,3], Associate Professor Larisa Haupt[1,2,3]
[1]Centre for Genomics and Personalised Health (CGPH) - QUT, Brisbane, AUSTRALIA, [2]ARC Training Centre for Cell and Tissue Engineering Technologies - QUT, Brisbane, AUSTRALIA, [3]Max Planck Queensland Centre for the Materials Sciences of Extracellular Matrices, Brisbane, AUSTRALIA

Biography:
Duy is a dedicated PhD candidate at Queensland University of Technology, working under the supervision of Associate Professor Larisa Haupt, Distinguished Professor Lyn Griffiths, and Dr Rachel Okolicsanyi. His research focuses on developing treatment-responsive models for neurodegeneration, specifically targeting Alzheimer's disease. Duy employs advanced techniques such as cell and stem cell culture, molecular and fluorescent microscopic techniques to identify novel pathological factors transported via tunnelling nanotubes. He is also pioneering biomarker and isolation techniques for these nanotubes in neuronal progenitor stem cells. Duy holds a first-class honours MSc in Pharmaceutical Sciences from Technological University Dublin.

Recent research underscores the critical impact of heparan sulfate proteoglycans (HSPGs) in neurogenesis and Alzheimer's disease (AD) pathogenesis through their modulation of various signalling pathways, including TGFβ, Wnt, AKT, Notch, and Shh. Understanding the interplay between HSPGs, signalling pathways, AD pathogenesis, and neurogenesis holds promise for developing novel therapeutic strategies.

Aim: This research investigated the multifaceted involvement of HSPGs in AD pathogenesis and neurogenesis, focusing on interactions with key signalling pathways. This study highlighted the potential of HSPGs as therapeutic targets by examining the in vitro effect of their analogue, heparin, at varying concentrations on immortalised human neural progenitor stem cell (hNPC) models.

Method: hNPCs, ReNcell VM (RVM – ventral mesencephalon) and ReNcell CX (RCX – frontal cortex), were explored following the addition of heparin at 1 µg/mL and 10 µg/mL over 7 days of treatment. Analyses included cell proliferation assays, gene expression assays, Western blotting, immunocytochemistry, and live cell imaging.

Results: Data indicated the largest increase in cell proliferation at 1 µg/mL and the greatest decrease at 10 µg/mL following addition of heparin in both RVM and RCX cells. Additionally, 1 µg/mL increased gene expression of AD protection markers and decreased AD pathological markers, with 10 µg/mL having the opposite effect. At 1 µg/mL, a balanced expression of self-renewal, neuronal, and glial markers was observed; however, at 10 µg/mL, RVM cells shifted toward glial and RCX cells toward neuronal lineages. At both concentrations, TGFβ, Wnt, AKT, Notch, and Shh signalling pathways were upregulated, correlating with membrane-bound HSPGs (syndecans and glypicans).

Conclusions: HSPGs play vital regulatory roles in neurogenesis and AD pathogenesis in hNPCs. Heparin at 1 µg/mL emerged as a potential therapeutic agent for AD, particularly against tauopathy and amyloidogenesis, and a key factor in neural differentiation in stem cell models.

POSTER PRESENTATIONS

P13

Unveiling the Neural Potential of Human Mesenchymal Stem Cells in 3D Differentiation Models

Mrs Sofia Petersen[1], Ms Tayla Elliott[1], Dr Rachel Okolicsanyi[1,2,4], A/Prof Larisa Haupt[1,2,3,4]
[1]Stem Cell and Neurogenesis Group, Genomics Research Centre, Centre for Genomics and Personalised Health, School of Biomedical Sciences, Queensland University of Technology, 60 Musk Ave., Kelvin Grove, Queensland 4059, Australia, Kelvin Grove, Australia, [2]Centre for Biomedical Technology, Queensland University of Technology, 60 Musk Ave., Kelvin Grove, Queensland 4059, Australia, Kelvin Grove, Australia, [3]ARC Training Centre for Cell and Tissue Engineering Technologies, Queensland University of Technology, Australia, , Kelvin Grove, Australia, [4]Max Planck Queensland Centre for the Materials Sciences of Extracellular Matrices.

Biography:
Sofia is a 3rd year PhD candidate with the Neurogenesis and Stem Cell Group at the Centre for Genomics and Personalised Health at Queensland University of Technology. Sofia's PhD project aims to explore the roles of heparan sulfate proteoglycans (HSPGs) in the induction of human mesenchymal stem cell (hMSC) induced neurospheres in 3D cell culture. The ease of isolation and extensive in vitro expansive potential of hMSCs make them an ideal cell model for investigating neurodegenerative diseases such as Alzheimer's disease (AD). Her project focuses on the roles of HSPGs and cellular signalling pathways in neurodegeneration and neural lineage-specific differentiation.

Introduction: Alzheimer's Disease (AD), characterised by the accumulation of amyloid-beta, leads to progressive cognitive decline and memory loss. Current treatments offer symptomatic relief, lacking curative solutions. Human mesenchymal stem cells (hMSCs) exhibit potential for AD therapy due to their extensive self-renewal and neurogenic capabilities. Heparan sulfate proteoglycans (HSPGs), including syndecans (SDC; SDC1-4) and glypicans (GPC; GPC1-6) influence neurogenesis and may guide neural lineage specification. We investigated hMSC neural differentiation potential in 3D cultures, focusing on growth factors that modulate neurogenesis, including brain-derived neurotrophic factor (BDNF) and platelet-derived growth factor (PDGF).
Methods: hMSCs were cultured following established protocols. Neural induction was initiated in ultra-low attachment plates, with cells subsequently transferred to 4kPa LunaGel™ matrices. Cultures were treated with neuroinductive media containing BDNF, PDGF, and heparin (+hep). Day 14 and 28 gene expression data was analysed using Q-PCR.
Results: In 3D cultures, SDC1 was found to be downregulated under BDNF, BDNF+hep, and PDGF+hep conditions, while SDC3 was upregulated following BDNF treatment. GPC1 and GPC4 showed increased expression in 3D cultures treated with BDNF+hep and PDGF+hep. Neural lineage markers were significantly ($p<0.05$) upregulated in 2D PDGF cultures; however, initial calcium assays showed no neuronal firing in the 3D cultures.
Discussion: The data suggests that hMSCs cultured in 4kPa 3D environments are predisposed toward glial lineages. Increased expression of stemness markers in the 3D cultures indicates a maintained pluripotent state, while glial marker upregulation supports glial lineage commitment. The absence of calcium signalling highlights limitations in achieving full neural maturation under the current conditions.
Conclusion: This study demonstrated that 4kPa 3D hMSC cultures favour glial differentiation over neuronal differentiation. Further studies will focus on exploring 1kPa gels to better replicate

the neuronal microenvironment for hMSC differentiation toward neuronal lineages. These findings provide insight into hMSC neurogenic potential for future studies using 3D gel models.

POSTER PRESENTATIONS

P14

New insights into links between the microtubule cytoskeleton and pluripotent cell identity

Mr Oliver Anderson[1], Dr Jessica Greaney[1], Ms Israa Hameed[1], Dr Asma Aberkane[1], Dr Sue Mei Lim[1,2,3,4], Professor Jose M Polo[1,2,3,4], A/Prof Jennifer Zenker[1]
[1]Australian Regenerative Medicine Institute, Clayton, Australia, [2]Adelaide Centre for Epigenetics, University of Adelaide., Adelaide, Australia, [3]Department of Anatomy and Developmental Biology, Development and Stem Cells Program, Monash Biomedicine Discovery Institute., Adelaide, Australia, [4]South Australian immunoGENomics Cancer Institute, University of Adelaide, Adelaide, Australia

Biography:
Oliver is a PhD student in the Zenker lab at the Australian Regenerative Medicine Institute at Monash University, where he uses cutting-edge fluorescence microscopy to explore the relationship between pluripotency and the microtubule cytoskeleton, utilising human induced pluripotent stem cells as a model.

AIM: Pluripotency is the ability of cells to adopt any cellular identity of the adult organism. To reach their final identity as a differentiated cell, genetic, epigenetic, and metabolic regulations are essential. However, significantly less is understood of the role of cell biology in the establishment and execution of pluripotency.
The internal structure of a cell is directed by its microtubule cytoskeleton, a framework that assists in signalling and organelle trafficking, as well as bestowing cellular identity. We aimed to explore the hugely understudied links between the microtubule cytoskeleton and pluripotency.

METHODS: Cutting-edge live and fixed-cell imaging techniques were utilised on human induced pluripotent stem cells (hiPSCs). CRISPR/Cas9 was used to deplete the microtubule-organising protein CAMSAP3 to assess its role in hiPSCs.

RESULTS: We discovered that following the transition to primed pluripotency, pluripotent cells in vitro utilise a non-centrosomal CAMSAP3-dependent microtubule organisation. High temporal resolution imaging demonstrated the directional growth of microtubules from an apical CAMSAP3 cap to the basal region of the cell. Despite the similarity to naïve pluripotent cells in utilising CAMSAP3, the microtubule organisation of primed hiPSCs differs in its widespread apical localisation and molecular composition, suggesting a signature of a more differentiated state. Trilineage differentiation of hiPSCs induced a switch from non-centrosomal to centrosomal microtubule organisation. When comparing genetic lineage markers, CAMSAP3-depleted hiPSCs showed altered expression profiles during differentiation.

CONCLUSIONS: This research has illuminated previously unknown relationships between the microtubule cytoskeleton and pluripotent identity of hiPSCs, and assists to fill out the picture of how microtubules are arranged at all points in human development. Uncovering the dynamics of cellular architecture in pluripotent cells using live imaging may allow us to better manipulate hiPSCs, and shed light on the fundamental cell biological differences between pluripotent stages.

POSTER PRESENTATIONS

P15

Harnessing the plasticity of enteric glia for cell and gene therapy for Hirschsprung Disease

David Lai[1], Dr. Jing Zhao[1], Gunes Yildiz[1], Annette Bergner[1], Dr. Marlene Hao[1], Dr. Lincon Stamp[1]
[1]University of Melbourne, Parkville, Australia

Biography:
Master's student at the Stamp/Hao Lab at the University of Melbourne

Aim: The enteric nervous system (ENS) is a complex network of neurons and glia that are essential for many key functions of the gastrointestinal tract. Defects in the ENS can result in enteric neuropathies. Traditionally, enteric glia were thought to play primarily supportive roles within the ENS. However, recent studies suggest that these glial cells have proactive functions, even acting as neural stem cells capable of differentiating into enteric neurons, particularly when the system is under stress. This evolving understanding of glial function could significantly impact therapeutic approaches. One promising approach is the manipulation of the RET receptor tyrosine kinase - glial cell line-derived neurotrophic factor (GDNF) signaling pathway, key for normal ENS development, to promote glial cell proliferation and neurogenesis. In this study, we investigated the effects of varying RET-GDNF signaling on murine primary enteric glia cells.

Methods: Adult enteric glia were isolated from the small intestine of Sox10creERT2;GCAMP mice by dissociation and cell sorting, and cultured in media supplemented with four different levels of GDNF. After two weeks of growth, the cultures were fixed, and immunohistochemistry was performed to examine the differences in glial cell characteristics, including proliferation and differentiation into neurons, across the varying GDNF concentrations and at different cell densities.

Result: This study is the first to test the effect of varying GDNF concentrations on isolated adult enteric glia cells. Our findings suggest that enteric glial cells can undergo proliferation and neurogenic differentiation in the presence or absence of GDNF in vitro, however the concentration of GDNF appears to play a role in modulating the glial-neuron differentiation fate decision.

Conclusion: These findings provide valuable insights into the potential outcomes of manipulating RET signaling in the mature ENS and offers novel new therapeutic targets for treatment of enteric neuropathies.

POSTER PRESENTATIONS

P16

Inflammatory cytokines trigger the activation of the Kynurenine Pathway of Tryptophan Metabolism: Effects on embryonic mouse neural stem cell proliferation, health and NAD state

Dr. Michael Lovelace[1], Dr. Ben Summers[1], Dr. Kazuo Suzuki[1], Prof. Bruce Brew[1]
[1]St. Vincent's Centre for Applied Medical Research, Sydney, AUSTRALIA

Biography:
I am a developmental neuroscientist with a keen interest in the physiology of the developing nervous system, neural stem cells including a role of purinergic receptors e.g. P2X7 and Kynurenine Pathway in development and diseases such as Multiple Sclerosis. A very experienced microscopist experienced in widefield, confocal and super-resolution (SIM, GSD and TIRF), for localisation of molecules & relation to function. I am founder and manager of St. Vincent's AMR Live Imaging Core Facility (LIF).

Aims: New regulators of neural stem cell (NSC) proliferation underpin future cell therapies, while understanding NSC vulnerabilities during disease states could explain why innate repair in neurodegenerative/neuroinflammatory diseases fails. The kynurenine pathway (KP) regulates essential amino acid tryptophan (TRP)'s bioavailability, notably induced by stimuli including interferons – which are components of a cell's antiviral defences. In neurodegenerative diseases the KP becomes dysregulated, producing high levels of metabolites like potent neurotoxin Quinolinic acid (QUIN). QUIN metabolism by enzyme quinolinate phosphoribosyltransferase (QPRT) into cofactor NAD is rate-limiting, favouring QUIN accumulation during chronic KP activation.

Methods: We characterized KP/NAD synthesis gene expression via real-time PCR, and hypothesized KP modulation by interferons alters embryonic mouse (emNSC) proliferation, neurosphere size (assessed by microscopy) and cell health (Muse flow cytometry assays).

Results: Tryptophan-2,3-dioxygenase (TDO2) is the master regulator of the initial step of TRP catabolism, showing substantial basal expression (65.45±27.45 (*1000 versus beta-actin) while modestly upregulated by interferons (type II family member IFN-gamma->2.7-fold)/type I IFN-beta->2.1-fold). IFN-gamma slightly upregulates alloenzyme indoleamine-2,3-dioxygenase (IDO1, 4.1±0.38); while IFN-beta has no effect. NSCs varyingly express all KP genes basally including QPRT; upregulated by both IFNs.
While IFN-gamma significantly increased mean neurosphere size (10IU/mL, 287.3±32.73µm; p=0.0032; 100IU/mL, 305.7±10.64µm; p=0.0005) versus controls (180.5±42.1µm), it increased oxidative stress/caspase-activation, suggesting compromised cell health. Conversely, 50nM KYNA significantly increased neurosphere size (307.5±11.45µm; p=0.0004) while retaining cell health. Strikingly, both IDO-1/TDO2 are intrinsically linked with NAD metabolic state, as siRNA-knockdown with IFN-gamma co-treatment resulted in increased NAD+/NADH ratio, associated with an active metabolic state.

Conclusions: We undertook the first KP/NAD gene expression characterization. emNSCs are vulnerable to deleterious effects of interferons-particularly IFN-gamma, which preferentially reduces (while IFN-beta enhances) enzyme expression linked with producing protective KP metabolites KYNA/Picolinic acid. Selective KP inhibition, or increasing KYNA could minimize cell

death, improve regeneration during inflammation, and optimize NSC proliferation therapeutically.

POSTER PRESENTATIONS

P17

Spatially charting the developing fat-tailed dunnart

Ms Monika Mohenska[1,2,3], Ms Kellie Wise[2,3], Ms Elly D Walters[2,3], Dr Ning Liu[2,3], Dr Michael J Roach[2,3], A/Prof Owen JL Rackham[4], Dr Anja S Knaupp[1], A/Prof Fernando J Rossello[5], A/Prof Luciano G Martelotto[2,3], Dr Oliver Griffith[6], Prof Jose M Polo[1,2,3]

[1]Department of Developmental Biology, Biomedical Discovery Institute, Monash University, Melbourne, AUSTRALIA, [2]Adelaide Centre for Epigenetics, The University of Adelaide, Adelaide, AUSTRALIA, [3]South Australian immunoGENomics Cancer Institute, The University of Adelaide, Adelaide, AUSTRALIA, [4]The University of South Hampton, South Hampton, UK, [5]Murdoch Children's Research Institute, Melbourne, AUSTRALIA, [6]School of Natural Sciences, Macquarie University, Sydney, AUSTRALIA

Biography:
Monika Mohenska is a PhD student at Monash University and also works under Professor Jose Polo at the Adelaide Centre for Epigenetics and SAiGENCI, at The University of Adelaide. Her research focuses on the epigenetic mechanisms of cellular reprogramming, pluripotency, and developmental biology in model and non-model organisms. Her research contributes to understanding cell fate decisions and the underlying epigenetic changes.

Mammalian development differs tremendously across and within the various clades (Monotremes, marsupials and eutherian). For example, monotremes lay eggs, whilst marsupials and eutherians develop a placenta, which exhibits varying degrees of invasiveness to the maternal tissue. Assessing marsupial biology offers valuable insights to developmental processes and can contribute to the future of conservation efforts as well as novel medical advancements in reproductive medicine. However, despite their importance, the development of most marsupial species remains elusive. To address this, we used the dunnart (sminthopsis crassicaudata) as a marsupial model to study early stages of development. We applied a spatial transcriptomic technique to consecutive FFPE sections of a near term dunnart embryo to capture, analyse and visualise its gene expression in 3D. We present the first high-resolution spatial map of the dunnart embryo. Notably, our preliminary findings reveal that cortical markers are expressed in neural progenitors even before cortex formation, offering key insights into the evolutionary conservation and divergence between marsupial and non-marsupial mammals. This study not only establishes a foundation for future investigation of marsupials, but also paves the way for the application of cutting-edge spatial techniques on various non-model organisms. By shedding light on marsupial development, our work contributes to the broader field of evolutionary developmental biology, and offers insights for discoveries in biomedical research.

POSTER PRESENTATIONS

P19

Investigation of transcription factor Tead1 in hydrocephalus and neural development

Miss Alexandra Pelenyi[1], Professor Michael Piper
[1]The University of Queensland, St Lucia, Australia

Biography:
Alexandra Pelenyi is a PhD student at the Piper Lab in the School of Biomedical Sciences at The University of Queensland. The Piper Lab focuses on revealing the fundamental mechanisms that underpin neurogenesis within the neocortex, hippocampus and cerebellum. Alexandra's project is investigating the function of the Hippo signalling pathway transcription factor Tead1, and its role in neural development in the murine mouse brain. Additionally, Tead1 is implicated in the developmental disease hydrocephalus. Her project encompasses the functional mechanism in which Tead1 is involved in this neural disease.

Hydrocephalus is a severe neurological condition characterised by swelling of the cerebral ventricles and increased intracranial pressure, often resulting in brain damage. The mechanisms behind hydrocephalus are not well understood, however dysfunction of neural progenitors is implicated. The Hippo pathway is a critical developmental signalling pathway and controls cell proliferation, differentiation, and cell identity. Hippo signalling converges on the TEA-domain (TEAD) transcription factor family, which activate when Hippo signalling is switched off. The role of Hippo signalling during neural development has not been extensively studied. However, mutation of the Hippo pathway gene Yap in the mouse results in hydrocephalus. Tead1 is expressed in the developing mouse brain, but its potential involvement in hydrocephaly is unknown.
We investigated a conditional knockout mouse that eliminates Tead1 from NSCs at embryonic day (E) 7.5 and observed hydrocephalus postnatally. To begin probing the underpinnings of this phenotype we have mapped the spatiotemporal expression of Tead1 in the brain at E14, E16, and E18 and postnatal day (P) P5 and P20, using immunofluorescence. In the embryo, we identify Tead1 expression in neural progenitors and ependymal cell along the ventricles, and Tead1 expression persists in ependymal cells postnatally. Ongoing studies are focused upon exploring the cellular and molecular role of Tead1 with respect to neural development and congenital hydrocephalus, and its possible interplay with Yap and the Hippo pathway during these events.

P18

A 2D in-vitro model of early-stage placental development

German Atzin Mora Roldan[1], Alexa Mae Gill[1], Karoline Swiatczak[1], Dulce Medina[1,2], Professor Jose Polo[1]

[1]Adelaide Centre for Epigenetics, School of Biomedicine, Faculty of Health and Medical Sciences, The University of Adelaide, Adelaide, South Australia, Australia. Adelaide, AUSTRALIA, [2]The South Australian Immunogenomics Cancer Institute, Faculty of Health and Medical Sciences, The University of Adelaide, Adelaide, South Australia, Australia. , Adelaide, AUSTRALIA

Biography:

German Mora is a Grant-Funded Researcher in ACE, where he works on the generation of improved in vitro models of early placentation, reprogramming, and differentiation. He has more than 10 years of experience with iPSCS. Born an raised in Mexico, during his bachelor degree, he co-created the first iPS cell lines from Mexican donors derived from dental pulp cells. He obtained his PhD at UNAM. Then, he became a full-time researcher at the department of genetics at Instituto de Oftalmologia "Conde de Valenciana" working in differentiation of pluripotent cells from patients carrying relevant mutations into eye field lineage organoids.

