id #66243

Conserved Epigenetic Regulatory Logic Infers Genes Governing Cell Identity

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Understanding genetic control of cell diversification is essential for establishing mechanisms controlling biological complexity. We analyzed 111 NIH epigenome roadmap data sets to identify distinguishing features of genome regulation associated with cell-type specification. We show that the a priori deposition of H3K27me3, which we call a gene's repressive tendency (RT), provides a genome-wide enrichment for genes governing fundamental mechanisms underlying biological complexity in cell differentiation, organ morphogenesis and drivers of disease. We tested the ability to infer regulatory genes controlling theoretically any somatic cell by interfacing genome-wide RT values with cell-specific genome-wide sequencing data. Using more than 1 million genome-wide data sets from diverse omics platforms including bulk and single cell RNA-seq, CAGE-seq, ChIP-seq and quantitative proteomics, we identify cell-type specific regulatory mechanisms underlying diverse cell-states, organ systems and disease pathologies. Since regulatory control of cell identity is highly evolutionarily conserved across species, we demonstrate that this computational logic enriches for cell- type specific regulatory genes from species across the animal kingdom including chordates and arthropods. Lastly, we use this computational inference approach for novel gene discovery. Analysis of single cell RNA-seq data from in vitro human iPSC cardiac differentiation predicted *SIX3* as a novel transcription factor controlling derivation of definitive endoderm, which we confirmed by *SIX3* genetic loss of function using CRISPRi hPSCs. Moreover, analysis of transcriptional data from heart development of the invertebrate chordate *Ciona robusta*, predicted *RNF220* to underlie tunicate heart field formation. This was confirmed with CRISPR knockout in vivo showing that *RNF220* loss of function results in pharyngeal muscle morphogenesis defects. This study demonstrates that the conservation of epigenetic regulatory logic provides an effective strategy for utilizing large, diverse genome-wide data to establish quantitative basic principles of cell-states to infer cell-type specific mechanisms that underpin the complexity of biological systems.

id #66485

The Vector and Genome Engineering Facility (VGEF): Customized service for your research needs

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The mission of the Vector and Genome Engineering Facility (VGEF), an innovative academic scientific core facility of the Children's Medical Research Institute, is to support basic and translational research in vector-based gene therapy and genome engineering. In addition to being a service facility that provides investigators the access to the latest viral-vector and genome engineering technologies, VGEF is also active in developing new, and improving existing, technologies that will empower its customers and the wider scientific community.

The VGEF facility offers customized preparations of lentiviral (LV), adenoviral (Ad), and adeno-associated viral (AAV) vectors, at purities and production scales tailored to the needs of individual projects to facilitate both *in vitro* and *in vivo* experimentation, as well as a range of ready-to-use AAV and LV stocks.

The VGEF also offers wide range of genome-editing services in immortalized lines and stem cells of human and animal origin. VGEF closely follows and utilizes the latest advances in genome editing technologies. Currently, VGEF uses a multitude of plasmid- and vector-based CRISPR/Cas systems based on active or inactive nucleases or nickase for precise genome or epigenetic modifications. We are also expending our services into RNA editing, endonuclease free editing using AAV-driven homology directed repair (HDR), as well as into editing with the use of Cas9 protein and Cas9 mRNA expression methods.

In alignment with our goal to promote vector-based and genome engineering technologies, we offer consultation in study design and technical support in the selection of appropriate tools for specific experimental needs, as well as hands-on training opportunities. Beyond our academic and commercial services, we also engage in collaborative studies that are aligned with our research focus in AAV biology, vector development and genome engineering.

id #66556

Using hiPSC-derived retinal and inner ear organoids to model Usher pathophysiology.

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Human communication and perception of the environment are mostly conveyed by information perceived through the ear and the eye. A number of different inherited conditions impair both sight and hearing. The most common of these inherited conditions is Usher syndrome a collection of severe autosomal recessive disorders. The sight loss is caused by gradual and progressive loss of photoreceptor cells and the hearing loss is caused by defects in the inner ear hair cells. Thus far there are no successful treatments for the retinal disorder and only a proportion of cases can benefit from cochlear implants. Here we use induced pluripotent stem (iPS) cells to model Usher2a retina and Usher1b inner ear diseases *in vitro*. The generation of a culture system to efficiently differentiate diseased sensory cells will aid the development of new treatments.