The placenta is a temporary fetal organ that plays a vital role in development. It is mainly composed of two differentiated cell lines: syncytiotrophoblasts (ST) and extravillous trophoblasts (EVT). Most current 2D in vitro models are comprised of cells differentiated from trophoblast stem cells (TSCs) or induced TSCs into STs or EVTs. This limited their ability to capture early stages of interaction between these two cell lineages. To overcome these limitations, we established a 2D model aimed to capture trophoblast differentiation and cellular interactions in an environment closely resembling in-vivo conditions through the incorporation of iTSCs, endometrial fibroblasts (EFs), and vascular cells (HUVECs). iTSCS were seeded in a 2D monolayer comprised of EFs and HUVECs. The differentiation process was conducted by alternating the culture medium daily, replacing it with ST and EVT media, to simulate a gradient. Cells were harvested and fixed at specific time points (Days 2, 4 ,6 ,8) The process of differentiation was captured through brightfield and confocal imaging. Observations were substantiated by qPCR and flow cytometry. The results highlighted physical changes in iTSCs throughout differentiation and confirmed presence of STs and EVTs in a simultaneous manner. The results show synchronised prescensce of ST and EVT, as well as interaction with other cell lineages. Further, validation of our results could lead to advancements of a currently limited model of early placentation.

POSTER PRESENTATIONS

P20

Investigating The Effect of Culture Time on Inflammatory Phenotype Retention in Stem-Cell Derived Pediatric Intestinal Organoids.

Miss Lucy Porter[1,2], Dr Diana Micati[1,2], Ms Khanh Ha Do[1,2], Dr Andrew Pattison[1,2,3], Dr Eva Chan[1,2,4,5], Dr Wing Hei Chan[1,2], Dr Stuart Archer[1,2,3], Dr Edward Giles[4,5,6], Professor Helen Abud[1,2]
[1]Department of Anatomy and Developmental Biology, Monash University, Clayton, Australia, [2]Development and Stem Cells Program, Monash Biomedicine Discovery Institute, Clayton, Australia, [3]Monash Bioinformatics Platform, Clayton, Australia, [4]Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Clayton, Australia, [5]Department of Molecular and Translational Sciences, Monash University, Clayton, Australia, [6]Department of Paediatrics, Monash University, Clayton, Australia

Biography:
Lucy Porter is a current medical and Honours student at Monash University, with a particular interest in translational research. She is currently involved in research work with Monash University's Department of Anatomy and Developmental Biology, and Eastern Health Clinical School.

Aim: Crohn's Disease (CD), the most common subtype of Inflammatory Bowel Disease (IBD) in children, is an idiopathic chronic disease thought to arise from an interplay of environmental, microbial, and immune factors in genetically predisposed individuals. In CD, the integrity of the small intestinal epithelium is disrupted, facilitating antigenic invasion of intestinal tissue and triggering activation of pro-inflammatory signalling pathways. Despite the well-established relationship between epithelial disruption and CD onset and progression, the mechanisms behind this pathological change remain unclear. Emerging research conducted on murine models, immortalized cell lines, and, more recently, organoid models, suggests loss of epithelial integrity may stem from transcriptional dysfunction in genes governing the cellular and junctional composition of the small intestine. However, to-date, limited research has investigated these transcriptional shifts in pediatric samples, nor whether stem-cell derived intestinal organoids faithfully maintain inflammatory signatures in culture.

Methods: Organoid cultures were established from pediatric terminal ileal biopsies donated by CD and control patients. Bulk RNA sequencing was conducted on RNA extracted from CD and non-IBD-derived organoid lines at early (passage 2-4) and late passages (passage 5-8). Gene set enrichment analysis (GSEA) was utilised to identify the relationship between organoid inflammatory status and expression of key genes related to the cellular and junctional functionality of the intestinal epithelium.

Results: At early passages, CD-derived organoids exhibited significant downregulation in transcriptional signatures associated with Paneth, goblet, enterocyte, and transit-amplifying cell function, as well as loss of signatures responsible for maintaining desmosome and adherens junction integrity. However, at late passages, CD-derived organoids failed to retain a unique transcriptional signature to non-IBD-derived organoids.

Conclusion: Stem-cell derived pediatric organoids may retain disease-specific transcriptional signatures at early passages. However, this signature appears to be lost with prolonged culturing, highlighting the influence of culture time on transcriptional variation in organoid cultures.

POSTER PRESENTATIONS

P21

Derivation of cynomolgus monkey induced trophoblast stem cells via nuclear reprogramming

Miss Elly Walters[1,2,3], Dr Jia Tan[3], Dr Naiara Bediaga[1,2], Dr Sue Mei Lim[3], Miss Monika Mohenska[1,2,3], Dr Guizhi Sun[3], Dr Yu Bo Yang Sun[3], Dr Joseph Chen[3], Miss Qiaoyu Chen[3], Prof Xiaodong Liu[4], Prof Jose Polo[1,2,3]

[1]Adelaide Centre for Epigenetics, University of Adelaide, Adelaide, Australia, [2]South Australian immunoGENomics Cancer Institute, University of Adelaide, Adelaide, Australia, [3]Department of Anatomy and Developmental Biology, Biomedical Discovery Institute, Monash University, Monash, Australia, [4]Westlakes University, Zhejiang Province, China

Biography:

Elly Walters completed an Advanced Bachelor of Science-Research (Hons) with First Class Honours at Monash University under the supervision of Prof. Jose Polo. Her honours work was centred around modelling placental development in non-human primates using somatic cell reprogramming, directed differentiation, and organoid formation. Still using the same induced pluripotency based models she is now completing her PhD in Adelaide and is looking at early development and placentation in model and non-model organisms using a combination of tissue culture and molecular techniques.

The placenta is a unique organ developed during pregnancy that serves as a maternal-fetal interface to ensure proper growth and development of the fetus. Dysfunction of the placenta can lead to defective development of the fetus and pregnancy failure, therefore understanding placental development is crucial. Phylogenetically, non-human primates are very similar to humans, and this is especially true for placentation, making them an excellent model species for studying early primate placentation. Although studying placentation in non-human primates is possible, it is still extremely difficult due to logistical and ethical barriers. As an alternative, trophoblast stem cells (TS cells), can be derived from blastocysts or first trimester placentas, these TS cells have shown to be of great utility to model placenta biology and disease. However, they still require access to non-human blastocyst or early placenta. Here we report the generation of cynomolgus monkey induced trophoblast stem cells (iTS cells) via OCT4, SOX2, KLF4, c-MYC (OSKM)-mediated reprogramming of fibroblasts. The cynomolgus monkey iTS cells are capable of long-term proliferation and demonstrate key features of trophoblast lineage using whole genome transcriptional profiling in comparison to de novo TS cell counterparts. Functionally, the iTS cells can differentiate into the *in vivo* subtypes; syncytiotrophoblasts and extravillous cytotrophoblast cells. Furthermore, we have shown that cynomolgus monkey iTS cells can self-organise to form a trophoblast organoid as their human counterparts. As iTS cells are more easily accessible than TS cells derived from primary placental tissues or non-human blastocyst, iTS cells represent a valuable and tractable in vitro stem cell model that could be applied widely to assist in the understanding of primate placentation.

POSTER PRESENTATIONS

P22

Applying stomics to pluripotent stem cell derived organoids

Ms Maria Rosaria Nucera[1,2,3], Natalie Charitakis[1,2,3], Ryan Leung[1,3], Anna Leichter[1,2], Emma Scully[1,2], Ritika Saxena[1,2,3], Alexander Maytum[1], Michelle Scurr[1], Dr Gulcan Sarila[1], Rebecca Sutton[1], Lynn Rowley[1], Dr Natasha Tuano[1], Dr Holly Voges[1,2], F Ahmad, Denis Bienroth[1,2], Hieu Nim[1], Micheal See[1], X Sun[7], Ka Leung Li[7], B Yang[7], Vallari Sawant[1,2,3], Merzena Walkiewicz[1,2], Prof David Eisenstat[1,2,3], Prof Andrew Elefanty[1,2,3], David Elliot[1,2,3], Alejandro Hidalgo-Gonzales[1,2,3], Marie Faux[1,3], Melissa Little[1,2,3], S Lamande[1,2,3], Richard Millis[1,2,3], E Ng[1,2,3], Enzo Porrello, Fernando Rossello[1,2,3,5], Ed Stanley[1,2,3], J Vanslambrouck[1,2,3], R Werder[1,2,3], S Velasco[1,2,3], Mirana Ramialison[1,2,3,4,6]

[1]Stem Cell Medicine Department, Murdoch Children's Research Institute, Parkville, Australia, [2]The Novo Nordisk Foundation Center for Stem Cell Medicine, reNEW Melbourne, Murdoch Children's Research Institute, Parkville, Australia, [3]Department of Paediatrics, University of Melbourne, Parkville, Australia, [4]Australian Regenerative Medicine Institute, Monash University, Melbourne, Australia, [5]Department of Clinical Pathology, University of Melbourne, Melbourne, Australia, [6]System Biology Institute, Clayton, Australia, [7]BGI Australia, Brisbane, Australia

Biography:
Maria Rosaria Nucera is a PhD student in the Neural Stem Cell lab and the Transcriptomic and Bioinformatic lab in MCRI.

Spatial transcriptomics (ST) is a novel, disruptive technology that permits exploration of gene regulatory networks with spatial and temporal resolution. The insight provided by ST platforms promises to shed new light on developmental biology across an array of tissue types including organoids. In particular, organoids offer the opportunity to model development and disease while overcoming many of the limitations of animal models or 2D cell cultures. As organoids can be cultured from genetically engineered stem cell and patient cell lines, they permit the detailed analysis of the transcriptional perturbations that underlie disease pathogenesis. More specifically, organoids display 3D organisation in vitro, making them prime candidates for exploration with ST.

There have been relatively few studies using ST to investigate organoids due to their small size and the limitations of commercially available ST technologies. Furthermore, they have been limited to exploration of a single type of organoid at a time. Here we present the use of STOmics, the first ST platform to offer sub-cellular resolution while capturing transcriptome-wide information, to profile an array of organoids including: brain, heart, kidney, lung, cartilage and blood. We successfully obtained spatial information and identified areas of optimisation that will improve the application of STOmics technology to a wide variety of organoid models. We provide guidelines for profiling multiple organoids from a single chip to maximise data capture. In summary, by providing a systematic overview of ST data across these varied tissue types, this study illustrates the power of ST to improve our understanding of human organoids.

POSTER PRESENTATIONS

P23

RUNX1 expression tracks human haematopoiesis from haemogenic endothelium to haematopoietic stem cell

Ritika Saxena[1,2,3], Alexander Maytum[1,2,3], Tanya Labonne[1,3], Gulcan Sarila[1,2,3], Chantelle Inguanti[1,3], Hasindu Edirisinghe[1,3], Ed Stanley[1,2,3], Elizabeth Ng[1,2,3], Andrew Elefanty[1,2,3]
[1]Murdoch Children's Research Institute, Parkville, AUSTRALIA, [2]Department of Paediatrics, Faculty of Medicine, Dentistry and Health Sciences, University of Melbourne, Parkville, AUSTRALIA, [3]The Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), Murdoch Children's Research Institute, Parkville, AUSTRALIA

Biography:
Final year PhD Student with Prof Andrew Elefanty, A/Prof Elizabeth Ng and Prof Ed Stanley working on blood stem cell generation for therapy.

The ability to generate haematopoietic stem cells (HSCs) derived from human induced pluripotent stem cells would significantly impact on medicine and biotechnology. However, generation of HSCs capable of multi-lineage reconstitution and long-term engraftment has been challenging, in part because we do not understand the haemogenic process in the embryo that leads to HSC generation from an endothelial precursor. RUNX1 is a critical early marker of the hemogenic endothelium and master regulator of haematopoiesis, that gives rise to HSCs via an endothelial to hematopoietic transition (EHT). By understanding more about the expression and regulation of RUNX1, we can begin to unravel the EHT.

We generated stem cell reporter lines detecting RUNX1 expression and differentiated the cells into endothelial and haematopoietic cells in vitro, benchmarking the expression of key cell surface markers. We used spatial transcriptomics to correlate the expression of key haemogenic genes with RUNX1. Finally, we evaluated the functional capacity of haematopoietic cells generated in vitro by transplantation into immunodeficient mice.

RUNX1+ cells first appeared as a subset of CXCR4+ arterial endothelium, clearly indicating the emergence of haemogenic cells from an arterially patterned precursor. Reculturing RUNX1+ cells isolated from differentiating endothelium gave rise to CD45+ cells, confirming their hematopoietic potential. RUNX1 expression correlated with HLF, MECOM, MYB and KCNK17 expression in our cultures across the hemogenic window. Functional evaluation of CD34+ haematopoietic cells generated mice that showed high level multilineage engraftment with a donor derived bone marrow HSC compartment enriched in RUNX1+ cells, recapitulating key aspects of normal haematopoietic development and validating the reporter *in vivo*.

These studies highlight the critical role of RUNX1 at both ends of the EHT – marking hemogenic endothelium and emerging HSCs. We can now work to identify factors enhancing HSC generation from RUNX1+ hemogenic endothelium, bringing us closer to clinical translation.

POSTER PRESENTATIONS

P24

Blood Vessel Networks Generated from Human Stem Cells

Dr Connor Sherwood[1,2], A/Prof. Tony Pang[1,2]
[1]Westmead Hospital, Westmead, AUSTRALIA, [2]University of Sydney, Sydney, Australia

Biography:
Connor Sherwood is a postdoctoral research scientist working as part of the Surgical Innovations Unit at Westmead Hospital. His current research priority is developing cutting-edge ex vivo models of how cancer tissue interacts with blood vessels. He is also involved in biobanking and biomarker discovery programs being run by the Surgical Innovations Unit, and is affiliated with the University of Sydney as an Adjunct Lecturer in the Sydney Medical School.

Aim: To generate blood vessel networks from human induced pluripotent stem cells (iPSC).

Method: Human iPSC are aggregated into embryoid bodies in suspension culture. These embryoid bodies are exposed to an optimized chemical cocktail which promotes first mesoderm induction, and then vascular lineage commitment. This produces floating aggregates of differentiated human endothelial cells and pericytes. These aggregates are then embedded in a gel matrix to facilitate sprouting of blood vessels from the aggregates into the gel. Fixed blood vessel networks undergo immunofluorescence staining and are imaged on a confocal fluorescence microscope.

In an additional experiment, differentiated aggregates were embedded in a gel inside a chamber which was custom-fabricated to produce a gradient in the concentration of key growth factors (VEGF-A and FGF-2). Interference phase contrast microscopy was utilized to observe growth patterns in the blood vessel network over time in response to this chemical gradient.

Result: Interference phase contrast microscopy of blood vessel networks show morphological features which are consistent with angiogenic tip cells (white arrows). Confocal microscopy of blood vessel networks following immunofluorescence staining demonstrates the presence of differentiated CD31+ endothelial cells (red arrows) and PDGFR-β+ pericytes (green arrows). These endothelial cells and pericytes have spontaneously assembled into tube-like structures in vitro. These results suggest successful differentiation of human induced pluripotent stem cells into mature blood vessel networks.

Furthermore, blood vessel networks show distinct growth patterns when exposed to a gradient in the concentration of growth factors in the culture medium. Specifically, blood vessel networks show preferential growth towards the region of high growth factor concentration.

Conclusion: Mature blood vessel networks containing both major capillary-bed cell types were differentiated from human iPSC. The growth patterns of these blood vessel networks were responsive to artificially generated growth factor gradients.

POSTER PRESENTATIONS

P25

Harnessing germline stem cells as tools for marsupial conservation

Dr Gerard Tarulli[1], Ms Dorthea Hansen[1], Ms Christina Alsterberg[1], Mr Patrick Tatt[1], Ms Nicola Kolaitis[1], Ms Sara Ord[2], Dr Stephen Frankenberg[1], Prof. Andrew Pask[1]
[1]The University of Melbourne, Parkville, Australia, [2]Colossal BioSciences, Dallas, The United States of America

Biography:
I am researcher leader of the stem cell division in TIGRR lab at The University of Melbourne. TIGRR lab is developing technologies necessary to de-extinct the Tasmanian tiger, such as somatic cell reprogramming, in-vitro gametogenesis and assisted reproduction technologies. Through this effort, marsupial conservation can engage contemporary approaches to species restoration including cloning, ART and genetic modification. These advances are sorely needed to preserve Australia's declining marsupial populations.

AIM: Australian marsupial species are in significant decline, and their conservation is limited by the absence of advanced technologies such as genetic restoration. This work aimed to fill this gap by developing methods to culture marsupial spermatogonial stem cells (SSCs) and generate induced pluripotent stem cells (iPSCs) for differentiation into germ cell lineages.

METHODS: Using fat-tailed dunnarts, a close living relative of several endangered marsupials, iPSCs were generated using transposase- or virus-based reprogramming. iPSCs were assessed for hallmarks of pluripotency, including expression of pluripotency markers and germ layer gene expression after differentiation. Further, iPSCs were differentiated into primordial germ cell-like cells (PGCLCs) under conditions developed for mouse and human iPSCs. Finally, testis tissue digestion and culture protocols were developed for primary dunnart SSCs.

RESULTS: Stable dunnart iPSC lines were successfully generated and expressed core pluripotency genes, and genes associated with germ lineages in a differentiation assay. Of 64 pluripotency genes conserved amongst iPSCs from several eutherian species, dunnart iPSCs showed high expression of 44. Nine were repressed and the remaining 11 unchanged. Exposure of dunnart iPSCs to PGCLC differentiation conditions induced upregulation of PRDM1, PRDM14, and ITGA6 that are hallmarks of PGCLC differentiation in human and mouse iPSCs. Further, transciptomic profiles of dunnart PGCLCs were highly similar to mouse and human PGCLCs. Digestion and culture of primary dunnart testis demonstrated that medium containing bFGF and GDNF allows survival and expansion of dunnart spermatogonia in vitro. These germ cells can be maintained in continuous culture for >14 weeks and survive multiple passages.

CONCLUSIONS: This study has developed the first protocols to enable survival and expansion of marsupial SSCs in vitro. Together with the successful generation of dunnart iPSCs and their early germline differentiation, this study provides critical foundations for successful generation of genetically modified Australian marsupials in the future.

POSTER PRESENTATIONS

P26

Developing a platform of quality-controlled, validated stem cell models for researchers within a multi-user facility

Dr Johana Tello Velasquez[1], Ms Robyn Yeh[1], Associate Professor Renee M Whan[2], Dr Alison Ferguson[1]
[1]UNSW Australia, 3DCF, Mark Wainwright Analytical Center, Sydney, Australia, [2]UNSW Australia, Mark Wainwright Analytical Center, Sydney, Australia

Biography:
Johana Tello Velasquez is a neurobiologist with expertise in the culture and profiling of stem cells and primary neuronal-glial cells. She completed her PhD in neuroscience at Griffith University where she developed a screening process to identify natural compounds that enhance olfactory glia as a therapeutic for Spinal Cord Injuries.

Aims: Advancements in cell culture techniques and the development of 3D models to recapitulate specific organs, biological processes, and disease states have enabled creation of more physiologically relevant models of human development and disease. However, developing these models is often inaccessible and technically, time and cost intensive. The 3D Culture Facility (3DCF) within the Mark Wainwright Analytical Centre at UNSW aims to provide a platform in which models including organoids and explant cultures will be readily accessible to researchers to support acceleration and translation of research outcomes.

Methods: The 3DCF is structured through three phases. Phase 1 focuses on establishing a pilot cohort of hESC, iPSC, differentiated cell lines and organoids that will be accessible to researchers. Phase 2 aims to develop a multi-user facility that will enable collaborative research efforts across multiple disciplines and include integration of advanced technologies and methodologies for organoid generation. Phase 3 will accelerate these capabilities by incorporating high-throughput technologies for automated culturing, imaging, analysis and manufacturing of organoids.

Results: The facility has successfully maintained quality-controlled iPSCs (n=2), enabling the creation of a diverse pilot cohort of organoids. This includes embryoid bodies, definitive endoderm, and endothelial cells, which have been differentiated into cerebral organoids, lung progenitors and lung organoids. The facility has also established a patient-derived airway cell line cultured at the air-liquid interface (ALI) and assisted researchers in testing novel hydrogels.

Conclusion: The 3DCF aims to provide a platform for generating robust, reproducible and validated stem cell models in a collaborative environment supporting UNSW faculties, external medical institutes and hospital partners. Future expansions will include a broader range of PSC-derived organoids, and patient-derived healthy and disease-state organoids and explant cultures. The facility plans to integrate liquid handling robotics, automation, image and analysis strategies, and biomanufacturing to accelerate research and translate findings into clinical applications.

POSTER PRESENTATIONS

P27

A dual-reporter human pluripotent stem cell line for live-cell imaging of human immune responses

Dr Zhengqi Cheng[1], **Professor Christine Wells**[1]
[1]University of Melbourne, Melbourne, Australia

Biography:
Christine Wells is Chair of Stem Cell Systems at the University of Melbourne. She is a senior stem cell researcher with over 20 years in myeloid biology. She has authored over 120 peer reviewed publications, collectively cited >28,000 times. Her team develops both computational and stem-cell derived models of human macrophages.

Aim: Macrophages are phagocytic immune cells at the front line of host defense against microbial infections, they recognize pathogen-associated molecular patterns (PAMPs) through pattern recognition receptors (PRRs). TLR-MyD88-NFkB and TLR-TRIF-IRF pathways are key signalling pathways that regulate responses to external or intracellular ligands. Previous NFkB reporters have tagged the p65 subunit, however recent proteomics analyses identify cREL and p50 as the main NFkB subunits in human monocytes and macrophages. In this study, developed a dual reporter cell line to study the dynamics of cREL and IRF activation in macrophages responding to a panel of bacteria commensal to human gut.

Method: PB001.1 iPSC cells were engineered with a dual reporter cREL-YFP/mCherry-OAS2 was used as a screening system to monitor NFkB pathway activation dynamics and IRF pathway upregulation in individual cells. iPSC-derived macrophages were differentiated and challenged with 16 PRR ligands.

Results: iPSC-derived macrophages express phenotypic macrophage markers of CD14, CD45, CD68 and HLA-DR. The cells were responsive to bacterial ligands and whole, heat killed bacteria. LPS induced dynamic c-Rel nuclear translocation peaks at 15min and 45min. The percentage and extent of macrophages responding to IRF pathway activation in translational level was ligand type dependent.

Conclusions: Our results suggest that the iPSC-derived macrophages serve as a useful model to study inflammatory pathways. Future studies are aimed at identifying interventions that can modify cREL and IRF-dependent inflammatory outputs.

POSTER PRESENTATIONS

P28

Macrophages influence epithelial responses to respiratory viral infections in human stem cell models of the lung

Hannah Baric[1,2], Katelyn Patatsos[1,2], Dr Declan Turner[1,2], Sahel Amoozadeh[1,2], Kathleen Strumila[1,2], Tanya Labonne[1,2], Dr Ed Stanley[1,2], **Dr Rhiannon Werder**[1,2]
[1]Murdoch Children's Research Institute, Melbourne, AUSTRALIA, [2]Novo Nordisk Foundation Centre for Stem Cell Medicine, reNEW Melbourne, Melbourne, Australia

Biography:
Dr Rhiannon Werder is a Team Leader at Murdoch Children's Research Institute, spearheading research utilising stem cell models of the lung to transform the discovery of new therapies for respiratory disease. She received a PhD from the University of Queensland in 2017. Dr Werder was awarded a NHMRC CJ Martin Early Career Fellowship to undertake postdoctoral work at a world-leading stem cell institute (Center for Regenerative Medicine, Boston University). Dr Werder now leads a multidisciplinary team at MCRI, combining expertise in stem cell biology and immunology, to develop new therapies for respiratory infections and chronic lung diseases.

AIMS: Respiratory viral infections are a leading cause of illness in humans and can become deadly when they reach the alveolar region, causing pneumonia. There are few vaccines or effective antivirals for these infections, partly due to the lack of human-relevant models. The aim of this project is to create a new model from stem cells to study pneumonia pathogenesis that incorporates alveolar epithelial cells, the first cells targeted by viruses, with macrophages, the resident immune cells in the lungs.

METHODS: Using established directed differentiation approaches, induced pluripotent stem cell (iPSC)-derived alveolar epithelial cells and iPSC-derived macrophages were created and then incorporated into a physiologically relevant air-liquid interface platform. Co-cultures were characterised by immunostaining, flow cytometry and gene expression analyses. Co-cultures or mono-cultures were infected with respiratory syncytial virus (RSV) and responses monitored across five days.

RESULTS: Sustained alveolar epithelial-macrophage co-cultures were reproducibly established. Co-culture did not negatively impact the identity or function of either cell type. Following co-culture, macrophages localised to the apical surface of the alveolar epithelial cells, and time lapse imaging revealed dynamic macrophage movement. Production of the macrophage supportive cytokines, CSF1 and CSF2, by iPSC-derived alveolar epithelial cells was sufficient to sustain the macrophages in co-culture. Indeed, neutralisation of epithelial-derived CSF1 impaired survival and activation of macrophages. Co-culture with macrophages significantly augmented epithelial antiviral responses to RSV and protected epithelial barrier integrity. Moreover, following infection, macrophages harboured RSV, suggesting roles for macrophages in both boosting immune responses and clearing virus. Finally, macrophages accelerated epithelial wound repair.

CONCLUSION: We have established the first co-culture at ALI of the human alveolar epithelium and macrophages from iPSCs. Epithelial-macrophage crosstalk in these cultures alters immune responses to viral infection and repair. In future studies, we will use this advanced stem-cell platform to screen for new therapeutic targets against respiratory viruses.

P29

Accessing Cord Blood Units for Research: Expanded Consent Options and Pathways towards Cellular Therapies

Dr Keren Abberton[1], Ms Junann Wish-Wilson[1], Ms Tricia McDonald[1], A/Prof Ngaire Elwood[1]
[1]MCRI, Parkville, Australia

Biography:
Dr Keren Abberton (BA, M. Rep. Sci. PhD (Monash University)) joined the MCRI as a Scientific Project Officer for the Cord Blood Research Group in 2016. She received her PhD from Monash in 2000 and completed a postdoctoral position at the University of Rochester New York. She has worked in a broad range of fields ranging from Physiology, angiogenesis, tissue engineering and Stem cells, with a passionate Interest in ethics and Governance She developed the protocols for reconsent of cord blood donors as well as helping develop QA standards guidelines for iPSC cell lines.

AIMS: The potential for Cord Blood (CB) as Advanced Therapeutic Medicinal Products and in basic research has increased significantly over recent years. Public cord blood banks (CBB) can play a key role in facilitating research. This study examines the provision of CB from the BMDI CBB for use in basic and clinical research.

METHODS: The AusCord Common Consent for Cord Blood Collection, Banking and Use (2016) provides donors the option to consent for their CB to be used for HREC-approved research. This was expanded in 2023, allowing CB to be used for clinical indications beyond blood disorders, and with the option for CB to be used for commercial use. With consent, CB units that do not meet the criteria for processing or banking are currently provided by the BMDI CBB to 14 research groups who completed the application process. Each group must provide a project outline, a copy of their ethics application and approval letter and a statement outlining predicted requirements for CB, the number of CB units required and the time period for which it is required. A separate process exists for accessing cryopreserved CB units that may not or may be on the bone marrow donor registry, and which can be used for laboratory or clinical research/trials, respectively.

RESULTS: The new consent form shows a strong uptake with 187 of 194 donors (96%) consenting to research, and 174 (90%) consenting to release for commercial use. Basic research areas where CB is being provided includes diabetes, novel cancer treatments, T-cell development, malaria and trisomy 21. Red cells and plasma are additional available CB components.

CONCLUSIONS: Cord blood and its components is an important source material for basic and clinical research. Public CBB, and their donors, play an important role facilitating the advancement of research and development of new therapies.

POSTER PRESENTATIONS

P30

Transient reduction of electrophysiological activity enhances energy reserves for cellular repair and protecting human midbrain neurons.

Robert Adams[1,2], Dr Bridget Milky[1,2], Paris Mazzachi[1,2], Amal Abdisharid Ali[1,2], Professor Glenda Halliday[4], Professor Glenn King[5], Dr Tim Sargeant[1,3], Professor Cedric Bardy[1,2]
[1]Laboratory for Human Neurobiology, South Australian Health and Medical Research Institute (SAHMRI), Adelaide, AUSTRALIA, [2]Flinders Health and Medical Research Institute, College of Medicine and Public Health, Flinders University,, Adelaide, Australia, [3]Lysosomal Health in Ageing, SAHMRI, Adelaide, Australia, [4]School of Medical Sciences, Faculty of Medicine and Health and the Brain and Mind Centre, University of Sydney,, Camperdown, Australia, [5]Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia, [6]Australian Research Council Centre of Excellence for Innovations in Peptide and Protein Science, The University of Queensland, Brisbane, Australia

Biography:
Robert Adams is a final year PhD student studying relationships between energy metabolism, cellular waste clearance mechanisms, and electrophysiological function in human neuronal cultures. His work has been performed in the context of neurodegeneration in Parkinson's disease and Sanfilippo syndrome with the aim of advancing our understanding of how energy deficiencies damage neuronal functions and survival.

Aging-induced metabolic decline disrupts neuronal repair functions, accelerates cell death and exacerbates brain disorders. In Parkinson's Disease (PD), dopaminergic neurodegeneration in the substantia nigra overloads the electrical activity of the remaining neurons, creating a vicious cycle that depletes energy reserves and amplifies cell loss. However, compensating for such high metabolic demand might effectively slow PD progression. We hypothesised that transiently lowering the electrophysiological activity of neurons would redistribute scarce energy reserves for cellular repair, enhancing neuroprotection.

We generated midbrain dopaminergic neuronal cultures from human embryonic stem cells and exposed them to broad-acting neuronal ion channel modulators (ICMs; e.g., tetrodotoxin and glutamate receptor blockers) or optogenetic inhibition (e.g., Halorhodopsin) to inhibit electrophysiological activity and raise energy levels in the form of increased adenosine triphosphate availability (ATP). To investigate if ICMs were protective against metabolic stress, rotenone was used to impair mitochondrial function, lowering both ATP levels and lysosome functions. Neurons were exposed to H_2O_2 to explore whether ICMs protected against neurodegenerative oxidative stress.

Our study shows that broad-acting neuronal ICMs or optogenetic inhibition can increase ATP levels, enhance lysosomal function when mitochondria are impaired, and protect against oxidative stress-induced neurotoxicity. Additionally, we identified substantia-nigra-specific ion channels that, when modulated, raise ATP levels in human midbrain neurons.

This work lays the foundation for developing neuron-type-specific ICMs or optogenetics to treat neuronal energetic stress in PD and other neurological disorders.

POSTER PRESENTATIONS

P31

Gene Editing for Usher Syndrome Type 2A: Unravelling Photoreceptor Degeneration Mechanisms and Exploring Therapeutic Potential using Retinal organoids

Dr Deborah Aubin[1,5], Dr Hani J Kim[2,5], A/Professor Pengyi Yang[2,5], Dr Mark Graham[3,5], A/Professor Leszek Lisowski[4,5,6,7], A/Professor Anai Gonzalez-Cordero[1,5]
[1]Stem Cell Medicine Group - Children's Medical Research Institute, Westmead, AUSTRALIA, [2]Computational Systems Biology - Children's Medical Research Institute, Westmead, Australia, [3]Proteomics Unit - Children's Medical Research Institute, Westmead, Australia, [4]Translational Vectorology Research Unit - Children's Medical Research Institute, Westmead, Australia, [5]chool of Medical Sciences, Faculty of Medicine and Health, The University of Sydney, Sydney, Australia, [6]Australian Genome Therapeutics Centre, Children's Medical Research Institute and Sydney Children's Hospitals Network, Westmead, Australia, [7]Military Institute of Medicine, Laboratory of Molecular Oncology and Innovative Therapies, Warsaw, Poland

Biography:
Dr. Deborah Aubin completed her PhD with highest honors in 2019 from the University of Paris-Saclay, France, where she modeled Neurofibromatosis type 1 using pluripotent stem cell-derived Schwann cells at ISTEM. She then joined the Collège de France Institute, working with Prof. Alain Prochiantz on the pathophysiology of ALS using human iPSC-derived motor neurons. In January 2022, Dr. Aubin became a postdoctoral researcher in the Stem Cell Medicine Group under A/Prof. Anai Gonzalez Cordero. Her current work focuses on modeling Usher syndrome type 2A with iPSC-derived retinal organoids to develop innovative genetic therapies.

Usher syndrome is a prevalent autosomal recessive disorder characterized by sensorineural hearing loss and progressive vision loss, with USH2A gene mutations being the predominant cause of Usher2, one of the most common inherited retinal diseases. Effective treatments are currently lacking, as standard adeno-associated virus (AAV) vector gene augmentation therapy is not feasible given the large size of the USH2A gene (15kb transcript).
Our study leverages pluripotent stem cell technology to form retinal organoids, providing a valuable model for studying disease mechanisms and testing therapeutic strategies. We derived retinal organoids from patients with a common USH2A mutation, specifically the deep intronic variant (c.7595-2144A>G), to gain insights into photoreceptor degeneration and explore gene editing strategies.
Through comprehensive molecular characterization using scRNA sequencing, we identified novel disease biomarkers and observed reduced levels of USHERIN and other Usher2 complex-associated proteins in affected organoids. Notably, we discovered significant mitochondrial dysfunction, evidenced by reduced mitochondrial respiration and increased reactive oxygen species production. These findings suggest that mitochondrial dysfunction plays a crucial role in Usher2A pathology.
To address this, we designed a single AAV vector to deliver genome editing machinery targeting the c.7595-2144A>G mutation. By treating Usher2A retinal organoids and assessing the cutting efficiency with various guide RNA (gRNA) combinations, we found increased cutting efficiency with prolonged treatment duration. Moreover, our comparative analysis of different AAV capsid variants identified a novel in-house developed capsid variant that showed improved gene delivery efficacy in vitro in photoreceptor cells.
Our study offers insights into the mechanisms driving photoreceptor degeneration in Usher2A, highlighting mitochondrial dysfunction as a key pathological feature. This underscores the

potential of combining stem cell-derived retinal organoids with gene editing approaches as a therapeutic avenue. These findings pave the way for further research and development of effective treatments to combat blindness in Usher syndrome patients.

POSTER PRESENTATIONS

P32

Vascular Phenotype Switching In Induced Pluripotent Stem Cell-Derived Models of Spontaneous Coronary Artery Dissection

Dr Monique Bax[1,2], Ms Keerat Junday[1,2], Dr Muhammad Alsherbiny[1], Dr Stephanie Hesselson[1], Ms Emily Hurley[1], Prof David Muller[3], Ms Ingrid Tarr[1,2], Dr Lucy McGrath-Cadell[1,2,3], Dr Siiri E Iismaa[1,2], A/Prof Eleni Giannoulatou[1,2], Prof Robert M Graham[1,2,3]
[1]Victor Chang Cardiac Research Institute, Darlinghurst, AUSTRALIA, [2]School of Medicine, University of New South Wales, Kensington, AUSTRALIA, [3]St Vincent's Hospital, Darlinghurst, AUSTRALIA

Biography:
Dr Monique Bax is an award-winning stem cell researcher working in vascular bioengineering and vascular disease modelling. As a Senior Post Doctoral Scientist at the Victor Chang Cardiac Research Institute, Sydney, Dr Bax uses induced pluripotent stem cells to research the major cause of myocardial infarcts in women under 50 (SCAD). She has lead a team to generate the largest stem cell biobank from patient blood cells; Using these stem cells, she generates vascular cells to understand the molecular mechanisms of this disease.

Spontaneous coronary artery dissection (SCAD) accounts for ~25% of myocardial infarcts in women under 50 yo. Characterised by haematoma development within the coronary artery wall, likely from microvessel rupture, the coronary artery dissects from within. As patients lack traditional cardiovascular risk factors, the cause of this potentially lethal disease is poorly understood. No specific preventative therapeutics are available. Onset is influenced by genetic background and environmental stressors.
Aim: Phenotype induced pluripotent stem cell (iPSC)-derived vascular models of SCAD compared to controls, to capture the biological complexity at the cellular level.
Methods: A SCAD iPSC biobank was generated using Sendai virus reprogramming, and an iPSC characterisation pipeline. Relevant coronary artery cell types, endothelial cells (iECs) and vascular smooth muscle cells (iVSMCs), were differentiated, and proteomic analysis performed (N=4-5/line/cell type) using a SWATH approach (SCIEX 6600 Triple-TOF-MS). Data was analysis with ExpressAnalyst, and IMPaLA pathway analysis. Live cell tracking was performed using an in-incubator imager (Incucyte). Statistical analyses as appropriate.
Results: We generated the largest SCAD biobank (21 genetically different lines) from SCAD survivors, and age- and sex-matched controls. Phenotypic analysis of the iECs (CD31+, VWF+, VE Cadherin+) and iVSMCs (TAGLN+, αSMA+, SM-MHC+) suggested SCAD may arise due to dysfunction in both cell types. Proteomic analysis of SCAD iECs demonstrated an upregulation in a thrombus formation-associated protein (adjusted P=0.04) compared to controls. Live cell tracking showed significantly increased proliferation in SCAD cells (4.5±1.1 h) compared to controls (5.6±1.6 h) (P=0.05). SCAD iVSMC proteomic analysis revealed altered phenotype switching pathways, including the TGF-β pathway, consistent with morphological changes also detected.
Conclusion: Together, these results suggest that cellular activation underpins SCAD. Importantly, this process of activation can be therapeutically targeted. These models will be fundamental in assessing therapeutic efficacy in future drug screening, critically needed to identify the first SCAD-specific therapeutics for disease prevention.

POSTER PRESENTATIONS

P33

Modelling Rubinstein-Taybi Epigenetic Neurodevelopmental Disorder to Test and Develop New Therapeutic Strategies

Dr Rudrarup Bhattacharjee[1], Dr Suzan de Boer[2], Ms Dulce Medina Garcia[1], Dr German A Mora-Roldan[1], Dr Joseph Chen[2], Dr Sue Mei Lim[2], Dr Stefan White[3], Prof Jose M Polo[1]
[1]Adelaide Centre for Epigenetics, School of Biomedicine, and South Australian Immunogenomics Cancer Institute, University of Adelaide, Adelaide, Australia, [2]The Department of Anatomy and Developmental Biology, Biomedical Discovery Institute, Monash University, Clayton, Australia, [3]White-Fox Science Consulting, New Zealand

Biography:
Dr Bhattacharjee is a postdoctoral researcher in Polo Lab at the Adelaide Centre for Epigenetics, University of Adelaide. He specialises in genetics and biology of neurodevelopmental disorders (NDD) with extensive experience in generation and characterisation of clinically relevant diseases models. His PhD work involved the discovery novel role of a mRNA export related gene in transcription and DNA damage leading to defining a mechanism of x-linked THOC2 NDD syndrome. His current work focuses to understanding the mechanism of NDDs involving mutations in epigenetic modifiers in order to define and test new therapeutic targets.

Rubinstein-Taybi syndrome (RTS) is a rare childhood neurodevelopmental disease (NDD) caused by genetic mutations in CREBBP or EP300 genes. Children with RTS show intellectual disability and other cognitive deficits. As this is a NDD, historically it has been very difficult to study in vitro. However, we got access to fibroblasts from RTS patients, carrying a mutated CREBBP gene, which we used to generate iPSCs. Importantly, this will allow us to model the disease in vitro, both as 2D monolayer neural culture and 3D brain organoids, to systematically evaluate the cellular and molecular mechanisms underpinning RTS NDD. Our preliminary data from these patient iPSCs indicate that the cells replicate some neuronal defects such as sub-optimal differentiation, associated with the disease. . As mutations in the CREBBP gene impacts histone acetylation, we premise that FDA approved Histone Deacetylase inhibitors (HDACi) (epigenetic drugs that prevent deacetylation) can be used to attenuate RTS symptoms and associated co-morbidities, paving a way for the development of a treatment for affected children. We used one such FDA approved HDACi drug and showed restoration of the neuronal differentiation potential of RTS patient derived iPSCs. We have now expanded this study to our 3D brain organoid system, to evaluate the efficacy of this drug in developmental context. As the human brain is extremely plastic in the first years of life, we hypothesise that any intervention that may improve the cognitive functions of the brain, could impart significant benefits to the RTS children. Furthermore, as the drugs are already approved for their use in against cancer, the outcomes of this work could help fasten the trial of this “repurposed” drug to treat RTS for human patients.

POSTER PRESENTATIONS

P34

Bioengineering functional skeletal muscle derived from pluripotent stem cells

Kaitlyn Bibby[1,2], Rebecca McElroy[1,2], Associate Professor Richard Mills[1,2]
[1]Muscle Bioengineering, Murdoch Children's Research Institute, Melbourne, AUSTRALIA, [2]The Novo Nordisk Foundation Centre for Stem Cell Medicine, reNEW, Murdoch Children's Research Institute, Melbourne, Australia

Biography:
Kaitlyn is a research assistant within the Muscle Bioengineering lab at the Murdoch Children's Research Institute. Kaitlyn's work involves the optimisation of stem cell derived protocols including skeletal muscle protocols using 3D platforms.

Skeletal muscle comprises 40% of our total body weight and plays an important role in metabolism and locomotion. A major issue in studying muscle disorders is a lack of physiologically relevant in vitro human models. Advances in stem cell biology and bioengineering have led to 3D culture systems that are more comparable to *in vivo* human physiology. Here, we direct induced pluripotent stem cells (PSCs) to paraxial mesoderm followed by myogenic specification to generate PSC-derived myoblasts. Myoblasts express the myogenic marker CD56, MyoD, the transcription factor PAX7 and can freely differentiate and fuse in 2D to myotubes. Our team uses a 96 well micro-muscle screening platform to enable functional screening of bioengineered muscle. Using this platform, PSC-derived myoblasts are combined within a hydrogel, and subsequently differentiated to form bundles of myofibres. The resulting bioengineered muscle expresses striated titin and functionally responds to electrical stimuli. We aim to further optimize our serum-free differentiation protocols to produce more physiologically relevant skeletal muscle, for use in research applications such as disease modelling and drug discovery.