Usher2 patients account for almost half of all the Usher cases. We have generated a number of iPS cell lines from Usher2A patients and utilized our recently described 2D/3D protocol to differentiate these into retinal organoids. Immunohistochemistry and real time PCR analysis were used to assess the expression of cilial and Usher genes in controls and Usher2A retinal organoids. Transmission electron microscopy was used to evaluate photoreceptor ultrastructure. Photoreceptor degeneration was evaluated by Tunel staining.

Retinal organoid cultures generated photoreceptors containing synapses, connecting cilia, inner segments and outer segments. Usher proteins were present in iPSC-derived USH2a photoreceptor cells and electron microscopy did not demonstrate morphological abnormalities in cilia and outer segment formation. However, increased photoreceptor cell death was observed in Usher2a retinal organoids when compared to control organoids. We have now started to evaluate cellular metabolism pathways such ER stress, autophagy and oxidative stress to further understand the cause of degeneration *in vitro*.

Mutations in *Usher1* genes cause the most severe form of Usher. Usher1b inner ear organoids containing hair cells were successfully generated and characterised using immunohistochemistry. A number of AAV capsids were tested in vitro to establish hair cells transduction efficiency.

These data suggest that Usher iPSC-derived retinal and inner ear organoids represent a potential tool to model disease and therefore enhance our understanding USH pathophysiologyand the mechanism of degeneration. Most importantly, diseased retinal organoids will aid the development of new treatments such as drug screening and gene therapy.

id #66968

Immunosuppression Free Human Beta Cell Therapy for Type-1-Diabetes Using a Bioengineered Device.

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Introduction: β-cell replacement is an attractive treatment for type-1-diabetes (T1D), but toxic immunosuppressive drugs are needed. We aimed to deliver allogeneic β-cell therapies without anti-rejection drugs using a bioengineered device that combines microencapsulation of β-cells and 3D scaffolds printed using melt-electrospin-writing (MEW).

Methods: Mouse β-cell (MIN6) clusters, islets from QS mice and human islet-like clusters (ILC) differentiated for 28 days from embryonic stem cells (hESC) were encapsulated in 2.2% ultra-pure alginate. Viability and glucose stimulated insulin secretion were assessed. Cells were encapsulated and seeded within MEW scaffolds. Devices were transplanted subcutaneously in immune-deficient (NOD/SCID) or immune-competent (BALB/c) mice made diabetic with low-dose streptozotocin (n=6-8/group). Blood glucose level (BGL) and glucose tolerance were tested. Vascularity inside grafts was quantified over 4 weeks by 3D-doppler ultrasound. Once BGL normalized, grafts were removed for examination. Insulin and C-peptide in plasma, pancreata and grafts were measured by ELISA.

Results: Cell viability and insulin secretion were unaffected by encapsulation. Transplantation of encapsulated MIN6 within MEW scaffolds lowered BGL (from 30 ± 3 to 5 ± 2mmol/L) and improved glucose tolerance in diabetic NOD/SCID and BALB/c strains within 25-41 days. Long-term BGL normalization (60-100 days) in BALB/c mice was achieved with QS islets in a device pre-vascularised for 3 weeks. Inflammatory infiltration of neutrophils (myeloperoxidase$^+$), macrophages (CD68$^+$) and B-lymphocytes (CD19$^+$) were present on MEW scaffolds but not on microcapsules, which had infrequent pro-fibrotic walling (α-SMA$^+$). In diabetic NOD/SCID mice receiving 2000 ILC, human C-peptide was measurable and no teratoma was detected for at least 83 days. BGL were lowered to almost normal in NOD/SCID mice receiving encapsulated ILC intraperitoneally, with C-peptide levels being > in recipients of subcutaneous grafts.

Conclusion: Allogeneic β-cell therapy for T1D without immunosuppression can be delivered using our bioengineered device. It is safe to implant β-cells differentiated from hESC.

id #66989

Targeting KDM6A as a novel therapeutic strategy for treating craniosynostosis in children with Saethre-Chotzen syndrome

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The upper part of the mammalian skull (calvaria) is made up by five flat bones which are separated by open sutures during childhood. Craniosynostosis is a condition where calvarial sutures fuse prematurely, as a result of aberrant osteogenic differentiation of calvarial mesenchymal stromal cells on the suture sites. Saethre-Chotzen Syndrome (SCS) is one of the most common forms of craniosynostosis (occurring in ~1/25,000 births), which is caused by a loss-of-function mutation of *TWIST-1* gene. This condition could lead to skeletal deformities and neurological deficits. Currently, the only treatment for craniosynostosis involves the removal of the affected sutures and remodelling of the skull, which could lead to serious complications. Thus, an attempt to identify a novel drug therapy that eliminates the use of invasive surgery is paramount.

This project utilised the established SCS mouse model, Twist-$1_{del/+}$ heterozygous mutant mice, to investigate this condition further. The results show that the expression of an epigenetic enzyme, called KDM6A, is negatively regulated by TWIST-1 and is upregulated in SCS mouse model, compared to wild type control littermate mice. KDM6A has been shown previously by our laboratory to promote osteogenesis in bone marrow-derived mesenchymal stromal cells. This suggests that KDM6A could be a potential target to treat the aberrant osteogenesis seen in SCS patients. Thus, this study aims to determine the effectiveness of a pharmacological inhibitor of KDM6A, GSK-J4, in alleviating the aberrant increase of osteogenic differentiation in calvarial stromal cells and explants-derived from SCS mouse model.

The results demonstrate for the first time that GSK-J4 could inhibit the osteogenic potential of SCS mouse calvarial stromal cells and explants at concentrations up to 2μM with minimal toxic effects *in vitro*. Thus, GSK-J4 could be a potential therapeutic strategy for treating craniosynostosis in children with SCS. Further studies including analysis of the efficacy of GSK-J4 in *Twist-*$1_{del/+}$ mice via local administration *in vivo* are required to assess GSK-J4 as a treatment for craniosynostosis in SCS.

id #66993

Nature trumps nurture: Use of naturally liver-tropic adeno-associated viruses as vector platforms for liver-directed human gene therapy

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Despite unequivocal recent successes in AAV-mediated liver-directed gene therapy trials, many liver diseases theoretically amenable to treatment with gene therapy are still beyond the reach of contemporary AAV technology, which is constrained by suboptimal hepatocyte targeting. The aim of this study is therefore to develop AAV vectors with superior hepatotropism. Our previous discovery and functional characterisation of conserved master hepatic transcription factor binding sites in the 3'-untranslated region of the prototypical human AAV isolate, AAV2, signify its intimate association with the human liver. However, vectors utilising the AAV2 capsid in a liver-directed gene therapy trial resulted in unexpectedly low therapeutic efficacy. This was consistent with our subsequent observations of inefficient human hepatocyte transduction in the xenograft FRG mouse model, despite robust targeting of human hepatocyte-derived cell lines. Upon discovering that most primate AAVs evolved to infect the liver, we resolved the paradox of AAV2 human hepatotropism by amplifying AAV capsid sequences directly from human liver samples. Comparison with the prototypical AAV2 capsid revealed functionally validated differences in the heparan sulfate proteoglycan binding domains, thereby attributing inefficient transduction of primary human hepatocytes by the originally culture-isolated AAV2 (henceforth "ciAAV2") to culture adaptation. The development of AAV vectors using capsids isolated directly from liver samples, and naturally evolved to traffic to the liver and target human hepatocytes, harnesses the power of viral evolution and circumvents cell culture attenuation of hepatotropism. In humanised FRG mice, several of the novel and sero-diverse wildtype liver-isolated capsids vectorised to date already functionally outperform the most human liver-tropic bioengineered AAV capsids – LK03 and AAVS3 – currently in gene therapy clinical trials targeting the human liver, but still fall short of the most human hepatotropic capsids our groups have developed. We are further optimising their performance and manufacturability to develop a set of superior, clinically translatable capsids.

id #67008

Wound healing promoting activity of tonsil-derived stem cells on 5-fluorouracil-induced oral mucositis model

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Background: We first determined the efficacy of lesional injection of tonsil-derived mesenchymal stem cells for the treatment of 5-fluorouracil induced oral mucositis.