POSTER PRESENTATIONS

P35

An automated protocol for generating microglia-containing cortical organoids

Miss Jordan Elli Clarke[1], Mrs Angela Connelly[2], Dr. Maciej Daniszewski[1], Ms Yumiko Hirokawa[1], Prof. Alice Pébay[1,3], Prof. Murray Cairns[4,5], Dr. Maria Di Biase[1,6,7]
[1]Stem Cell Disease Modelling Lab, Department of Anatomy and Physiology, The University of Melbourne, Australia, [2]Central Cardiovascular Regulation Laboratory, Department of Anatomy and Physiology, The University of Melbourne, Australia, [3]Department of Surgery, Royal Melbourne Hospital, Melbourne Medical School, The University of Melbourne, Melbourne, Australia, [4]School of Biomedical Sciences and Pharmacy, University of Newcastle, Newcastle, Australia, [5]Centre for Brain and Mental Health Research, Hunter Medical Research Institute, Newcastle, Australia, [6]Systems Neuroscience Lab, Melbourne Neuropsychiatry Centre, Department of Psychiatry, The University of Melbourne, Australia, [7]Psychiatry Neuroimaging Laboratory, Department of Psychiatry, Brigham and Women's Hospital, Harvard Medical School, Boston, United States

Biography:
Jordan completed her Master of Biomedical Science (2017) under Assoc. Prof. Max Lim at the St Vincent's Institute in Melbourne. She's held several positions in stem cell-focused disease-modelling laboratories, where her translational work with organoids gained her a Kellaway Excellence Award and an international patent. She was awarded the Australian Rotary Health Jonathan Ceddia Memorial PhD Scholarship (2023) and is currently a second year PhD candidate under Dr. Maria Di Biase and Prof. Alice Pébay at the University of Melbourne. Her PhD studies are focused on establishing patient iPSC-derived cortical organoids to model synaptic pruning and neuroanatomical development in schizophrenia.

AIMS: The use of induced pluripotent stem cell (iPSC)-derived cortical organoids as an elaborate human model system provides indispensable opportunities for studying neuropsychiatric and neurological disorders. While cortical organoid protocols are constantly advancing, there remain some key limitations. Here, we aim to derive an improved protocol for generating iPSC-derived cortical organoids that contain a physiologically relevant ratio of functional microglia, architectural organisation of the cortical laminae, and more accurately recapitulate *in vivo* corticogenesis.
METHODS: Organoids were generated via seeding patient-derived iPSCs simultaneously with matched hemopoietic stem cells (HPCs) or early microglia progenitors (eMPs) using a BioTek MultiFlo FX fitted with AMX modules. The AMX system performed high-precision media changes throughout the neural induction process. A series of variable media compositions enabled concurrent neural differentiation and maturation, and proliferation and differentiation of progenitors towards a microglial fate. The long-term culturing of organoids in spinning bioreactors enhanced oxygenation and nutrient exchange, enriching organoid development.
RESULTS: Automation of neural induction enhanced inter-organoid consistency. Expression of proliferative (Ki67) and forebrain and neural progenitor markers (EMX1, PAX6 & NESTIN) by D20 indicated successful neural induction. At D40, both HPC- and eMP integrated organoids expressed early neuronal and cortical differentiation markers (TUJ1, MAP2, SOX2, NFM, TBR1 & CTIP2), displayed rosette-like formations and were positive for astrocytes (GLAST) and microglia (IBA-1, PU.1, CD11b & TREM2). D80 organoids exhibited early organisation of layer-specific markers (Reelin, SATB2, CTIP2, TBR1), mature uniform expression of neuronal and astrocyte markers, and ramified morphology of microglia (IBA-1+).
CONCLUSION: By using this protocol to generate organoids that recapitulate cortical development, we will gain insights into neurodevelopmental alterations in schizophrenia. Our patient-derived models enable direct links between in vitro models and *in vivo* disease states,

making this platform invaluable for translational research and the development of improved, personalised therapeutics.

POSTER PRESENTATIONS

P36

Generation of iPSC-derived adipocytes to investigate the role of epicardial adipose tissue in atrial fibrillation.

Lauren Cook[1,2], Dr Jordan Thorpe[1,2], Dr Matthew D Perry[1,3], A/Prof Adam P Hill[1,2]
[1]Victor Chang Cardiac Research Institute, Darlinghurst, Australia, [2]School of Clinical Medicine, Faculty of Medicine and Health, UNSW Sydney, Australia, [3]School of Biomedical Sciences, Faculty of Medicine and Health, UNSW Sydney, Australia

Biography:
Lauren is a PhD student at VCCRI using iPSC-derived engineered heart tissues to investigate mechanisms of obesity-induced atrial fibrillation.

Atrial fibrillation (AF) is the most common clinically relevant arrythmia, affecting more than 5% of Australians aged over 55 and significantly increasing risk of stroke and heart failure. AF is associated with a variety of environmental risk factors, a prominent example being obesity. Obesity is a growing epidemic and is proposed to increase AF risk due to an increased volume of epicardial adipose tissue (EAT) which can directly interact with the myocardium. However, the specific cellular mechanism of this process requires further investigation. To model EAT at a cellular level, human-induced pluripotent stem cells (hiPSCs) can be utilised to derive adipocytes, providing a potentially limitless cell source. However, current protocols lack efficiency, maturity and specificity. The aim of this project is to generate and characterise iPSC-derived adipocytes to investigate the role of adipose tissue in a model of AF.
Adipocytes were differentiated from iPSCs using two protocols: an established method via the mesenchymal stem cell (MSC) pathway and a novel approach via a second heart field fibroblast progenitor pathway. The resulting adipocytes were characterised and compared to EAT-like adipocytes, examining lipid droplet size and number.
Adipocytes derived from the fibroblast pathway exhibited increased lipid droplet number and size, demonstrating improved differentiation efficiency compared to the MSC method. This new differentiation method more closely mimics natural EAT development, offering a promising approach to generate epicardial-like adipocytes to study the effects of obesity-induced AF.
IPSC-derived adipocytes can be cocultured with iPSC-derived atrial-specific cardiomyocytes to model the effects of EAT on cardiac. Overall, this approach will provide insight into EAT-mediated structural and electrical remodelling in an atrial model with potential for scalability and high-throughput experimentation to facilitate drug studies to target AF.

POSTER PRESENTATIONS

P37

Betacellulin is a central target for Schizophrenia

Dr Agustin Cota-Coronado[1], Associate Professor Rachel Hill[1], Dr Andrew Gibbons[1], Associate Professor Joseph Rosenbluh[2], Professor Suresh Sundram[1,3]
[1]Department of Psychiatry, Monash University, Melbourne, Australia, [2]Monash Biomedicine Discovery Institute, Melbourne, Australia, [3]Monash Medical Centre, Melbourne, Australia

Biography:
Research Fellow and Leader of the Human Stem Cell Laboratory at Monash University, specializing in human pluripotent stem cell biology, neurodifferentiation, and disease modeling. As an Early Career Researcher (3 years post-PhD), I focus on optimizing in vitro protocols for authentic neuronal subtypes and understanding molecular mechanisms in neuropsychiatric disorders. I integrate clinical findings with advanced stem cell models and multi-omics to identify disrupted pathways in health and disease. Recently, I established Monash's first human stem cell program in Psychiatry and serve on the ECR organizing committee for Stem Cell Research Victoria, affiliated with ASSCR and Monash Neuroscience EMCR.

Several members of the epidermal growth factor (EGF) family have been implicated in the pathology of schizophrenia. One of these EGF-related ligands, Betacellulin (BTC), plays a significant role in neural stem cell proliferation and differentiation. We have shown that BTC protein levels are lower in the post-mortem dorsolateral prefrontal cortex of individuals with schizophrenia compared to healthy controls. However, as this reflects a post-diagnostic state, how such BTC deficits affect neural specification and neurodevelopment remains unclear.

Aim: This study aims to investigate the effects of reduced BTC on neurogenesis using two experimental paradigms with human induced pluripotent stem cells (iPSCs).

Methods: a) We differentiated iPSCs into GABAergic progenitors using a small molecule-defined protocol and silenced BTC expression via siRNA at the neural progenitor stage (Day 14). BTC suppression was validated through qPCR and confocal imaging. b) We created constitutive iPSC-BTC knockout (KO) lines employing CRISPRc to study BTC's role in neurodevelopment and mature neuronal specification. Novel iPSC lines were validated using FACS, western blotting, and confocal imaging.

Results: Temporal BTC loss in GABAergic progenitors led to significantly reduced proliferation and decreased newborn neuronal DCX-positive cells, which are crucial for neurogenesis. Structural impairments at the neuronal connectivity level and compromised neural rosette formation were also observed.

Conclusions: This study is the first to demonstrate that BTC is directly involved in developmental and structural abnormalities postulated to underlie schizophrenia and related psychiatric disorders. The findings highlight the BTC-EGF system as a novel target for schizophrenia treatment. Additionally, the newly developed iPSC lines serve as invaluable tools for immediate drug screening efforts aimed at mitigating early and late neuronal deficits in schizophrenia.

POSTER PRESENTATIONS

P38

Making mature muscle: Investigating metabolic and developmental pathways for improving the maturation of bioengineered muscle

Dr Callum Dark[1,2,4], Kaitlyn Bibby[1,2], Dr Natasha Tuano[1,2], Rebecca McElroy[1,2], Dr Tabitha Cree[1,2], A/Prof Richard Mills[1,2,3]

[1]Murdoch Children's Research Institute, Melbourne, Australia, [2]Novo Nordisk Foundation Center for Stem Cell Medicine, reNEW, Melbourne, Australia, [3]Department of Pediatrics, The University of Melbourne, Melbourne, Australia, [4]Department of Anatomy and Physiology, The University of Melbourne, Melbourne, Australia

Biography:
Callum Dark is a Senior Research officer in the Muscle Bioengineering group at MCRI. He completed his PhD at Monash University in 2020 where he looked into the functional characterisation of ADHD-associated gene variants using the Zebrafish as an animal model. Later in 2020 he began a postdoc at the Peter MacCallum Cancer Centre, using Drosophila to investigate the mechanisms underlying cancer cachexia. He now works at MCRI, where he uses stem cells to model genetic muscle disease in both 2D and 3D culture systems.

Recent developments in three-dimensional culture systems for bioengineered muscle have provided a strong foundation for high-throughput disease modelling and compound screening. Despite this, bioengineered tissues still fail to recapitulate many aspects of adult muscle such as transcriptional and functional outputs, with tissues often displaying a foetal phenotype. Therefore, to better model muscle disease, we need tissue culture systems that can mature bioengineered muscle to the equivalent of childhood and beyond. We are building upon current differentiation protocols to derive human skeletal myofibers from induced pluripotent stem cells (iPSCs), to form both 2D cultures and 3D human skeletal micro muscles (hμMs). Utilising an iPSCs line containing an mScarlet tagged reporter of the sarcomeric Z-Disc protein alpha-actinin-2 (ACTN2), we can visualise increased expression and organisation of ACTN2 as muscle fibres mature. In addition, functional analysis of hμMs allows us to measure contractile force of the tissues, including twitch strength, tetanus strength and twitch/tetanus ratios. Preliminary analyses have demonstrated that altering culture conditions can improve muscle maturation, including factors such as fusion timing, small molecules, hormones, B-27 supplement levels, and fatty acid provisions. To further investigate novel factors that may improve muscle tissue maturation, we will perform screens in both 2D and 3D culture, examining the effects of metabolic and developmental factors during different stages of myofiber development. From this study, we aim to identify culture conditions that can produce hμMs that are more functionally mature, and thus be a more representative platform for investigating muscle disease.

POSTER PRESENTATIONS

P39

Optimisation of a whole genome CRISPR-Cas9 screen in PD-iPSC derived dopamine neurons

Dr Isabelle de Luzy, Miss Wenna Chen, Dr Alexander Henderson, Dr Marco Rosso, Dr Sanjoy Mehta, Professor Ting Zhou, Dr Nathalie Saurat, Dr Gabrielle Ciceri, Dr Lorenz Studer
[1]Memorial Sloan Kettering Cancer Centre, New York, United States

Biography:
Dr. Isabelle de Luzy completed her PhD doctorate at the Florey Institute of Neuroscience and Mental Health and more recently her post-doctoral studies at Sloan Kettering Institute in New York under the guidance of Lorenz Studer and as a part of the Aligning Science Across Parkinson's consortium (funded by the Michael J Fox Foundation). Her studies have focused on advancing the therapeutic capacity and safety of stem cell derived dopamine neurons to restore motor function in PD and more recently to understand the age-related genetic contributions associated to the pathogenesis of PD.

Aging is one of the biggest risk factors for Parkinson's disease (PD). Despite this, relatively little is known about the age-related genetic contributions that lead to the pathogenesis of PD. To identify candidate genes, we will perform a whole genome CRISPR-Cas9 knockout screen as a readout of PD-neuron death. Candidate genes will be screened to identify age-related modifiers that trigger phenotypic penetrance in PD. Here, we have engineered three independent hiPSC lines with a strong PD risk mutation (GBA, LRRK2 or A53T) and confirmed appropriate differentiation into mature DA neurons at equivalent efficiency. To maintain a pure neuronal culture, we identified an appropriate dose of mitomycin C that reduces undesirable proliferative cells without compromising DA neuron viability. We next engineered two independent Cas9 expressing hPSC line, either inducible or constitutive Cas9 expression, to compare their functional capacity to knockout genes of interest in mature dopamine neurons. To confirm functionality, we transfected cells with a lentiviral gRNA targeting TdTomato. Both Cas9 expressing hPSC lines displayed a substantial and comparable reduction in TdTomato expression in PSCs. However, upon differentiation an increasing proportion of resistant TdTomato-positive cells could be observed in the inducible Cas9 line, indicating a loss of Cas9 functionality. This finding was supported by a reduction in Cas9 protein expression. While surprising, similar results have been observed in hPSC-derived microglia and cortical neurons and we speculate this is due to transgene silencing. Importantly, the functionality of the constitutive Cas9 line was comparable between undifferentiated PSCs and PSC-derived DA neurons. We validated the quality and representation of a new CRISPR knockout pooled gRNA library (Gattinara) and confirmed successful transduction and puromycin selection in DA neurons. These findings demonstrate an optimised workflow to perform a whole genome knockout screen in neurons that can identify novel genes related to PD pathophysiology.

POSTER PRESENTATIONS

P40

Understanding the cellular environment of the heart to advance the development of cardiac therapies.

Dr Sebastian Bass-Stringer[1,2], Ms Yinghan She[1], Ms Hayley Pointer[1], Dr Adam Piers[1], Dr Kevin Watt[1,2], Professor James Hudson[3], A/Prof David Elliott[1,2], Professor Enzo Porrello[1,2]
[1]MCRI, Melbourne, Australia, [2]The University of Melbourne, Melbourne, Australia, [3]QIMR, Brisbane, Australia

Biography:
Sebastian is an early career research officer in the Heart Regeneration and Disease laboratory at MCRI. He completed his PhD at the Baker Heart and Diabetes Institute with a focus on the generation of novel AAV gene therapeutics to treat heart failure. He is currently generating and using molecular tools to understand the roles of the major cell types within the cardiac environment. His primary interests include heart disease, molecular tool development and generation of novel therapeutics.

AIM: Recent studies have demonstrated the importance of multicellular interactions in the generation of functionally mature engineered heart tissues derived from pluripotent stem cells (iPSCs). Non-cardiac cells comprise 60-70% of cells in the heart, understanding of how these cell types promote functionality in cardiac tissue remains limited. The aim of this project was to:
1. Generate a tool capable of selective, inducible ablation of the major cardiac cell types (cardiomyocytes, smooth muscle cells, fibroblasts & endothelial cells), in multicellular cardiac tissue derived from iPSCs.
2. Determine how the absence of specific non-cardiomyocyte cell populations impact function of iPSC-derived multicellular cardiac tissue.

METHOD: The ablation tool was generated using CRISPR-cas9 gene editing to knock-in a bacterial gene Nitroreductase (NTR) and a Green Lantern (GL) reporter into cell type specific genes (cardiomyocytes: TNNT2, fibroblasts: PDGFRa, smooth muscle cells: MYH11, endothelial cells: FLT1) in iPSCs. iPSCs were differentiated into cardiac organoids and treated with metronidazole (MTZ) to induce ablation. Cell-type ablation, specificity and changes in function were evaluated.

RESULTS: Reporter expression was confirmed to be specific to the targeted cell type through independent cell markers. Following induction with MTZ, ablation of specific cell types ranged between 75-100% for all targets. Untargeted cell types were shown to be preserved in ablated tissue. No toxicity or change in function was observed in wildtype tissue treated with MTZ. Ablation of cardiomyocytes led to complete loss of contraction. Ablation of smooth muscle cells significantly reduced contractile force and acceleration of contraction. Ablation of endothelial cells did not impact contractile parameters. Ablation of fibroblasts increased rate of contraction.

CONCLUSION: Here we describe a broadly applicable tool that ablates specific cell types in an inducible manner. Efficacy of the tool was validated in cardiac tissue and the impact of ablating different non-cardiac cell types on contractile parameters was demonstrated.

POSTER PRESENTATIONS

P41

Stem cell modelling of mitochondrial disease-linked cardiomyopathy

Dr Ann Frazier[1,2], Dr Yau Chung Low[1,2], Cameron McKnight[1,2], A/Prof David Stroud[1,3,4], A/Prof David Elliott[1,2,5,6], A/Prof Diana Stojanovski[3], Prof David Thorburn[1,2,4]
[1]Murdoch Children's Research Institute, Melbourne, AUSTRALIA, [2]Department of Paediatrics, The University of Melbourne, Melbourne, AUSTRALIA, [3]Department of Biochemistry and Pharmacology and The Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Melbourne, AUSTRALIA, [4]Victorian Clinical Genetics Services, The Royal Children's Hospital, Melbourne, AUSTRALIA, [5]The Novo Nordisk Foundation Center for Stem Cell Medicine (reNEW), Murdoch Children's Research Institute, Melbourne, AUSTRALIA, [6]Melbourne Centre for Cardiovascular Genomics and Regenerative Medicine, The Royal Children's Hospital, Melbourne, AUSTRALIA

Biography:
Dr Frazier is an experienced mitochondrial biologist with over 15 years of research focussed on this organelle powerhouse. Her studies are aimed at improving the diagnosis and treatment of mitochondrial disease, encompassing a range of biochemistry, cell biology and genomics techniques. Through the development of various cellular models of mitochondrial disease, including pluripotent stem cell systems, she hopes to improve our understanding of disease mechanisms and develop systems for screening promising therapeutic options.

AIMS: Mitochondrial diseases show significant clinical and genetic heterogeneity, making the generation of models that encompass the breadth of mechanisms and pathology difficult, and there are currently no validated treatments. We are therefore using clinically relevant stem cell models of mitochondrial disease to investigate disease pathomechanisms and develop screening platforms, with a particular focus on mitochondrial cardiomyopathy.
METHODS: Knockout (KO) models were generated in human pluripotent stem cells (hPSCs) using CRISPR-Cas9, targeting mitochondrial disease genes with biallelic loss-of-function variants identified in patients representing both primary and secondary oxidative phosphorylation (OXPHOS) disorders: NDUFS6- OXPHOS complex I (CI) subunit; SURF1- OXPHOS complex IV (CIV) assembly factor; AGK- mitochondrial carrier protein biogenesis; and TAFAZZIN- cardiolipin maturation.
Matured contractile cardiomyocytes (CMs) differentiated from these hPSC models were subjected to biochemical and functional studies including analysis of OXPHOS and mitochondrial function, label-free quantitative proteomics, transcriptomics and assessment of cardiac parameters.
RESULTS: Analysis of OXPHOS and other mitochondrial complexes identified defects that were exaggerated in the CMs of all models. Proteomics revealed global increases in mitochondrial protein abundance in NDUFS6-KO, SURF1-KO and AGK-KO CMs, indicative of compensatory mitochondrial proliferation, along with reductions of mitochondrial proteins associated with their specific functional pathways. TAFAZZIN-KO CMs showed reduced mitochondrial protein abundance and broader changes in metabolic proteins. Variations in cardiac-specific markers and stress proteins indicated a cardiomyopathy phenotype, particularly in NDUFS6-KO and AGK-KO CMs, supported by transcriptomic studies and proteomic analysis of AGK patient heart biopsies.
NDUFS6-KO and SURF1-KO CMs exhibited abnormal calcium handling and decreased contractile force (CardioExcyte). Changes in contractility were also observed in AGK-KO and TAFAZZIN-KO 3D-cardiac organoids. Structural abnormalities were revealed by immunocytochemistry of CMs aligned on nano-patterned surfaces.

CONCLUSIONS: Our findings support the utility of these hPSC cardiac models for uncovering disease mechanisms and facilitating preclinical treatment studies across a range of disease categories.

POSTER PRESENTATIONS

P42

Preclinical iPSC drug screen and machine learning predict neuroprotective agents for a form of childhood dementia

Dr Zarina Greenberg[1], Ella McDonald[1,2], Alejandra Noreña Puerta[1], Manam Inushi De Silva[1,2], Cade Christensen[1], Robert Adams[1,2], Jenne Tran[1,2], Paris Mazzachi[1,2], Sebastian Loskarn[1], Siti Mubarokah[2], Megan Maack[6,7], Kris Elvidge[6,7], Mark Hutchinson[3,8], Kim Hemsley[2], Lisa Melton[6], Nicholas Smith[4,5], Cedric Bardy[1,2]

[1]Laboratory for Human Neurophysiology and Genetics, South Australian Health and Medical Research Institute (SAHMRI), Adelaide, AUSTRALIA, [2]College of Medicine and Public Health, Flinders University, Adelaide, Australia, [3]School of Biomedicine, University of Adelaide, Adelaide, South Australia, [4]Department of Neurology and Clinical Neurophysiology, Women's and Children's Health Network, Adelaide, Australia, [5]Paediatric Neurodegenerative Disease Research Group, Discipline of Paediatrics, Faculty of Health and Medical Sciences, University of Adelaide, Adelaide, Australia, [6]Sanfilippo Children's Foundation, Sydney, Australia, [7]Childhood Dementia Initiative , Sydney, Australia, [8]Institute for Photonics and Advanced Sensing, University of Adelaide, Adelaide, Australia

Biography:

Zarina is an early/mid-career researcher in the Laboratory for Human Neurophysiology, Genetics and Stem cells at the South Australian Health and Medical Research Institute, investigating the development and optimization of neuronal disease models. She completed a PhD in Neurobiology at University of South Australia, specialising in neurodevelopment and cell fate specification. Her current research now focuses on using human induced pluripotent stem cells and neural derivatives to investigate Sanfilippo syndrome. Zarina has been working on reprogramming, differentiating and characterising iPSC-derived neurons and integrating high-throughput cell phenotyping protocols to use for drug screening.

Childhood dementia is an umbrella term for rare paediatric neurological conditions with symptoms and pathophysiology overlapping the common adult forms of dementia. Sanfilippo syndrome or Mucopolysaccharidosis type IIIA (MPSIIIA) is a progressive neurodegenerative form of childhood dementia caused by a genetic mutation in lysosomal enzyme sulfamidase. Currently, there is no cure for MPSIIIA with the primary area of research focusing on treating enzyme deficiency, however evidence demonstrates that downstream neurophysiological impairments can drive neurological symptoms and accelerate neurodegeneration. Here, we developed a patient-derived iPSC preclinical model to screen 63 repurposed drugs used in adult neurodegenerative and CNS disorders and target downstream neuropathological pathways. We found that our iPSC-derived patient neurons revealed lysosomal dysfunction and heparan sulfate build-up, characteristics of MPSIIIA. Furthermore, patient-derived neurons were particularly prone to stress, astrocytic reactivity, and chronic neurodegeneration. Combining machine learning, high-content imaging and single-cell transcriptomics, we found nine compounds that ameliorated MPSIIIA phenotypes within two weeks of treatment in vitro. Our study introduces a novel preclinical model for childhood dementia and reveals repurposable agents that may help slow neurodegeneration and neuroimmune dysregulation in children with MPSIIIA and related disorders.

POSTER PRESENTATIONS

P43

Creating a micro-exon splicing map of early human brain development with genetically engineered pluripotent stem cell derived brain organoids

Ms Pallavi Gupta[1,2,3], Dr Hannah Leeson[3], Dr Ishaan Gupta[2], Prof Ernst Wolvetang[3]
[1]UQ-IITD Research Academy, [2]The Indian Institute of Technology - Delhi, India, [3]The University of Queensland, Australia

Biography:
Pallavi Gupta comes from Lucknow, India, where she completed her schooling. She received her dual Bachelor / Master of Science in Biological Sciences from the IISER, Bhopal. She was admitted to the 4-year joint PhD program of University of Queensland, Brisbane, Australia and Indian Institute of Technology, Delhi, India as a part of the UQ-IITD Research Academy in August 2021. Her work involves the use of stem cells and tissue culture, genetic engineering and bioinformatics. She is open to exploring and learning new tools and techniques. She is also an animal lover and an amateur wildlife photographer.

Micro-exons are exons, 3-27nt in length, that modulate protein-protein interactions and are specifically spliced-in in neurons, affecting mRNAs involved in neuronal development and synaptic function. Differential inclusion of micro-exons has been reported in the post-mortem brains of individuals diagnosed with Autism Spectrum Disorder (ASD). This was attributed to a reduced expression of the splicing factor SRRM4. However, when, during development, mis-splicing occurs in ASD and which cell types are affected remains unclear. Brain organoids, derived from human embryonic or induced pluripotent stem cells, recapitulate early human brain development to a large extent and can therefore be used to elucidate the extent and impacts of dysregulated micro-exon splicing. In support of this idea, our bioinformatic analyses of in-house brain organoid models of neurodevelopmental disorders identified consistent patterns across several conditions, including Down Syndrome (DS), Ataxia-Telangiectasia (AT), and Hypomyelination with Brainstem and Spinal cord involvement and Leg Spasticity (HBSL). To systematically investigate the role of micro-exon splicing we utilized the CRISPR-Cas9 system to knockout (KO) the micro-exon splicing factors SRRM4 and its paralog, SRRM3, in H9 embryonic stem cells and established clonally derived lines with validated frameshift mutations. We are currently utilizing these lines and their isogenic controls to generate cortical brain organoids that will be subjected to full-length single-cell RNA sequencing (scRNA-seq) across multiple timepoints during brain organoid maturation. This systematic analysis should unravel how disruptions in SRRM4 and SRRM3 function are associated with the pathophysiology of neurodevelopmental disorders and elucidate which mRNA species are affected in each cell type across development.

POSTER PRESENTATIONS

P44

Optimizing the *in vitro* neuron microenvironment to mitigate phototoxicity in live-cell imaging

Cassandra Hoffmann[1], Dr Simon Maksour[2], Jordan Clarke[3], Dr Maciej Daniszewski[3], Professor Alice Pébay[3,4], Professor Paul Gleeson[5], Professor Mirella Dottori[6], Dr Ellie Cho[7], Professor Andrew Zalesky[1,8], Dr Maria Di Biase[1,3,9]

[1]Melbourne Neuropsychiatry Centre, Department of Psychiatry, The University of Melbourne, Melbourne, Australia, [2]School of Chemistry and Molecular Bioscience and Molecular Horizons, University of Wollongong, Wollongong, Australia, [3]Department of Anatomy and Physiology, The University of Melbourne, Parkville, Australia, [4]Department of Surgery, Royal Melbourne Hospital, The University of Melbourne, Parkville, Australia, [5]Department of Biochemistry and Pharmacology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Australia, [6]School of Medical, Indigenous and Health Sciences and Molecular Horizons, University of Wollongong, Wollongong, Australia, [7]Biological Optical Microscopy Platform, University of Melbourne, Parkville, Australia, [8]Department of Biomedical Engineering, The University of Melbourne, Parkville, Australia, [9]Department of Psychiatry, Brigham and Women's Hospital, Harvard Medical School, Boston, United States

Biography:
Cassandra Hoffmann is a PhD candidate at the Melbourne Neuropsychiatry Centre within the University of Melbourne. Her research focuses on using computational techniques to model the organisation of in vitro neuronal systems.

AIM: Long-term imaging formats are ideal for capturing the dynamic formation of neuronal networks in vitro, yet fluorescent techniques are often constrained by the impact of phototoxicity on cell health. The current investigation optimised a culturing protocol to reduce light-induced cell damage by comparing the protective effects of three culturing factors: extracellular matrix (human- and murine-derived laminin), culture media (Neurobasal and Brainphys Imaging media), and seeding density (1×10^5 or 2×10^5 cells per cm^2).

METHOD: Cortical neurons were differentiated from human embryonic stem cells by viral transduction of Neurogenin-2, then imaged in different microenvironments over 33 days. Alongside viability and digital polymerase chain reaction quantification, an automated image analysis pipeline was developed to characterise network morphology and organisation. Main and interaction effects between culturing factors were examined with a general linear model.

RESULT: Under fluorescent light irradiation, we found that Brainphys Imaging medium supported neuron longevity to a greater extent than Neurobasal medium with either source of laminin, denoted by significant main effects in viability ($p = 0.0052$) and neurite outgrowth ($p < 0.0001$). Brainphys Imaging medium also promoted significantly higher self-organisation in terms of somata clustering ($p < 0.0001$) and neurite fasciculation ($p < 0.0001$). In contrast, combining Neurobasal medium and human laminin perturbed neuron outgrowth, revealed by a two-way interaction effect between media and laminin ($p < 0.0001$). Furthermore, a higher seeding density fostered somata clustering ($p = 0.0024$), but showed no advantages relative to lower density in extending cell viability ($p = 0.4929$).

CONCLUSIONS: These findings suggest an interaction between species-specific laminin and culture media, which is positively mediated by photoprotective compounds found in Brainphys Imaging medium. Therefore, Brainphys Imaging medium alongside either murine or human

laminin is recommended to reduce phototoxicity in experiments using long-term optical probes to characterise mechanisms in neuronal health and disease.

POSTER PRESENTATIONS

P45

Using patient-derived stem cell models to study childhood onset Facioscapulohumeral muscular dystrophy (FSHD)

Dr Peter Houweling[1,2], Ms Vanessa Crossman[1], Ms Kathrin Mattes[1], Dr Natasha Tuano[1,3], Ms Rebecca McElroy[1,3], Dr Chantal Coles[1], Dr Katy de Valle[1,2], Prof Peter Jones[4], A/Prof Richard Mills[1,3], Dr Ian Woodcock[1,2]

[1]Murdoch Childrens Research Institute, Parkville, Australia, [2]Royal Children's Hospital, Parkville, Australia, [3]Novo Nordisk Foundation for Stem Cell Medicine, , Australia, [4]University of Nevada, Reno, United States of America

Biography:

Dr Peter Houweling (PhD) is a Senior Research Fellow and Team leader in the Muscle Research group at the Murdoch Children's Research Institute (MCRI). He obtained his PhD from the University of Sydney and completed his post doctoral training under Prof Kathryn North at the Institute for Neuroscience and muscle Research (INMR), at the Westmead Children's Hospital and MCRI. In 2018 he was promoted to team leader and his group now focuses on using induced pluripotent stem cell (iPSCs) lines from patients with genetic muscle diseases to model disease and test new treatments.

Facioscapulohumeral muscular dystrophy (FSHD) is a progressive degenerative disease characterised by weakness and wasting of the neck, head and shoulder muscles. It is the most common autosomal-dominant neuromuscular disorder, with an estimated prevalence of 1 in 8,000 individuals worldwide. FSHD is caused by the misexpression of the transcription factor double homeobox 4 (DUX4). While symptoms typically present in the 2nd decade of life, presentation in early childhood correlates with a more rapid disease progression.

At the Murdoch Children's Research Institute (MCRI) we are in the third year of a childhood onset FSHD longitudinal outcome study (iFSHD-LOS) which includes the collection of clinical natural history data and the biobanking of peripheral mononuclear cells (PBMC's) for the generation of induced pluripotent stem cell (iPSCs) lines for disease modelling and drug screening.

The aim of this project was to generate and characterize patient specific iPSC based skeletal muscle models of children with early onset FSHD to study disease mechanisms and screen potential novel treatments/therapeutic options.

METHODS: Direct differentiation of FSHD patient and healthy control (n = 4 / genotype) iPSC lines to skeletal muscle progenitors (myoblasts) and terminally differentiated muscle fibers (myotubes) in 2-dimensional cultures we assessed using 1) immunohistochemistry to determine myotube area, volume and abundance; 2) electrical pulse stimulation (EPS) to induce muscle contraction and 3) transcriptomic (RNA-sequencing) analyses to define the disease specific features of FSHD muscle in a dish.

RESULTS: Our preliminary analyses showed that patient-derived myoblasts and myotubes have a reduced muscle volume, abundance and altered transcriptomic profile that segregates with disease and correlates with severity.

CONCLUSION: Our results support the use of iPSC-derived skeletal muscle cultures to study FSHD. This program provides a link between laboratory-based research and clinical management and will aid in the identification of new pathways to treat FSHD in the future.

POSTER PRESENTATIONS

P46

What makes us breathe? Uncovering the neural rhythm generators for involuntary breathing using human pluripotent stem cells

Dr Kevin Law[1], Prof Clare Parish[2], Prof Lachlan Thompson[1,2]
[1]University of Sydney, Camperdown, AUSTRALIA, [2]The Florey, Parkville, AUSTRALIA

Biography:
Dr Kevin Law is an early-career postdoctoral researcher in the Stem Cells & Neurotherapeutics laboratory at the Charles Perkins Centre. Dr Law is passionate about using human pluripotent stem cells to study respiratory neurobiology, answering key questions like how do we breathe. He also has a strong interest in investigating brain repair using stem cell-derived neural cells, having completed his PhD at The Florey in 2023, with a focus on advancing stem cell therapy for brain repair in stroke and Parkinson's disease to the clinic. Dr Law is currently an EMCR committee member of ASSCR.

The rhythm of breathing is vital for mammalian life, required for daily activities and during sleep. The central nervous system (CNS) cells responsible for initiating and autonomously maintaining breathing rhythm are the preBotzinger complex (preBotC) neurons, residing in a caudal/hindbrain region called the brainstem. Although the development of these neurons has been investigated in animal studies, how these essential preBotC neurons develop in humans have yet to be investigated.

AIM: Here, we aimed to establish one of the world's first human pluripotent stem cell (hPSC) protocol to differentiate preBotC neurons for the study of their development in humans.

METHODS: Using a human induced pluripotent stem cell line, we inducted neural fate in vitro by dual-SMAD signalling inhibition, followed by screening and titrating a panel of small molecules to tune the rostrocaudal and dorsoventral patterning. After patterning, cells were matured for immunocytochemical characterisation of their neuronal identity.

RESULTS: At 6DIV, we found that retinoic acid was necessary to activate the expression of the homeobox gene required for the correct rostrocaudal patterning in hindbrain development. At 12DIV, we found the combination of caudalising and ventralising small molecule correctly generated progenitors expressing a marker of respiratory neural progenitor, whereas the absence of one or multiple patterning small molecules did not generate the respiratory neural progenitors. Following maturation or the progenitors, we successfully generated neurons expressing markers of the preBotC identity.

CONCLUSION: Here, we presented a preliminary protocol for one of the world's first generation of preBotC neurons by modulating rostrocaudal and dorsoventral patterning. Establishing this hPSC protocol of preBotC neuron differentiation will enable future gene editing and disease modelling studies to further understand breathing and the rhythm of breathing in humans, as well as therapeutically supporting patients living with CNS-related respiratory disorders.

POSTER PRESENTATIONS

P47

The PSEN1 H163R mutation in an iPSC-retinal model of Alzheimer's disease causes detectible disease-associated features involving ß-amyloid pathways.

Dr Grace Lidgerwood[1], Dr Damián Hernández[1], Ms Jenna Hall[1], Ms Anne Senabouth[2], Dr Mehdi Mirzaei[3], Dr Allison van de Meene[4], Prof Celeste Karch[5], Prof Alison Goate[5,6], Prof Joseph Powell[2,7], Prof Alice Pébay[1,8]

[1]Department of Anatomy & Physiology, University Of Melbourne, Parkville, AUSTRALIA, [2]Garvan-Weizmann Centre for Cellular Genomics, Garvan Institute of Medical Research, Sydney, AUSTRALIA, [3]Macquarie Medical School, Faculty of Medicine, Health and Human Sciences, Macquarie University, Sydney, AUSTRALIA, [4]Melbourne Advanced Microscopy Facility, The University of Melbourne, Parkville, AUSTRALIA, [5]Dominantly Inherited Alzheimer Network (DIAN), Department of Psychiatry, Washington University School of Medicine, St. Louis, USA, [6]Department of Genetics and Genomic Sciences, Ronald M. Loeb Center for Alzheimer's Disease, Icahn School of Medicine at Mount Sinai, New York, USA, [7]UNSW Cellular Genomics Futures Institute, University of New South Wales, Sydney, AUSTRALIA, [8]Department of Surgery, Royal Melbourne Hospital, The University of Melbourne, Parkville, AUSTRALIA

Biography:
Dr Lidgerwood is a Research Fellow in the Stem Cell Disease Modelling Unit at the University of Melbourne. Over the past 10 years, her focus has been on the development of large-scale disease models of retinal degenerative diseases from patient-derived stem cells. Coupled with advances in -omics and imaging technologies, results of her research have shed light on some of the important determinants of macular degeneration and Alzheimer's disease.

AIMS: The retina, which is part of the central nervous system, has gained increasing attention as a potential non-invasive model to study Alzheimer's disease (AD), especially as clinical studies have reported some visual symptoms can precede the onset of cognitive decline. We therefore aimed to establish a retinal culture of AD using a PSEN1 mutant and isogenic CRISPR control iPSC line to determine if an AD signature could be detected in a cellular retinal model.

METHODS: A PSEN1-mutant iPSC line from a patient with clinically diagnosed familial AD (H163R) and a CRISPR-corrected isogenic control iPSC line (Hernández et al 2024) were differentiated into retinal cells using previously published methods (Lidgerwood et al 2016, 2018, 2021). Extensive analysis using ELISA, microscopy (electron, in-house automated fluorescent quantitative pipeline), and -omics approaches (sc-RNASeq and LC MS/MS) were utilised to explore if an AD signature could be detected in our retinal iPSC model of the disease.

RESULTS:We found that there were significantly higher levels of toxic β-amyloid1-42 in the retinal pigment epithelium layer (RPE) by ELISA, and this was further validated using our microscopy plaque-detection pipeline, which found that plaque volumes were statistically larger in the diseased RPE than CRISPR controls. The proteomics and sc-RNASeq analysis identified upregulation in pathways associated with β-amyloid and AD, including regulation of neurofibrillary tangle and amyloid fibril formation (>100 fold and 43-fold increase respectively). Interestingly, we were also able to uncover a novel relationship between the H163R mutation in PSEN1 mutant RPE and melanosome biogenesis using electron microscopy and our -omics analysis – a relationship that has not yet been described in a human model.

CONCLUSIONS: These novel findings bring to the fore a pathologically relevant model of neurodegeneration associated with AD in an *in vitro* retinal model to that may have clinical translation in the future.

P48

Developing a model of incomplete penetrance in arrhythmogenic cardiomyopathy using patient-derived induced pluripotent stem cells

Ms Serena Li[1,2], Doctor Matisse Fox[3], Doctor Samantha B Ross[1,2], Ms Mira Holliday[1,2], Doctor Richard D Bagnall[1,2,4], Doctor Seakcheng Lim[1,2], Professor Christopher Semsarian[1,2,5]
[1]Agnes Ginges Centre for Molecular Cardiology at Centenary Institute, The University of Sydney, Sydney, Australia, [2]Faculty of Medicine and Health, The University of Sydney, Sydney, Australia, [3]School of Medicine and Dentistry, Griffith University, Gold Coast, Australia, [4]Bioinformatics and Molecular Genetics Group at Centenary Institute, The University of Sydney, Sydney, Australia, [5]Department of Cardiology, Royal Prince Alfred Hospital, Sydney, Australia

Biography:
Serena Li is a research assistant in the Agnes Ginges Centre for Molecular Cardiology at Centenary Institute. Her research focuses on studying the effects of disease-causing variants in genetic heart diseases using induced pluripotent stem cell models derived from patients.

Aim: Arrhythmogenic cardiomyopathy (ACM) is a genetic heart disease characterised by fibrofatty replacement of the myocardium, leading to arrhythmias and sudden cardiac death. Clinically normal individuals with pathogenic variants in plakophilin-2 (PKP2) are advised to follow the same lifestyle restrictions as symptomatic patients. This study aims to explore phenotypic differences in patient-specific cellular models of ACM.

Method: We generated induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) from two members of a three-generation family with an ACM-causing c.2146-1G>C splice variant in PKP2. The ACM phenotype was assessed in iPSC-CMs, one of whom is phenotype positive, PKP2+/- (G+/P+), and the other who is phenotype negative, PKP2+/- (G+/P-) and compared to an unaffected control. To induce lipid accumulation and an adult-like metabolism in diseased iPSC-CMs, the cells were supplemented with a five-factor adipogenic media for 7 and 30 days. Cells were stained with Nile Red and Phalloidin to assess lipid accumulation in iPSC-CMs.

Result: The patient-derived ACM iPSC-CM model is currently in development. To date, we are assessing the phenotype of these lines, relating both to lipid accumulation and susceptibility to arrhythmias using an exercise model. In 2D cultures, we found similar levels of lipid accumulation between gene carriers of the PKP2 variant that are phenotype positive and phenotype negative. We will continue to assess the phenotype by using 3D culturing methods.

Conclusions: Development of this ACM model will help us understand disease mechanisms behind incomplete penetrance and aid in risk stratification in families with ACM. This will help in improving patient care in those who carry a disease-causing variant but do not have an ACM phenotype.