Methods: Oral mucositis was induced in hamsters by administration of 5-fluorouracil (day 0, 2, 4) followed by mechanical trauma (day 1, 2, 4). The experimental groups included MT (mechanical trauma only), 5-FU+MT (mechanical trauma with 5-fluorouracil administration), TMSC (mechanical trauma with 5-fluorouracil administration, tonsil-derived mesenchymal stem cells injection),

DEXA (mechanical trauma with 5-fluorouracil administration, dexamethasone injection), and saline (mechanical trauma with 5-fluorouracil administration, saline injection).

Results: On day 10, gross and histologic analyses showed that nearly complete healing and epithelialization of the cheek mucosa of the TMSC group, whereas the other groups showed definite ulcerative lesions. Compared with the MT and DEXA groups, CD31 expression was greater in the TMSC group on days 10 and 14. In addition, the TMSC group showed higher expression of TGF-β, and NOX4 on day 10 compared with the other groups.

Conclusion: Intralesional administration of tonsil-derived mesenchymal stem cells may accelerate wound healing of 5-fluorouracil induced oral mucositis by upregulating neovascularization and effective wound contraction.

id #67426

Pentose phosphate pathway is required for L-proline-mediated transition of mouse embryonic stem cells to early primitive ectoderm-like cells

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L-proline stimulates transition of mouse embryonic stem cells (ESCs) to early primitive ectoderm-like cells (EPLCs) by activating signalling pathways. We show changes in cellular metabolism are also required for the transition: the L-proline-mediated transition depends on the pentose phosphate pathway (PPP) which stimulates glycolysis, resulting in increasing anaerobic metabolism. The role of the PPP in the ESC-to-EPLC transition was assessed by culturing ESCs with L-proline to produce EPLCs with or without 6-aminonicotidamide (6AN) and dichloroacetate (DCA). 6AN is a competitive substrate of glucose-6-phosphate dehydrogenase, the rate-limiting enzyme of the PPP. DCA inhibits pyruvate dehydrogenase kinase, alleviating inhibition of pyruvate dehydrogenase to allow pyruvate to enter mitochondrial respiration, thus decreasing the rate of anaerobic metabolism via glucose. When 6AN or DCA was added to ESCs along with L-proline, it prevented the decrease in doubling time and reduced the percentage of differentiated colonies expected to occur with L-proline alone. 6AN or DCA also prevented L-proline-mediated upregulation of EPLC markers *Fgf5* and *Dmnt3b*. Addition of L-proline to ESCs increased anaerobic metabolism rate but addition of 6AN or DCA prevented this increase. Therefore, an increase in anaerobic metabolism involving the PPP along with L-proline-mediated activation of signalling pathways is required for L-proline-mediated ESC-to-EPLC transition.

id #67536

RUNX1 and Group F SOX genes are required at distinct stages for human haematopoietic development

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Detailed understanding of the genetic regulation of haematopoiesis is essential for blood cell therapies and to faithfully model haematologic disease. Unlike in the mouse, this knowledge is lacking in humans. To address this, we followed endothelial (*SOX17*) and haematopoietic (*RUNX1C*) development using in vitro differentiation of human pluripotent stem cells (hPSCs). Mimicking yolk sac haematopoiesis, we identified SOX17-CD34+CD43- endothelial-like cells as the major source of haematopoietic progeny while SOX17+CD34+CD43- cells mostly formed endothelium. Deletion of RUNX1 permitted a single wave of GFI1/1B-dependent yolk sac-like primitive erythropoiesis, but no yolk sac myelopoiesis or aorta-gonad-mesonephros (AGM)-like haematopoiesis. Deletion of *SOX17* or all Group F SOX genes (*SOX7*, *SOX17*, *SOX18*) did not influence yolk sac-like haematopoiesis, but severely perturbed the development of AGM-like vessels and reduced their haemogenic capacity. This phenotype was largely mediated by reduced NOTCH signaling and recapitulated by inhibiting γ-secretase. Our data extend mouse studies, identifying unique and distinct requirements for *RUNX1* and *SOX* genes during human haematopoiesis.