POSTER PRESENTATIONS

P49

Reduced connexin-43 expression, slow conduction, and repolarisation dispersion in a model of hypertrophic cardiomyopathy

Dr Seakcheng Lim[1,2], Dr Melissa Mangala[3,4], Ms Mira Holliday[1,2], Dr Henrietta Cserne Szappanos[6], Dr Samantha B. Ross[1,2], Ms Serena Li[1,2], Dr Jordan Thorpe[3,4], Ms Whitney Liang[3,4], Ms Ginell N. Ranpura[1,2], Professor Jamie I. Vandenberg[3,4], Professor Christopher Semsarian[1,2], A/Prof Adam P. Hill[3,4], Professor Livia C. Hool[3,6]

[1]Agnes Ginges Centre for Molecular Cardiology, Centenary Institute, The University of Sydney, Sydney, Australia, [2]Faculty of Medicine and Health, The University of Sydney, Sydney, Australia, [3]Molecular Cardiology and Biophysics Division, Victor Chang Cardiac Research Institute, Darlinghurst, Australia, [4]St. Vincent's Clinical School, University of NSW, Sydney, Australia, [5]Department of Cardiology, Royal Prince Alfred Hospital, Sydney, Australia, [6]School of Human Sciences, The University of Western Australia, Crawley, Australia

Biography:
Dr Seakcheng Lim completed her PhD in 2017 in regenerative medicine at the University of Western Sydney, focusing on elucidating the mechanisms of pluripotency in embryonic stem cells and embryonic development. She joined the Agnes Centre for Molecular Cardiology in 2018, and currently uses high-throughput platforms to screen and characterise cardiomyocytes generated from patient-derived induced pluripotent stem cells to understand the electrophysiological mechanisms of inherited heart diseases.

Aim: Hypertrophic cardiomyopathy (HCM) is an inherited cardiac disorder characterised by left ventricular hypertrophy ≥ 15 mm, resulting in arrhythmias, heart failure, and sudden cardiac death. In this study, we used an induced pluripotent stem cell model to identify the molecular basis of electrophysical changes resulting from HCM.

Method: We modelled HCM using cardiomyocytes from induced pluripotent stem cells (iPSC-CMs) derived from a patient carrying the HCM disease-causing Arg403Gln variant in myosin heavy chain 7 (MYH7403/+) compared with CRISPR-corrected isogenic iPSC-CMs (MYH7-C+/+). To study the electrophysiological phenotypes in iPSC-CM monolayers were screened on a high-throughput multi-electrode array (MEA) platform. Western blotting and immunocytochemistry were used to identify key protein changes in HCM cardiomyocytes.

Results: For the first time, we show significant slowing of conduction velocity and an increase in local spatial dispersion of repolarisation - both well-established substrates for arrhythmia - in monolayers of HCM cardiomyocytes. Analysis of rhythmonome protein expression in Arg403Gln cardiomyocytes revealed dramatically reduced connexin-43, sodium channels, and inward rectifier channels – a three-way hit that combines to reduce electrotonic coupling between HCM cardiomyocytes and slow cardiac conduction.

Conclusion: Our data represents a novel, biophysical basis for arrhythmia in HCM, that is intrinsic to cardiomyocyte electrophysiology. Later in the progression of the disease, these proarrhythmic electrical phenotypes may be accentuated by fibrosis and myocyte disarray to contribute to sudden death in HCM patients.

POSTER PRESENTATIONS

P50

Exploring cell senescence in iAstrocytes from C9orf72 ALS patients

Alexandra Maximova[1], Dr Michael Sullivan[1], Dr Eryn Werry[2,3], Professor Michael Kassiou[3]
[1]School of Medical Science, The University of Sydney, Darlington, Australia, [2]Central Clinical School, The University of Sydney, Darlington, Australia, [3]School of Chemistry, The University of Sydney, Darlington, Australia

Biography:
Alexandra completed her Bachelor of Medical Science (Pharmacology) at the University of Sydney in 2018. She then joined the Kassiou Drug Discovery Group for her Honours year in 2019, exploring drug design in oxytocin signalling. Wanting to learn more about the world of stem cell research, she submitted her PhD in 2024, focusing on senescence characterisation in iPSC-derived glial cells in amyotrophic lateral sclerosis.

Aims: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative condition with no cure or effective treatment, and an average lifespan of 3-5 years following diagnosis. Cell senescence is a non-proliferative state that cells enter due to prolonged stress, while remaining metabolically active. Under chronic conditions, this can lead to increased inflammatory and oxidative stress in healthy neighbouring cells. Senescent cells also dysregulate signalling pathways allowing them to evade apoptosis, making them a difficult target for drug design. Evidence of senescence in ALS has recently increased through post-mortem studies and the development of animal models of ALS, such as utilising the superoxidase 1 mutation. However, the most common genetic mutation associated with ALS – hexanucleotide repeat expansion in the C9orf72 gene – is not well modelled, creating obstacles for drug translation. iPSC-based modelling circumvents this challenge, allowing determination of differences between ALS and healthy donors to help identify potential new drug targets. This study aimed to characterise senescence in iPSC-derived ALS astrocytes in the absence of motor neuron degeneration, and to explore underlying molecular pathways and cell autonomous pathomechanisms.

Methods: Three experimental assays were used to characterise cell viability, proliferation, and senescence expression in iPSC-derived astrocytes (iAstrocytes) developed from fibroblast lines donated by ALS patients carrying C9orf72 hexanucleotide repeats (n=3) and age-matched healthy controls (n=3). To explore some molecular pathways underlying iAstrocyte senescence in ALS, we performed western blotting for the Bcl-xL protein, which helps senescent cells evade apoptosis.

Results: ALS iAstrocytes showed 2-fold and 4-fold significantly reduced cell viability and proliferation ($p<0.05$), respectively, while displaying 2-fold significantly more senescent cells compared to controls ($p<0.05$). ALS iAstrocytes showed 2-fold significantly higher Bcl-xL expression compared to controls ($p<0.05$).

Conclusions: These data confirmed the presence of senescence in human ALS astrocytes and identified a potential target for further molecular pathway exploration and drug design.

POSTER PRESENTATIONS

P51

Hyperactive synaptic circuits in neurons derived from children with a form of dementia

Ms Paris Mazzachi[1,2,10], Ms Ella McDonald[1,2,10], Dr Zarina Greenberg[1], Ms Alejandra Noreña Puearta[1], Dr Jenne Tran[1], Ms Manam Inushi De Silva[1,2], Mr Cade Christensen[1], Mr Robert Adams[1,2], Mr Sebastian Loskarn[1], Ms Helen Beard[2], Dr Michael Zabolocki[1], Ms Meera Elmasri[1,2], Ms Megan Maack[6,7], Dr Kris Elvidge[7], Professor Mark R. Hutchinson[8], Dr Cara O'Neill[9], Professor Kim Hemsley[2], Dr Lisa Melton[6], Dr Nicholas Smith[4,5], Professor Cedric Bardy[1,2]

[1]Laboratory for Human Neurophysiology and Genetics, South Australian Health And Medical Research Institute, Adelaide, AUSTRALIA, [2]Flinders Health and Medical Research Institute, College of Medicine and Public Health, Flinders University, Adelaide, AUSTRALIA, [3]School of Biomedicine, University of Adelaide, Adelaide, AUSTRALIA, [4]Department of Neurology and Clinical Neurophysiology, Women's and Children's health Network, Adelaide, AUSTRALIA, [5]Paediatric Neurodegenerative Disease Research Group, Discipline of Paediatrics, Faculty of Health and Medical Sciences, University of Adelaide, Adelaide, AUSTRALIA, [6]Sanfilippo Children's Foudnation, Melbourne, AUSTRALIA, [7]Childhood Dementia Initiative, Sydney, AUSTRALIA, [8]Institute for Photonic and Advanced Sensing, University of Adelaide, Adelaide, AUSTRALIA, [9]Cure Sanfilippo Foundation, Columbia, UNITED STATES OF AMERICA, [10]Authors contributed equally.

Biography:

Paris Mazzachi is a final year PhD student at the Laboratory for Human Neurophysiology, Genetics and Stem Cells under the supervision of Professor Cedric Bardy. She has extensive skills in electrophysiology, where she utilises whole-cell patch clamping to investigate disease-specific changes in neuronal function across neurodevelopment. Combining this with single-cell RNA sequencing, she explores potential mechanisms that may underlie abnormalities in neuronal function in disease. Her research currently focuses on the role of synaptic dysfunction in paediatric and adult neurodegenerative disorders using human induced pluripotent stem cell derived neuronal models and explores how modulating their electrical activity may be neuroprotective.

Alterations in synaptic homeostasis are linked to cognitive and behavioural impairments in adult and pediatric brain disorders. However, synaptic dysfunction in childhood dementias is poorly understood. Here, we matured cortical synaptic circuits in vitro from induced pluripotent stem cells (iPSCs) of children with Mucopolysaccharidosis Type IIIA (MPSIIIA), one of the most prevalent childhood dementias. We found no differences in action potential firing or neurite morphology between MPSIIIA and healthy neurons. However, long-term maturation revealed excitation/inhibition imbalances caused by hyperactive excitatory synapses and dysregulated gene expression linked to synaptic homeostasis. A drug screen with whole-cell patch-clamping, multi-electrode arrays and high-content confocal imaging identified a cocktail of repurposed drugs effective in restoring excitation/inhibition balance. This study validates the use of preclinical human neural models in vitro to detect functional phenotypes in childhood dementias and expedite drug discovery targeting synaptic dysfunction, which may improve cognition for patients with MPSIIIA and other neurodegenerative disorders.

POSTER PRESENTATIONS

P52

Identifying new therapies for Facioscapulohumeral muscular dystrophy using a phenotypic screening approach

Miss Rebecca McElroy[1,6], Dr Natasha Tuano[1,6], Miss Kaitlyn Bibby[1,6], Dr Timothy Johanssen[2,6], Dr Henry Beetham[2,6], Dr Alejandro Hidalgo-Gonzalez[2,6], Dr Peter Houling[3], Dr Kevin Watt[4,6], Doctor Sara Howden[5,6], Associate Professor Richard Mills[1,6]

[1]Muscle Bioengineering, Murdoch Children's Research Institute, Melbourne , Australia, [2]Stafford Fox Stem Cell-based Drug Discovery Facility, Melbourne , Australia, [3]Muscle Research, Murdoch Children's Research Institute, Melbourne , Australia, [4]Heart Regeneration and Heart Disease, Murdoch Children's Research Institute. , Melbourne , Australia, [5]iPSC Derivation & Gene Editing Facility, Murdoch Children's Research Institute, Melbourne , Australia, [6]Novo Nordisk Foundation Center for Stem Cell Medicine, reNEW Melbourne, Melbourne , Australia

Biography:

My name is Rebecca McElroy, my background is primarily in lung disease immunology and immunological clinical trials. However, I'm currently working in the Muscle Bioengineering group at Murdoch Children's Research Institute in Melbourne. I am working on developing a 2D drug screening platform that can be used to screen for compounds that could improve the disease phenotype of patients with FSHD.

Facioscapulohumeral muscular dystrophy (FSHD) is the most common autosomal-dominant neuromuscular disorder, with an estimated prevalence of up to 1 in 8,000 individuals. FSHD is characterised by a progressive degeneration and weakness of skeletal muscle in the neck, head and shoulders due to misexpression of the transcription factor double homeobox 4 (DUX4). Whilst the life expectancy of patients with FSHD is normal, the deterioration of muscle mass and function limits independent mobility, reduces quality of life and significantly increases the risk of secondary health complications. The identification of targeted therapeutics for FSHD remain elusive. We have recently developed an iPSC derived model of FSHD via the expression of the DUX4 transcription factor. Differentiation of iPSC to myotubes and activation of DUX4 through a tetracycline-inducible system result in myofiber degeneration. Using this high-throughputscreening approach, we aim to identify treatments that inhibit/prevent the functional decline in skeletal muscle after DUX4 expression.

POSTER PRESENTATIONS

P53

Phosphoproteomic analysis of the signalling networks controlling human cardiomyocyte proliferation

Ms Yulia Mitina[1,2,6], Dr Yi Sing Gee[3], Dr Hannah Huckstep[1,6], Dr Rosie Hyslop[1,6], Ms Hayley Pointer[1,6], A/Prof Benjamin Parker[2,5], Dr Sean Humphrey[1,6], Prof Jonathan Baell[3], Prof Paul Stupple[3], Dr Kevin Watt[1,2,6], A/Prof David Elliott[1,4,6], Prof Enzo Porrello[1,2,6]
[1]Murdoch Children's Research Institute, Parkville, Australia, [2]Department of Anatomy and Physiology, University of Melbourne, Parkville, Australia, [3]Medicinal Chemistry, Monash Institute of Pharmaceutical Sciences, Parkville, Australia, [4]School of Biomedical Sciences and Department of Paediatrics, University of Melbourne, Parkville, Australia, [5]Centre for Muscle Research, The University of Melbourne, Parkville, Australia, [6]Novo Nordisk Foundation Centre for Stem Cell Medicine (reNEW), Murdoch Children's Research Institute, Melbourne, Australia

Biography:
Yulia Mitina is a medical doctor with specialized training in cardiovascular surgery. She completed her residency in cardiovascular surgery and worked as a surgeon, treating both pediatric and adult patients. Currently, she is a PhD candidate at Murdoch Children's Research Institute and in the Anatomy and Physiology Department at the University of Melbourne, where her research focuses on enhancing the regenerative potential of the heart in pediatric dilated cardiomyopathy. Ms Mitina also holds a Master's degree in Biotechnology and has a robust background in bioinformatics and clinical research. Her work is dedicated to advancing cardiac regeneration.

AIM: To identify the critical signalling networks governing human cardiomyocyte proliferation.
METHOD": Compound 6.28 was previously identified as a potent pro-proliferative agent in human iPSC-derived cardiomyocytes (Mills et al. 2017, 2019). A multifaceted approach was employed including confocal imaging of iPSC-derived cardiomyocyte proliferation using cell cycle activation markers, molecular docking for target prediction, DiscoverX KINOMEscan® Kinase Assay for target validation, thermal proteome profiling for binding partner identification, and phosphoproteomic profiling to elucidate signalling pathways associated with cardiomyocyte proliferation.
RESULT: Compound 6.28 exhibited a robust dose-dependent proliferative response in human iPSC-derived cardiomyocytes. Molecular docking simulations indicated chemical promiscuity of compound 6.28 (potential interactions with 113 unique protein targets). Using a cell-free kinase inhibitor screen, 31 of these targets were validated as high-affinity binders (selectivity score 0.362). Thermal proteome profiling of lysates from human iPSC-derived cardiomyocytes confirmed the Sterile-20 kinase MST2 as a direct binding partner of compound 6.28 (ΔTm = 6.445℃, $p < 0.05$). Phosphoproteomics profiling identified 55697 phosphosites, of which 2836 were altered in human iPSC-derived cardiomyocytes treated with compound 6.28 across several concentrations for 1hr (padj<0.05). Compound 6.28 led to a dose-dependent activation of Nrg1-ErbB, Hippo-YAP and cell cycle pathways. Downstream target analysis revealed decreased expression of the inhibitory S109 phosphosite on YAP1 (p=0.0024), a downstream effector protein of the MST2 kinase in the Hippo pathway.
CONCLUSIONS: Compound 6.28 directly binds to MST2, a core effector of the Hippo signalling pathway, in human iPSC-derived cardiomyocytes. Activation of human cardiomyocyte proliferation by Compound 6.28 alters the activity of several critical regenerative signalling networks including Nrg1-ErbB signalling and the Hippo pathway. Our findings identify inhibition of MST2 signalling as a critical event in the proliferative response of Compound 6.28 in human

cardiomyocytes, offering new insights into cardiomyocyte proliferation mechanisms and potential regenerative cardiac therapies.

POSTER PRESENTATIONS

P54

Transcriptome Analysis of ADHD Dopaminergic Neuron Derived From iPSC: Evidence of Signalling Disruption and Maturation Delay

Atefeh Namipashaki, Professor Mark Bellgrove, Dr Ziarih Hawi
[1]Turner Institute for Brain and Mental Health, School of Psychological Sciences, Monash University, VIC, Australia, Melbourne, AUSTRALIA

Biography:
I am PhD candidate at Monash University, focusing on modelling the impact of ADHD genes on dopaminergic-neuron function using stem cells. With master's in medical biotechnology, my research encompasses human neuronal and stem-cell culture techniques, neuronal-functional assays, CRISPR, and genome-wide approaches. I developed highly homogeneous functional dopaminergic neurons from iPSCs and am proficient in CRISPR-editing. My PhD journey has been marked by independence, problem-solving, and extensive collaborations.Passionate about stem-cell biology and neuroscience, I aim to pursue a career as an independent researcher in these fields. I am dedicated to advancing our understanding and application of stem-cell biology and neuroscience.

The most recent ADHD–GWAS meta–analysis highlighted the potential role of 76 genes enriched among genes expressed in early brain development and associated with midbrain dopaminergic neurons. However, the precise functional importance of the GWAS identified single nucleotide polymorphisms (SNPs) remain challenging. In contrast to GWAS, transcriptome analysis directly investigates the products of the gene by assessing the transcribed RNA. This allows to draw functional insights into gene expression paving the way for better understanding of the molecular mechanisms of the disorder. In this study, we performed the ADHD transcriptome profiling of highly homogenous dopamine neurons developed from ADHD and healthy induced pluripotent cells (iPSCs). Comparative gene expression analysis between the examined lines revealed several significant findings. The top differentially expressed genes (DEGs) were predominantly implicated in nervous system functions related to neuronal development and dopaminergic regulation. Notably, 29 of the DEGs overlapped with those identified by the ADHD – GWAS associated genes. These genes are overrepresented in biological processes including developmental growth regulation, axonogenesis, and nervous system development. In addition, gene set analysis revealed significant enrichment with meta categories such as ion channel activity, synaptic function and assembly, and neuronal development and cell differentiation. The dysregulation of multiple genes involved in processes related to neuronal development regulation indicate the importance of functional and structural alterations in the brain of individuals with ADHD. This was further highlighted by significantly reduced projections in the ADHD dopamine neurons at the mid–differentiation stage (day 14 in vitro), thus, supporting the delay maturation hypothesis proposed for ADHD. This study underscores the robustness of using iPSC– derived cell type–specific models and highlight the benefits of integrating genome and transcriptome analyses for biological discovery in ADHD.

POSTER PRESENTATIONS

P55

Using human stem cell-derived macrophages to examine the effects of platinum dissolution from cochlear implants

A/Prof Bryony Nayagam[1], Mr Steven Iem, Dr Jackie Ogier, Dr Ulises Aregueta Robles, Mr Dhyey Shah, Ms Marnie Maddock, Prof Mirella Dottori, Prof Laura Poole-Warren
[1]University of Melbourne, Melbourne, Australia

Biography:
Bryony's research aims to better understand the biology of hearing and vestibular function, to prevent the loss of key sensory cell types following inner ear damage. Her research spans basic auditory neurobiology, regenerative stem cell therapy, industry projects with Cochlear Ltd and human MRI. She collaborates with neuroscientists, surgeons, bioinformaticians, engineers, bioethicists, and patient advocacy groups. Bryony's team are examining the dissolution of platinum from cochlear implants in a range of in vitro and in vivo models, supported by an ARC Linkage grant with Cochlear Ltd, and academic partners UNSW and University of Wollongong.

AIMS: It has come to light over the last decade, that neural prostheses that use platinum stimulating electrodes, such as the cochlear implant, can cause platinum dissolution into surrounding tissues. The effects of this platinum dissolution are challenging to measure *in vivo,* and as such, an in vitro macrophage model was established to examine how changes in concentration, duration and platinum type affect human macrophage survival.

METHODS: Human pluripotent stem cells were differentiated into macrophages over 28 days using standard protocols. Differentiated macrophages were then exposed to a range of types of platinum including soluble $PtCl_4$, platinum particulates, or platinum sourced from a stimulating electrode (from Cochlear Ltd). Tested platinum concentrations ranged from 0.075 ng/mL to 19 µg/mL, and exposure durations from 24 h to 2 weeks in culture. Cell survival was measured using a Resazurin viability assay, and radical oxygen species (ROS) production was investigated as a measure of cellular stress.

RESULTS: All platinum types examined were toxic to cells, even at the lowest concentrations tested, after 2 weeks in vitro ($p<0.001$). Soluble $PtCl_4$ was found to be significantly more toxic than all other forms of platinum tested in this study. In addition, the application of $PtCl_4$ caused significantly greater accumulation of ROS compared to other platinum types ($p<0.001$), suggesting that redox stress contributes to cell death after soluble platinum exposure.

CONCLUSIONS: These experiments demonstrate a novel human in vitro model for examining macrophage biology in response to platinum exposure. Together with our *in vivo* experiments examining macrophage responses to platinum within the cochlea, these studies help to advance our understanding of immune cell interactions with neural prostheses.

POSTER PRESENTATIONS

P56

Single cell transcriptomics elucidates intra-cellular signalling networks involved in donor-host interactions following transplantation of hPSC-derived photoreceptor cells to restore visual function

Dr Michelle Sophie O'Hara-Wright[1], Dr Hani Kim[2], Prof Pengyi Yang[2], Assoc Prof Anai Gonzalez-Cordero[1]
[1]Stem Cell Medicine Group, Children's Medical Research Institute, University of Sydney, , ,
[2]Computational Systems Biology, Children's Medical Research Institute, University of Sydney, ,

Biography:
Michelle is a Post-Doctoral Research officer in the Stem Cell Medicine Group at CMRI, Westmead, and the University of Sydney.

The leading cause of incurable blindness worldwide is irreversible degeneration of photoreceptor cells in the retina. Retinal organoids derived from hPSCs offer a renewable source of transplantable photoreceptors for therapy. However, efficacious visual rescue is challenged by optimal cell integration, survival, and complex functional connectivity within the host retina.

Here, we characterise the molecular identity of hPSC-derived photoreceptor cells following transplantation into the end-stage blind mouse retina. This study aimed to elucidate donor-host cell-cell communication networks that promote hPSC-derived photoreceptors to integrate, mature and establish functional connections with host higher-order neurons.

Purified CRX+ photoreceptor precursor cells isolated from 17 week-old retinal organoids were transplanted into immunodeficient blind mice (Rd1/Foxn1nu), or CRX- non-photoreceptor cells to act as a negative control. Twelve weeks post-transplantation, scRNAseq was performed in transplanted and un-transplanted retinas, alongside age and batch-matched retinal organoids, offering an important in vitro comparison.

For the first time, transcriptomic libraries of transplanted retinas were established, identifying differential gene expression in transplanted photoreceptors compared to in vitro retinal organoids. We identify specific ligand-receptor interactions between donor and host retinal neurons using CellChat, including axon and synaptic-guidance pathways Neurexin, Slit and Ephrin. Moreover, cell-cell interaction networks indicated significantly strengthened incoming- and outgoing-signals in the highest-order host retinal neurons following transplantation of hPSC-derived photoreceptors. Finally, we validate these findings with histological analysis paired with behavioural rescue of vision.

This study therefore provides unequivocal evidence towards the feasibility and functionality of hPSC-derived photoreceptor cell transplantation. We uncover a wealth of novel cell-type specific molecular cues influencing survival, integration and maturation of transplanted photoreceptors. Moreover, by implementing robust controls, we identify molecular differences between photoreceptors within the *in vivo* and in vitro environment. This may allow for selection of cells with more favourable communication profiles, or modulation of the host microenvironment, overall enhancing transplantation outcome and efficacy.

POSTER PRESENTATIONS

P57

Modelling the influence of the autonomic nervous system in atrial fibrillation using induced pluripotent stem cell models

Sutapa Saha[1,2], Dr. Azadeh Zahabi[1,2], Dr. Jordan Thorpe[1,2], Assoc. Prof. Shiang Lim[3], Dr. Adam Hill[1,2]

[1]Victor Chang Cardiac Research Institute, Sydney, Australia, [2]School of Clinical Medicine, Faculty of Medicine and Health, UNSW Sydney, , Australia, [3]St Vincent's Institute, Melbourne, Australia

Biography:
Sutapa is a first-year PhD student at the Victor Chang Cardiac Research Institute. She is currently investigating the effect of the autonomic nervous system and its dysfunction on atrial cardiomyocytes and the arrhythmogenic mechanisms associated to cause atrial fibrillation.

Cardiac arrhythmias affect up to 5% of people worldwide, with atrial fibrillation (AF) being the most common sustained arrhythmia. Management of AF is a major clinical challenge since the mechanisms underlying the disease pathophysiology are diverse while effective management and therapeutics are limited. The development of in vitro engineered human atrial tissue models, containing multiple induced pluripotent stem cell (iPSC)-derived cell types, such as cardiomyocytes, cardiac fibroblasts and endothelial cells, is now facilitating the study of different factors, such as fibrosis, obesity, and inflammation, in the development of AF. Despite this progress, autonomic neurons are yet to be incorporated into in vitro models of AF even though dysregulated autonomic neuronal activity – sympathetic and parasympathetic – is known to play a role in pathogenesis of disease.

The overall aim of this project is to create an iPSC-derived engineered tissue model of AF that incorporates the autonomic nervous system to study the pathways that lead to arrhythmia and to screen for new therapeutics. As a first step, we aim to develop and optimise robust protocols for the differentiation of iPSC-derived sympathetic neurons (iPSC-symN) and identify conditions (media/bioengineering) to facilitate co-culture.

Preliminary characterisation of iPSC-symNs suggests successful differentiation in two different cell lines. Flow cytometry experiments show neurons positive for the sympathetic markers, PHOX2B and tyrosine hydroxylase (TH). Brightfield also reveals long axonal outgrowth by day 18, suggestive of a postganglionic sympathetic neuron morphology. Subsequent immunocytochemistry of these neurons at day 35 revealed PHOX2B, peripherin and TH expression.

Future work will involve optimising the differentiation protocol for purity and efficiency, as well as performing co-culture experiments to incorporate cardiomyocytes to observe neuronal innervation and consequent molecular interaction with cardiomyocytes. Successful development of an in vitro neuro-cardiac model will bring us closer to a well-rounded disease model of AF for drug screening.

POSTER PRESENTATIONS

P58

Innovative stem cell-based disease modeling: pioneering discoveries and future directions in RP11, ADOA and PMS disease

Mrs Priyanka Sain[1], Ms Caitlyn Richworth[1], Dr Wissam Chiha[1], Mr Sudhanshu Gupta[1], Dr Resmi Menon[1], Ms Maria Hoyos Carmona[1], Dr Suzy Juraja[1], Dr Richard Francis[1], Ms Bridget Flis[1], Ms Laura Florez[1], Ms Anna Mills[1], Dr Clarissa McDonagh[1], Ms Danie Champain[1], Mr Dean De Alvis[1], Dr Abbie Francis[1], Ms Tenielle George[1], Dr Janya Grainok[1], Dr Jessica Nichols[1], Dr Carla Jackson (née Mellough)[1]

[1]PYC Therapeutics Ltd, Perth, AUSTRALIA

Biography:

I am currently working as a Research Scientist in the Cell and Disease Modelling team at PYC Therapeutics, Perth. I completed my master's degree in biotechnology from the University of Queensland and currently engaged in cutting-edge research on rare monogenic diseases. With a focus on developing and refining iPSC-derived disease models, I am committed to creating innovative tools for therapeutic testing. My research aims to bridge the gap between laboratory discoveries and clinical applications, ultimately enhancing therapeutic options and improving patient outcomes.

The widespread utilisation of human induced pluripotent stem cells (iPSCs) has significantly advanced our capacity to model diseases with unparalleled precision. In-vitro patient-derived iPSC models have enabled the recapitulation of disease-specific genotypes and phenotypes in a dish. This facilitates a better understanding of disease mechanisms and progression, and act as a much better predictor of clinical success than non-human disease models, streamlining the development of precision therapeutics.

Our studies focus on leveraging iPSC technology to generate models for monogenic indications including inherited eye diseases, neurodevelopmental disease, and polycystic kidney disease. We have successfully established protocols to differentiate iPSCs into Retinal Pigment Epithelium (RPE), Retinal Ganglion Cells (RGC) and Glutamatergic neurons (GlutNs), representing pathologies such as Retinitis Pigmentosa 11 (RP11), Autosomal Dominant Optic Atrophy (ADOA) and Phelan-McDermid Syndrome (PMS).

Our iPSC models undergo rigorous quality control via immunocytochemistry, immunohistochemistry, flow cytometry and qPCR. Evaluation of these parameters enables the confirmation of consistent quality between patient-derived and gene-corrected/wildtype cells. Furthermore, our models exhibit disease-related hallmarks including altered expression of target gene and protein, as well as functional and morphological impairment. For example, RP11 patient-derived iPSC-RPE display disease-associated alterations in cellular morphology, and ADOA patient-derived iPSC-RGCs exhibit disrupted mitochondrial morphometry and mitochondrial fragmentation. In our iPSC-derived PMS model, SHANK3 protein, the key driver of PMS disease progression, is expressed at lower levels in patient-derived GultNs compared to unaffected controls, reflecting the haploinsufficient phenotype of the disease.

Robust disease modelling has enabled reliable and reproducible evaluation of preclinical efficacy of our therapeutics. These preclinical models de-risk the drug development process and establish a medically plausible basis for expecting the drug to be effective in these rare diseases. Increased conviction in lead therapeutic candidates because of utilising these preclinical models aids transition to the clinical stage and improves the likelihood of a successful outcome.

POSTER PRESENTATIONS

P59

Modelling SETBP1 Haploinsufficiency Disorder using CRISPR gene editing and neural differentiation of iPSCs

Dr Nicole Shaw[1], Mr Kevin Chen[2], Ms Kathryn Farley[2], Mr Mitchell Hedges[1], Clinical Professor Gareth Baynam[3], Associate Professor Timo Lassmann[2], Dr Vanessa Fear[1]
[1]Translational Genetics, Telethon Kids Institute, Perth, Australia, [2]Computational Biology, Telethon Kids Institute, Perth, Australia, [3]Rare Care Centre, Perth Children's Hospital, Perth, Australia

Biography:
Dr Shaw is a Research Officer at the Telethon Kids Institute, Perth, where she has been part of the Translational Genetics Team since completing her PhD in 2021. While Dr Shaw's research background is in airway cell biology and lung disease, her current role involves using CRISPR/Cas9 gene editing to introduce genetic variants into induced pluripotent stem cells to model patient disease in a variety of stem-cell derived culture models.

AIM: SETBP1 Haploinsufficiency Disorder (SETBP1-HD) is a neurodevelopmental disorder caused by heterozygous loss of function mutations in SETBP1 and characterised by mild to moderate intellectual disability, speech and language impairment, and mild motor developmental delay. The precise role of SETBP1 in neural development and disease pathogenesis remains elusive. Our primary aim was to investigate the molecular consequences of patient SETBP1 variants in a neural model of SETBP1-HD to elucidate underlying disease mechanisms. In addition, we aimed to characterise the effect of a SETBP1 variant of unknown significance (VUS).

METHOD: Two pathogenic variants, SETBP1 p.Glu545Ter and SETBP1 p.Tyr1066Ter, and one VUS, SETBP1 p.Thr1387Met, were introduced into the KOLF-2C iPSC genome using CRISPR/Cas9 gene editing. Neural differentiation was induced, and changes in stem and neural marker expression monitored using flow cytometry. Bulk RNA sequencing was performed on iPSC and neural progenitor cells (NPCs). Transcriptomics analysis was used to determine changes in gene expression across differentiation and between SETBP1 variant cells and SETBP1 wild-type control cells.

RESULT: Temporal differences in pluripotency and neural marker expression between SETBP1 variant iPSC lines were identified during neural differentiation. Gene set enrichment analysis demonstrated changes in the WNT pathway, RNA polymerase II pathway, and dysregulation in gene sets related to forebrain development. Additionally, GATA2 was identified as a central transcription factor in disease pathogenesis. Heterogeneity in molecular pathway dysregulation was observed between NPCs derived from the three SETBP1 variant iPSC lines.

CONCLUSIONS: Our study demonstrated the utility of using single base gene editing in iPSCs and neural disease modelling to provide a suitable model for human SETBP1-HD. Additionally, the substantial phenotypic overlap between the SETBP1 VUS and the pathogenic SETBP1 variants, provided supporting evidence towards reclassification of the variant as pathogenic.

POSTER PRESENTATIONS

P60

TRAPPC4 disorder: neuronal models of disease for therapeutic validation studies

Dr Nicole Van Bergen[1,2], Ms Riley Hall[1,2], Ms Vallari Sawant[1,2], Dr Jinchao Gu[3], Mr Tim Sikora[1], Dr Ben Rollo[3], A. Prof Silvia Velasco[1], Prof Jinkuk Kim[4], Prof John Christodoulou[1,2]
[1]Murdoch Children's Research Institute, Parkville, Australia, [2]University of Melbourne, Parkville, Australia, [3]Monash University, Prahran, Australia, [4]Korea Advanced Institute of Science & Technology, Daejeon, South Korea

Biography:
Dr Van Bergen is a Team Leader in the Genetics Theme at MCRI and a cell and molecular biologist with experience in functional genomics studies for rare diseases, stem cell modelling and developing new functional assays. Dr Van Bergen has extensive experience in screening and testing of treatments for rare neurodevelopmental disorders. Dr Van Bergen has an ongoing interest in developing treatments for rare genetic disorders, and leads several research programs for rare diseases.

AIMS: A rare neurodevelopmental disorder has been linked to a well-conserved splice site in the TRAPPC4 gene (c.454+3A>G), which causes mis-splicing of TRAPPC4 transcript and reduced levels of TRAPPC4 protein. Patients present with severe progressive neurological symptoms including seizures, microcephaly, intellectual disability and facial dysmorphism. TRAPPC4 is a core subunit of the TRAPP complex involved in intracellular transport, secretory and autophagic pathways. Little is known about neuronal disease pathophysiology and there are currently no effective treatments. We are developing iPSC-based neuronal and cortical organoid models to study TRAPPC4 disorder and identify specific interventions.

METHODS: We have generated stem cells from fibroblasts of two individuals with the same homozygous TRAPPC4 c.454+3A>G pathogenic variant and used CRISPR/Cas9 editing to generate heterozygous gene-corrected isogenic controls. Clones were tested for pluripotency, differentiation potential, genotyped and karyotyped. We are currently generating inducible neurons by insertion of NGN2 into the AAVS1 locus and differentiating iPSC to cortical organoids for disease modelling and therapeutic testing.

RESULTS: We have confirmed that all iPSC clones carry the expected TRAPPC4 variant, are karyotypically normal, express pluripotent markers and differentiate into three germ layers, using FACS and immunofluorescence endpoints. Patient cells have a significant reduction in the wild-type TRAPPC4 transcript and increased mis-splicing, resulting in >5-fold decrease in TRAPPC4 protein levels ($p<0.0001$).
In ongoing work, we are developing inducible neurons and cortical brain models of TRAPPC4, which are currently being characterised for growth rate, expression of neuronal markers, TRAPPC4-specific endpoints (transcript and protein), vesicle trafficking and electrophysiology.

CONCLUSION: These iPSC-based models will be used to understand disease mechanisms of TRAPPC4 disorder. We will also validate a potential therapy for TRAPPC4 patients that we are currently working up in patient fibroblasts.

POSTER PRESENTATIONS

P61

Bioengineering complex, functional kidney tissue with spatially organised proximal nephrons

Dr Jessica Vanslambrouck[1,2], Ker Sin Tan[1], Sophia Mah[1], Kathleen Dominic[1], Professor Melissa Little[1,2,3]

[1]The Novo Nordisk Foundation Centre for Stem Cell Medicine (reNEW), Murdoch Children's Research Institute, Parkville, Melbourne, AUSTRALIA, [2]Department of Paediatrics, Faculty of Medicine, Dentistry and Health Sciences, The University of Melbourne, Parkville, Melbourne, Australia, [3]The Novo Nordisk Foundation Centre for Stem Cell Medicine (reNEW), University of Copenhagen, DK-2200 Copenhagen, Denmark

Biography:
Dr Jessica Vanslambrouck is a Team Leader within the Kidney Regeneration Laboratory (MCRI, Melbourne, Australia) where she focuses on stem cell approaches to improve kidney disease treatment and understanding. Her research has pioneered patented approaches for creating kidney progenitors and kidney organoids using cellular reprogramming, stem cell differentiation, and 3D cellular bioprinting (Nature Cell Biology 2014, Kidney International 2019, Nature Materials 2021). Recently developing methodology to improve the accuracy of proximal tubules within stem cell-derived kidney tissue (Nature Communications 2022, Nature Protocols 2023), Dr Vanslambrouck's vision is to bioengineer functionalised kidney proximal tubules to improve kidney disease outcomes.

AIM: With limited treatment options and donor organ shortages, chronic kidney disease (CKD) is a leading cause of death globally, arising from common conditions such as diabetes and hypertension that damage the kidneys' blood filtration units (nephrons). While pluripotent stem cell (hPSC)-derived kidney organoids have attracted attention for disease modelling, drug screening, and supplementing kidney function in patients, their applications remain challenged by immaturity and spatial disorganization. This is particularly problematic for the proximal tubule (PT) segment of the nephron, critical for body homeostasis and hormone, vitamin, and energy production. Aiming to address this need for accurate PT, we refined hPSC differentiation to develop PT-enhanced kidney organoids, demonstrating superior infectious disease and toxicity modelling. Recently, our breakthroughs in the biophysical control of nephron spatial arrangement, improved PT patterning, tissue scale-up, and organ-on-a-chip culture have further advanced bioengineered PT functionalisation, complexity, and translation capacity.
METHOD: Kidney progenitors were generated from standard and fluorescent reporter hPSC lines by recapitulating the temporal signaling events of embryonic kidney development (Vanslambrouck et al. Nature Protocols 2023). PT-enhanced kidney tissue was generated via manual methodology or 3D-bioprinting in varying conformations. PT functionalisation and transferability was achieved through metabolic-directed maturation, organ-on-a-chip culture, scale-up via cryopreservation, and 3D-bioprinting of tissue sheets. Maturity and organisation was informed by high-content confocal imaging, transcriptional profiling, and transporter functional assays.
RESULT: hPSC-derived PT spatial arrangement, maturity, patterning, and function can be controlled via bioprinting conformation and culture media alterations, with appropriate metabolic guidance improving functional maturation. We also showed the generation of spatially-organised and maturing kidney tissues from cryopreserved progenitors, facilitating scale-up manufacture. Finally, such structures are transferrable to organ-on-a-chip systems.

CONCLUSIONS: These advances provide unique insights into controlling the spatial organisation of bioengineered tissue, while founding more accurate hPSC-derived PT for disease research and future treatment approaches to improve CKD outcomes.

POSTER PRESENTATIONS

P62

Characterization of a novel human Amyotrophic lateral sclerosis (ALS) three- dimensional (3D) Neurovascular Unit (NVU) in vitro cell model

Ms Stephani Viljoen[1,2], Dr Juliana C. S. Chaves[1,3], Mr Ian Peall[1,3], Associate Prof. Anthony R White[1,2], Dr Lotta Oikari[1,2]

[1]QIMR Berghofer Medical Research Institute, Brisbane, Australia, [2]The University of Queensland (UQ), Brisbane, Australia, [3]Queensland University of Technology (QUT), Brisbane, Australia

Biography:

Stephani Viljoen is a graduating Honours Student, with a bachelors degree in Biomedical Science, completing her studies through the University of Queensland. Based at QIMR Berghofer Medical Research Institute, with the Cellular and Molecular Neurodegeneration, Mental Health, and Neuroscience Program, Stephani completed her honours project on characterization of a novel human Amyotrophic lateral sclerosis (ALS) three- dimensional (3D) Neurovascular Unit (NVU) in vitro cell model, supporting the potential application of this model for mechanistic exploration and therapeutic testing in ALS.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder, characterized by the progressive deterioration of motor neurons (MNs), and impairment to the blood brain barrier (BBB) and supporting cells of the neurovascular unit (NVU). The structural and functional consequences of NVU pathology in ALS remains poorly defined, owing to the limitations of available disease models, which have poor translatability to living ALS patients. This project aims to compare a novel 3D model and traditional 2D cell culture platform for in vitro physiological replication of the NVU. For this analysis, we used an immortalized neural progenitor cell (NPC) line and human induced pluripotent stem cell (hiPSCs)-derived NPCs from an ALS patient and healthy control, cultured in a 2D culture system and 3D extracellular matrix model. Mixed populations of neurons and astrocytes, important cells within the NVU, were derived by spontaneously differentiating NPCs for a period of 21 days. Cells were characterized based on gene and protein expression, to compare the impact of each platform on cell differentiation. We further evaluated the ability of these systems to recapitulate the disease phenotype by analyzing inflammatory and oxidative stress markers associated with ALS pathogenesis. Preliminary results from this investigation have demonstrated the ability of this 3D system to promote efficient differentiation of NPCs to NVU cell types. These findings, alongside ongoing analyses, will ultimately help support the utility of a 3D in vitro approach for developing a comprehensive ALS NVU model, which can contribute to the therapeutic development and drug screening for ALS patients.

P63

Investigating the cell biology of childhood dementia using high-content imaging of organelles and macromolecules

Dr Emma Wilkinson[1], Elizabeth Read[1], Dr Sueanne Chear[1], Adelene Chaim[1], Professor Alex Hewitt[2], Dr Jana Talbot[1], Associate Professor Anthony L Cook[1]
[1]Wicking dementia research and education Centre, University of Tasmania, Tasmania, Australia, Hobart, Australia, [2]Menzies institute for Medical Research, Hobart, Australia

Biography:
Dr Wilkinson is a Postdoctoral research fellow at the Wicking dementia research and education centre (University of Tasmania) working in the Stem cell models Group. Dr Wilkinson is currently investigating multiple childhood dementia-causing diseases, and is testing potential novel therapeutic options for treating these diseases.

Aims: Childhood dementia, caused by over 100 rare genetic neurodegenerative disorders with varied progressions, affects an estimated 2,300 Australians. Globally, a child dies from childhood dementia every 11 minutes, which is comparable to childhood cancer. Lysosomal storage disorders (LSDs), constituting approximately 21% of childhood dementia cases, are associated with the accumulation of undegraded macromolecules, including complex lipids such as glycosphingolipids (GSLs), due to lysosomal dysfunction. Quantifying the difference in abundance and morphology of organelles and accumulating macromolecules in relevant cell types is crucial for assessing potential therapeutic agents.