id #67537

THE ROLE OF VENTX HOMEOBOX GENE DURING HUMAN HAEMATOPOIETIC DEVELOPMENT

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The *Ventx* genes are non-clustered homeobox transcription factors that confer a ventral phenotype on mesodermal cells in the developing embryo. *Ventx* genes are conserved in vertebrates but have been lost in rodents. In the human haematopoietic system, *VENTX* promotes myeloid differentiation and is expressed in some acute myeloid leukaemias (AML). Using a Doxycycline (DOX) inducible *VENTX* expression system, we found that *VENTX* overexpression in hPSC impaired mesoderm formation, but *VENTX* enforced expression after mesoderm commitment resulted in the emergence of an increased percentage of immature blood cells that co-expressed CD90 and CD34. These cells displayed high clonogenic capacity in methylcellulose, but only after DOX was removed from the media. This suggested that *VENTX* expression held cells in a non-proliferative state. Transcriptional profiling revealed increased expression of *HOXA* genes in haematopoietic cells following *VENTX* induction, consistent with their immature phenotype. Conversely, genes involved in myeloid differentiation were down regulated during *VENTX* overexpression, as were genes involved in proliferation, such as *MYC* and *MYB*. ATAC-sequencing demonstrated

that *VENTX* closes selected chromatin loci, in particular areas targeted by *HOXB13* and *CDX* transcription factors. We hypothesise that *VENTX* might act as a transcriptional suppressor during haematopoietic differentiation and we are currently investigating the genomic targets of *VENTX*, combining ATAC-seq and ChIP-seq with the transcriptional profiling. In summary, *VENTX* overexpression generated immature blood cells in a quiescent state, preventing their proliferation and differentiation into myeloid lineages.

id #67596

Utilising patient-specific retinal organoids to investigate the role of *SNRNP200* variants of unknown significance in severe early onset retinitis pigmentosa

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The consequence of variants in *SNRNP200*, implicated in dominant retinitis pigmentosa (RP), remain unknown. *SNRNP200* encodes for a component of the spliceosome complex. To investigate the effect of *SNRNP200* variants, we generated human retinal organoids (ROs) from a patient with severe early-onset RP found to harbour compound heterozygous missense variants in *SNRNP200*; an unusual case of autosomal recessive inheritance. Computer-assisted pathogenicity programs and other tools were used to predict the pathogenicity of candidate variants. Control and patient-derived induced pluripotent stem cells (iPSCs) were differentiated into ROs for 350 days. Immunohistochemical, qRT-PCR, and transmission electron microscopic analysis was performed. iPSCs and ROs were also subjected to single cell RNA-Seq and bioinformatic analysis. Several morphological differences in patient ROs were identified including (1) reduced neuroepithelial integrity, (2) vacuole accumulation within photoreceptors, and (3) abnormal mitochondrial morphology. Transcriptomic analysis revealed multiple genes associated with immunologic signatures, and genes associated with cell coenzyme biosynthesis, were significantly differentially expressed. By generating a human RO-based model of RP we have identified patient-specific differences in gene, protein and transcriptomic expression, neuroepithelial integrity, photoreceptor morphology and ultrastructure. These data show the potential of using ROs to assess functional consequences in patients affected by variants of unknown significance.

id #67638

Antisense Oligonucleotide-Mediated Exon Skipping to Treat Spinocerebellar Ataxia Type 3

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Spinocerebellar ataxia type 3 (SCA3) is a devastating neurodegenerative disease, which is one of nine polyglutamine disorders. Although SCA3 is pathogenically heterogeneous, the main feature is progressive ataxia, which in turn affects speech, balance and gait of the affected individual. There is currently no cure, nor effective treatment strategy for affected individuals. SCA3 is caused by an expanded polyglutamine tract found in ataxin-3, resulting in conformational changes that lead to toxic gain of function. This expanded glutamine tract is located at the 5' end of the penultimate exon (exon 10) of *ATXN3*. This study aims to use antisense oligonucleotide (AO) mediated exon skipping to develop a therapeutic strategy for the treatment of SCA3.