Methods: We used two patient-derived iPSC models from individuals with childhood dementia-causing LSDs (Tay-Sachs disease and CLN3 [juvenile Batten] disease), alongside isogenic gene-corrected controls, to discern morphological signatures associated with each disorder. We employed Cell Painting, a morphological profiling assay, that uses multiplexed fluorescent dyes to visualize multiple cellular components and organelles concurrently.
Alongside this, we are developing a lipid and lysosomal staining protocol, to identify further disease-relevant morphological signatures and for preclinical testing of novel therapeutic options. This multiplexes lysosomal staining (Lysotracker) with dyes for lipids known to be dysregulated in LSDs including GM1 ganglioside (Cholera Toxin Conjugates), and lipid droplets (LipidSpot).

Results: Applying the established Cell Painting methodology, we have identified morphological differences in multiple organelles, including mitochondria and endoplasmic reticulum, between disease models and their corrected controls in Tay-Sachs disease and CLN3: We are extending this analysis with the novel lipid and lysosomal staining panel to further explore disease-relevant differences.

Conclusions: Morphological changes linked to disease offers a valuable model for evaluating drug impacts on disease-specific cellular components. This panel will help assess the efficacy of therapeutics, such as the FDA-approved Miglustat, which inhibits glucosylceramide synthase (GCS) to reduce GSL levels. Furthermore, this approach provides a biologically relevant model for testing novel therapeutics in childhood dementia-causing diseases.

POSTER PRESENTATIONS

P64

Multicellular stem cell-derived human heart valve organoids to model Rheumatic Heart Disease

Serene Yeow[1,2,3], Ms Jessica Durrant-Whyte[1,2], Dr Adam Piers[1,2], Dr Hannah Frost[1,3], Prof Andrew Steer[1,3,4], Prof Enzo Porrello[1,2,3], Dr Holly Voges[1,2,3]
[1]Murdoch Children's Research Institute, Melbourne, Australia, [2]Novo Nordisk Foundation Center for Stem Cell Medicine, Murdoch Children's Research Institute, Melbourne, Australia, [3]Department of Paediatrics, School of Medicine, Dentistry and Health Sciences, The University of Melbourne, Melbourne, Australia, [4]Royal Children's Hospital, Melbourne, Australia

Biography:
Serene is a PhD student in the Heart Regeneration group within MCRI, under the supervision of Dr Holly Voges. Her PhD is interested in developing a stem cell-derived model of Rheumatic Heart Disease (RHD) to investigate why and how the heart valves are specifically targeted. RHD is an autoimmune sequelae from a human-strict bacteria Streptococcus pyogenes (Strep A), making human induced pluripotent stem cells an appealing tool for this study. Serene wa previously in the Strep A research space as part of the Australian Strep A Vaccine development, developing assays to assess vaccine efficacy and characterising circulating strains.

Rheumatic Heart Disease (RHD) is the leading cause of heart valve disease, primarily affecting young children. It is a highly human-specific autoimmune complication arising from untreated Streptococcus pyogenes infection, making it difficult to replicate in animal models. Existing research uses measures of systemic autoimmunity, characterising peripheral blood from patients for insights into the proinflammatory state of affected heart valves. Contemporary understanding of human valve biology highlights a dynamic tissue environment regulated by diverse resident cell populations. This suggests that identifying the initiating events leading to RHD pathology requires a disease model that better reflects the complexity of native valve microenvironment.

Our group has developed a 3-dimensional heart valve organoid derived from human induced pluripotent stem cells (hiPSCs). This model comprises valve interstitial and myeloid populations but currently lacks the surrounding endothelial cell layer present in native tissue. We aim to generate an endothelial-valve organoid that allows us to interrogate the interaction of the circulating autoimmune factors and heart valves during RHD initiation.

Cardiac endothelial cells (ECs) were derived from directed differentiation of hiPSCs and cocultured with human valve organoids. We were able to encapsulate our valve organoid with an endothelial layer as shown via live microscopy and immunofluorescent labelling of cell-specific markers. The valve interstitial matrix was not impeded by the surrounding endothelial layer in normal conditions. This was measured by tissue passive tension, a functional readout of matrix remodelling. Next, we will introduce RHD patient serum collected through the Melbourne Children's Heart Tissue Biobank into our complex endothelial-valve organoid to investigate pathogenic responses of different valve cell populations in the presences of RHD autoimmune environment.

Together, we have developed a multicellular heart valve organoid model that emulates the spatial organisation of the native valve tissue, providing a novel tool to study RHD pathogenesis and develop future therapies for patients.

POSTER PRESENTATIONS

P65

Investigating the Role of Hypoxia-Immune Tumour Microenvironment in Colorectal Cancer Using Patient-derived Organoids

Miss Ruobing Zhang[1,2], Dr Wing-Hei Chan[1,2], Dr Rebekah Engel[1,2,3,4], Dr Zhengqi Cheng[5,6], Dr Andrew D. Pattison[1,2,7], Dr Thierry Jardé[1,3,4], Prof Paul J. McMurrick[4], Dr Lochlan Fennell[1,3], Prof Christine A. Wells[5,6], Prof Helen E. Abud[1,2,3,4]

[1]Department of Anatomy and Developmental Biology, Monash University, Clayton, Australia, [2]Development and Stem Cells Program, Monash Biomedicine Discovery Institute, Clayton, Australia, [3]Cancer Program, Monash Biomedicine Discovery Institute, Clayton, Australia, [4]Cabrini Monash University, Department of Surgery, Cabrini HEALTH, Malvern, Australia, [5]Department of Anatomy and Physiology, The University of Melbourne, Parkville, Australia, [6]Stem Cell System, The University of Melbourne, Parkville , Melbourne, [7]Monash Bioinformatics Platform, Clayton, Australia

Biography:

Ruobing Zhang is a PhD candidate in the Stem Cells and Cancer laboratory led by Prof. Helen Abud at Monash Biomedicine and Discovery Institute. Her research focuses on modelling the tumour microenvironment of colorectal cancer that sets the stage to further investigate therapeutic strategies for patients. Ruobing completed her Master of Biomedical and Health Sciences degree at Monash University in 2021 and Bachelor of Science degree at The University of Sydney in 2018, double-majoring in Pharmacology and Molecular Biology and Genetics. Outside of lab, Ruobing also gained her industry experience from an internship at Johnson & Johnson in 2021.

AIM: Colorectal cancer (CRC) is the second most common cause of cancer death in Australia. Despite advances in current treatments, CRC patients still suffer from a high relapse rate. The crosstalk between the tumour and its surrounding microenvironment contributes to aggressive cancer phenotypes. The presence of hypoxia and tumour-associated macrophages (TAMs) are major features in the tumour microenvironment (TME) and contribute to poor patient survival. Existing work has laid the foundation for modelling tumour-stromal interactions in vitro, however, the literature discussing the modelling of the hypoxic niche in tumours remains limited. Thus, this project aims to unveil the role of the TME in CRC by developing a novel co-culture model incorporating macrophages into patient-derived organoids (PDOs) under hypoxia. We hypothesized that the crosstalk between tumour hypoxia and TAMs forms a positive feedback loop to promote CRC development via promoting tumour growth and metastasis.
METHODS: We investigated the transcriptomic and phenotypical effects of both acute and chronic hypoxia on PDOs and macrophages. A PDO-macrophage co-culture system was also established under normoxia and hypoxia.
RESULTS: We successfully introduced tumour hypoxia in PDOs and discovered that chronic hypoxic conditions promoted cancer cell growth. Transcriptional profiling showed an enrichment of cancer hallmarks including angiogenesis and epithelial-to-mesenchymal transition signatures and a downregulation of stem cell markers under hypoxia. Macrophages survived chronic hypoxia and were activated in response to hypoxic stress. We also observed direct PDO-macrophage interactions under normoxia and hypoxia.
CONCLUSION: Overall, our findings demonstrate that hypoxia promotes tumour growth, macrophage activation, and modulates PDO-macrophage interactions. This project will further dissect the effects of hypoxia-immune interactions on the molecular and cellular characteristics of CRC in a PDO-macrophage co-culture system. We have developed a novel

patient-derived CRC model that more accurately reflects the tumour microenvironment and sets the stage to further investigate therapeutic strategies for patients.

POSTER PRESENTATIONS

P66

Don’t call me “iPSC1”. Addressing identity and provenance in Australian Stem Cell Research

Dr Suzanne Butcher[1], Dr Noel Faux[2,3,4], Dr Stuart Lee[2], Mengqi Hu[1], Dr Daniel Russo-Batterham[2], Priyanka Nair-Turkich[2,5], Masoud Kamali[6], Thao Nguyen[2], Professor Christine Wells[1]
[1]Stem Cell Systems, The Department of Anatomy and Physiology, The University of Melbourne, Parkville, AUSTRALIA, [2]Melbourne Data Analytics Platform (MDAP), The University of Melbourne, Australia, [3]The Florey Department of Neuroscience and Mental Health, The University of Melbourne, Australia, [4]The Peter MacCallum Cancer Centre, , Australia, [5]School of Computing and Information Systems, The University of Melbourne, Australia, [6]Department of Infrastructure Engineering, The University of Melbourne, Australia

Biography:
Suzanne completed her PhD at the University of Melbourne in 2019 and has a background in computational biology, cell biology, and data curation. Suzanne is currently working with the Australian Stem Cell Registry team to engage with the broader stem cell community and curate a comprehensive Registry of Australian pluripotent cell lines which will enhance the visibility of Australian research and service the needs of the Australian research community.

AIM: Today’s research drives tomorrow’s clinic. The provenance of stem cell lines is important at a time when stem cell technologies increasingly inform disease diagnosis, drug efficacy/ safety profiles, or are themselves used in the clinic. To this end, registration of stem cell lines forms part of the ISCCR Standards for Human Stem Cell Use in Research (2023). Here we describe the design of a new registry of Australian pluripotent lines that aligns with FAIR Data Principles (Findable, Accessible, Interoperable, and Reusable) and can assist Australian researchers find the resources to support their research.

METHODS: The Registry was designed in consultation with researchers, clinicians and patient advocates. Lines are assigned a unique, persistent digital identifier using the international hPSCreg nomenclature. The information captured by the Registry includes a minimal mandatory set to facilitate provenance and reproducibility, and voluntary information about cell line characterisation and downstream applications. We have used a PubMed search to identify Australian hPSC lines, and cross-validated these against hPSCreg and other international databases to find lines that are partially or not registered. The information from methods in these publications was used to review experimental details about the derivation of Australian hPSC lines.

RESULTS: We identified hundreds of unregistered Australian lines in the public domain. To date, we have manually curated over 90 lines in our Registry prototype. Common nomenclature issues include lack of a unique identifier (“iPS1”, “Control1”), unclear methodology, incorrect linking of subclones to parental lines, and lack of transparency about acceptable uses.

CONCLUSION: The Australian Stem Cell Registry will provide a local resource to facilitate transparency within the Australian field and enhance the visibility of our research in the global sphere. We will continue to work with researchers to establish naming conventions and complete line provenance that supports reproducible stem cell research.

POSTER PRESENTATIONS

P67

How FAIR are hPSCs?

Ms Mengqi Hu[1], Prof Christine. A Wells[1]
[1]Stem Cell Systems, Department of Anatomy and Physiology, University of Melbourne, Melbourne, Australia

Biography:
I am a PhD candidate at the University of Melbourne, exploring how a national stem cell registry can promote FAIR data principles (Findable, Accessible, Interoperable, Reusable) in stem cell research. My research spans policy, humanity, and science, involving collaboration with stakeholders including researchers, clinicians, and industry professionals. I contribute to designing and developing the national stem cell registry, aiming to enhance the FAIR principals. My work seeks to improve awareness of best practices and support the governance of stem cell lines in Australia.

Over the past two decades, over 40,000 publications have described the use of human pluripotent stem cell (hPSC) lines. In this study, we asked how easy it was to find relevant data on the specific lines used in pluripotent stem cell research, and whether the community is consistently describing these in a way that meets the FAIR data principles of Findable, Accessible, Interoperable, and Reuseable. We reviewed publications from the last 12 months to determine whether the lines were FAIR-ly described, if the names were unambiguous, or if a registry generated a unique and persistent cell line identifier.

Despite ISSCR guidelines advocating for cell line registration, our study revealed a substantial gap between the number of lines described in publications and those registered. We used Australian-based researchers as an exemplar, analyzing data from scientific publications, stem cell registries, biobank websites, and other databases. Our study also included interviews with stem cell infrastructure developers from the United States, Europe, and Japan, enabling a comparative analysis with Australia. This revealed considerable disparities in hPSC visibility and registration practices across international jurisdictions. Globally, over half of the registered hPSCs in an international bank or registry are not associated with publication outputs. Australian researchers rely heavily on publications to share information about hPSC, but most publications lack clarity about which lines were used, and many lines have ambiguous names such as HDF1 or Control iPSC. Currently, registration of cell lines is not widely adopted among Australian researchers.

This study underscores the need for standardized registration to improve the traceability and utility of hPSCs. We advocate for greater adherence to the ISSCR guidelines to ensure the scientific community can fully leverage stem cell registries, promoting cell line reuse, fostering collaboration, and improving communication with donors and funding agencies regarding research progress.

POSTER PRESENTATIONS

P68

The research-teaching nexus

Dr Julia Young[1], Dr Sonja McKeown[1]
[1]Monash University, Clayton, AUSTRALIA

Biography:
Dr Julia Young is a senior Lecturer with the Department of Anatomy and Developmental Biology, and an adjunct Senior Research Fellow at the Hudson Institute of Medical Research. Julia runs undergraduate and post graduate Units in developmental and stem cell biology, while maintaining a research interest in reproductive development and basic stem cell biology. Julia undertook her PhD at Monash, then moved to the IGBMC in Strasbourg, France for post-doctoral training, before returning to Australia to join the burgeoning stem cell research field. Julia is passionate about integrating real world research experiences into her teaching to inspire students.

Aim: Higher education has always faced challenges in defining the responsibilities and roles of research-focussed academics in the undergraduate classroom. However, it is well-recognised that the research community has a critical role to play in the undergraduate teaching space, so clarity in communicating how this role can be integrated into the research workload through approaches to time-efficient and expertise-focussed learning delivery need to be clearly communicated to researchers.

Methods and Results: Bringing the expert knowledge and experience of the research community into the classroom is most effective when researchers integrate their own current research into their teaching. This teaching can include the presentation of research design, experimental approaches, data, data analysis, and the design of future research directions. Here, we will present a series of concrete examples of how stem cells researchers can translate their skills knowledge and experience efficiently and effectively into the teaching context. And will include detailed information on what is required for involvement in lecture series design and presentation, with a particular focus on how stem cells researchers can contribute their skills into the laboratory aspect of undergraduate teaching through the development of achievable experimental approaches to the interrogation of relevant current research questions

Conclusions: In joining the classroom, researchers gain exposure to a new cohort of potential future research students and the next generation of research scientists. Studies clearly show, too, that development of teaching materials and interaction with students often brings new perspectives to research questions and can often inspire new ideas and approaches. Finally, supporting and increasing researcher access to the research-teaching nexus will increase undergraduate student interaction and engagement by bringing enhanced relevance and currency into the classroom, and inspire the next generation of stem cells research scientists.

POSTER PRESENTATIONS

P69

Targeting colon cancer stem cells as new therapeutic approach in the treatment of colorectal cancer

Dr Reem ALHulais[1], A/Prof Stephen Ralph[1]
[1]School of Pharmacy and Medical Sciences, Griffith University, Gold Coast, Australia

Biography:
I recently obtained my Doctor of Philosophy on 28 March 2023 from the Griffith University, School of Pharmacy and Medical Sciences, Gold Coast. My PhD thesis Title: "Drug Targeting of Colorectal Cancers, Including Cancer Stem Cells", received excellent feedback. The first examiner gave me a first-class award for the quality of my research, and the second examiner indicated only minor corrections. Both stated that my work was of excellent quality and recommended its completion for publication. I am currently, in the process of finalising my thesis for publication in high-quality research or peer-reviewed journals.

Cancer stem cells (CSCs) are notoriously drug resistant and are well known for their ability to undergo self-renewal and differentiation into more mature cancer cells. To date, there has been a notable dearth of investigations regarding the exact role and functions of isolated populations of CSCs. This study developed a method for selectively enriching CSC populations to establish a basis for testing the effects of drugs on cancer metastasis. SW480 and CT26 parental wild-type (WT) cells were transfected with a vector encoding the octamer-binding transcription factor 4 (OCT4) promoter site regulating expression of enhanced green fluorescent protein (GFP). The most highly positive OCT4-GFP cell population after extensive rounds of sorting (the top ~1%–5%) could be further enriched by intermittent cycling involving alternating conditions of growth (between normoxia and anoxia). The highly enriched CT26 OCT4-GFPCSC population produced significantly greater tumour numbers with larger tumour sizes than did the CT26 WT inoculated mice. However, colorectal tumours formed by either cell types were significantly decreased (~50%) in numbers and volumes by celecoxib treatment. Significant levels of red blood cells were present in the peritoneal cavities of mice with the untreated colorectal tumours but greatly inhibited peritoneal angiogenesis was noted in the celecoxib-treated mice. Using these model systems for study will ensure that the role of CSC-enriched populations in tumour growth and metastasis and their therapeutic targeting can now be effectively conducted. The evidence obtained here also supports the potential for celecoxib to be repurposed and used in chemosensitising colorectal cancer cells, thereby rendering them more susceptible to standard chemotherapies such as doxorubicin and 5-fluorouracil.

POSTER PRESENTATIONS

P70

Identifying Genomic Drivers Of In Vitro Human Mesenchymal Stem Cells Proliferation Via Targeted Transcriptome Sequencing.

Mr. Nghia Nguyen[1], Sofia Petersen[1], Duy Nguyen[1], Martina Gyimesi[1], Rachel Okolicsanyi[2], Larisa Haupt[3]

[1]Stem Cell and Neurogenesis Group, Centre for Genomics and Personalised Health, Genomics Research Centre, School of Biomedical Sciences, Queensland University of Technology (QUT), , Australia, [2]Stem Cell and Neurogenesis Group, Centre for Genomics and Personalised Health, Genomics Research Centre, School of Biomedical Sciences, Queensland University of Technology (QUT); Centre for Biomedical Technologies, Queensland University of Technology (QUT); Max Planck Queensland Centre for the Materials Science of Extracellular Matrices, , Australia, [3]Stem Cell and Neurogenesis Group, Centre for Genomics and Personalised Health, Genomics Research Centre, School of Biomedical Sciences, Queensland University of Technology (QUT); Centre for Biomedical Technologies, Queensland University of Technology (QUT); Max Planck Queensland Centre for the Materials Science of Extracellular Matrices; ARC Training Centre for Cell and Tissue Engineering Technologies, Queensland University of Technology (QUT), Australia, , Australia

Biography:
Nghia is a MPhil student at the Stem Cell and Neurogenesis Group within the Genomics Research Centre at the Queensland University of Technology. Under his supervisors Associate Professor Larisa Haupt and Dr. Rachel Okolicsanyi, Nghia has been investigating the drivers of in vitro human mesenchymal stem cells proliferation and lineage specification via targeted transcriptome sequencing, with a focus on the role of key signalling mediators such as heparan sulfate proteoglycans (HSPGs) in the process.

AIMS: Human mesenchymal stem cells (hMSCs) are an exciting candidate for regenerative therapies due to their ease of isolation and multilineage potential. However, the necessary long-term in vitro expansion of hMSC populations may reduce their proliferative and lineage specification capacity. This in vitro aging of hMSCs may be driven by changes in signalling pathways such as NF-κB signalling, TGFβ signalling, and changes in key signalling mediators such as heparan sulfate proteoglycans (HSPGs). Identification of genomic changes within hMSCs as they age in vitro may lead to the possibility of maintaining proliferation in these cell populations, thus expanding their therapeutic capacity.
METHODS: In this study, we profiled two hMSC populations at key passages using targeted transcriptome sequencing. These passages represent hMSCs at the early phase of expansion (P+5), hMSCs at exponential phase of growth (P+7), and hMSCs at stationary phase of growth (P+13). Differential gene expression (DGE) analysis was used to associate gene expression with both the increase (at P+7) and decrease of hMSC proliferation (at P+13). Identified differentially expressed genes were subjected to gene set variation analysis (GSVA) and protein-protein interaction (PPI) analysis. Additionally, DNA methylation of P+5 and P+13 hMSCs were profiled to examine expression changes correlating with epigenetic changes.
RESULTS: DGE analysis showed temporary upregulation of genes associated with TGFβ signalling includng JUNB and NR4A1-3, with PPI analysis demonstrating interaction between differentially regulated genes with the NF-κB complex. Gene expression changes were mirrored by temporary upregulation of TNFα via NF-κB signalling and TGFβ signalling pathways at P+7. Methylation changes (hypermethylation of promoters) between P+5 and P+13 correlated with observed gene expression changes ($r = -0.3$, $p < 0.05$).

CONCLUSION: These findings highlight NF-κB and TGFβ signalling as potential drivers of proliferation and lineage specification capacity of hMSCs and provide potential targets of in vitro proliferation of hMSC populations.