Initial *in vitro* data using 2'-O-methyl AOs in patient cells show that it is possible to create an internally truncated protein, missing the toxic CAG repeat contained in *ATXN3* and still maintain normal function of the protein. Confirmatory data using the clinically relevant *phosphorodiamidate morpholino oligomer* (PMO) chemistry showed complementary positive results to 2'O-methyl data. Additionally, significant downregulation of both the mutant and wild-type protein was observed, allowing for a combination of benefits. However, PMO is widely considered to be a superior chemistry when compared to 2'-O-methyl, as they are chemically stable and have an excellent safety profile to date. Further data shows that PMO chemistry is longer lasting and significantly better tolerated by cells. Therefore, this study provides a possible therapeutic strategy to treat SCA3.

id #67734

Human iPSC-derived vascularised and innervated cardiac organoids for disease modelling

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Cardiovascular disease is the leading cause of death worldwide necessitating accurate human disease models to improve our understanding and facilitate preclinical trials. Cardiac organoid models can recapitulate the 3D microenvironment and integrate the different cell types found in native hearts. This project aims to deliver an advanced pre-clinical human model for modelling heart disease. Induced pluripotent stem cells (iPSCs) were differentiated into cardiomyocytes, endothelial cells, and sympathetic neurons and then combined to form vascularised and innervated cardiac organoids which were maintained for up to 4 weeks. Cardiac organoids exhibited spontaneous and synchronous contractions (168±7 bpm) at 1-week. Histology showed CD31+ endothelial networks and tyrosine hydroxylase+ neural networks interspersed throughout the organoid. Single-cell RNAseq confirmed organoid reproducibility and identified input cell types. Organoids significantly increased lactate dehydrogenase release in response to doxorubicin-induced cardiotoxicity ($P<0.05$) and simulated ischaemia-reperfusion injury ($P<0.05$) indicating the capability of the organoids to simulate standard cardiac responses to injuries. In conclusion, human iPSC-derived cardiomyocytes, endothelial cells and sympathetic neurons self-assembled within our proprietary organoid model to generate beating cardiac tissue with a microvascular and neural network. This in vitro human cardiac tissue will be an ideal pre-clinical human model to study and develop novel therapeutics for heart diseases.

id #67736

Modelling vascular endothelial cell dysfunction in Friedreich's ataxia using human iPSCs

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Friedreich ataxia (FRDA) is a hereditary neurodegenerative disorder which is frequently accompanied by cardiomyopathy which can result in premature mortality. Clinical reports have suggested that the associated cardiomyopathy may be linked to abnormalities of the small coronary arteries. This study aims to assess the endothelial and mitochondrial functions in endothelial cells derived from induced pluripotent stem cells derived from FRDA patients (FRDA-iPSCs) and CRISPR-corrected isogenic control iPSCs. Cardiac tissue of FRDA patients displayed focal interstitial fibrosis and increases in endothelial cell proliferation in some small blood vessels, creating a non-uniform intimal layer that partially occluded the vessels. Compared to isogenic controls, FRDA-iPSCs displayed a ~3-fold lower FXN mRNA and ~6-fold lower frataxin protein expression. CD31+ endothelial cells derived from FRDA- and isogenic control-iPSCs expressed endothelial cell markers (CD31 and VE-cadherin) and formed capillary-like tube structures on Matrigel. Compared to isogenic controls, endothelial cells derived from FRDA-iPSCs had higher mitochondrial membrane potential ($P<0.05$) and equivalent levels of mitochondrial superoxide under basal and oxidative stress conditions. In conclusion, endothelial cells derived from FRDA-iPSCs exhibit abnormal mitochondrial function. This *in vitro* pre-clinical vascular model may provide a valuable platform for future drug discovery to develop novel treatment for FRDA-induced cardiomyopathy.

id #67787

Identification of age-related transcriptional changes in hPSC-derived retinal pigment epithelium cells using single cell RNA-Seq.

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The retina is a complex, multi-layered tissue responsible for sensing and converting light photons to neural signals for visual recognition. The outermost cells of the tissue, the retinal pigment epithelium (RPE), performs a number of critical functions in the visual cycle, and is subjected to enormous daily stress to meet these various demands. Over time and particularly in disease, the RPE undergoes a number of well-characterised changes, however the molecular mechanisms involved in these changes are not completely understood. Using single-cell sequencing technology, we aim to assess the unique transcriptional changes that occur in biologically aged human pluripotent stem cell-derived RPE cells. The human embryonic stem cell (hESC) line H9 was differentiated to RPE using an adaptation of our published protocol (Lidgerwood *et al* 2016). Briefly, cells were differentiated for 60 days, passaged and maintained continuously until the characteristic pigmented, polygonal and homogeneous morphology was observed at day 30 (D30) post-passaging, at which point the first sample, designated "young" RPE, was harvested. The remaining culture was continuously maintained and harvested on day 365 (D365), designated "aged" RPE. Both samples at the

time of harvesting were live cell sorted using FACS and captured on the 10X Genomics Single Cell 3′ Chip. Sequencing libraries were generated with unique sample indices (SI) for each sample and mapped to the *Homo sapien* genome (GRCh38, Annotation: Gencode v29). Using standard processing and analysis, we identified 652 uniquely differentially expressed genes between the young vs aged hPSC-RPE, which were largely involved in response to metal ions, oxidation, trafficking, immune responses and mitochondrial function, according to (GO) biological processes. Together this data is the first to map the biological transcriptome of aged hPSC-RPE at the single cell level and may provide insight into the early molecular events that lead to RPE aging and age-related diseases.

id #67799

The use of endogenously expressed microRNA for targeted gene delivery to cancer.

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Targeted gene delivery relies on the ability to limit the expression of a transgene within a defined cell/tissue population. Exploiting genetic and phenotypic differences between cell types presents a window for targeted gene delivery. One genetic difference that may be exploited is differences in MicroRNA expression. MicroRNAs represent a class of highly powerful and effective regulators of gene expression that act by binding to a specific sequence present in the corresponding messenger RNA. Involved in almost every aspect of cellular function, many miRNAs have been discovered with expression patterns specific to developmental stage, lineage, cell-type, or disease stage. Exploiting the binding sites of these miRNAs allows for construction of targeted gene delivery platforms with a diverse range of applications. Here, we summarize our studies exploiting the differential expression of miRNA in cancerous verses healthy normal tissue to achieve targeted gene delivery. We will discuss the use of endogenous miR122 and miR199 to limit gene expression to hepatocellular carcinoma (liver cancer), pointing to the pros and cons of this approach. Additionally, we will discuss criteria that are important when choosing a particular miRNA for targeting and tips and tricks learnt when designing miRNA-regulated expression cassettes.

id #67802

Differentiation and proliferation characteristics of tonsil-derived mesenchymal stem cells according to age, gender and tissue source

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Introduction

Tonsil-derived mesenchymal stem cells (TMSCs) with capacities to differentiate into mesodermal, endodermal and ectodermal lineages have been wildly addressed for their practical applications in many disease animal models. TMSCs' differential and proliferation potency may be different according to the donors (donor dependency). However, no one have compared the stemness of TMSCs from donors with different ages or gender, or TMSC isolated from different parts of tonsil. In this study, we characterized TMSCs isolated from epidermis and stroma of tonsil for their proliferation rate, multi-potency and surface marker profile in gender-dependent and age-dependent manners.

Methods

We divided tonsil tissue into epidermis, stroma and both parts. TMSCs isolated from these parts were classified into male and female donors and sub-classified into before, within and after puberty period groups. Then we characterized TMSCs for their proliferation, multi-potency differentiation into adipocytes, osteocytes, chondrocytes and surface protein profile.

Results

Our data showed that TMSCs isolated from young donors grew faster than ones from old donors. Between male and female donors, TMSCs from male had higher proliferation than female. Within sub-classes both, epidermis and stroma, TMSCs from stroma showed the highest proliferation rate. Besides, the adipogenic potential of stroma-TMSCs was also higher than both or epidermis-TMSCs. Osteogenesis, chondrogenesis and surface protein profile of TMSCs will be investigated.

Conclusion

Our data suggested that donor age, gender and tissue niches can influence the quality and characteristics of TMSCs. Optimization these conditions might help giving rise to a more stable TMSC source for therapeutic applications in the future.

id #67805

Modelling the neurodegenerative disorder Huntington's disease by direct cellular reprogramming of adult human fibroblasts

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder characterised by the progressive loss of GABAergic medium spiny neurons (MSNs) in the striatum. The study of neurodegenerative disorders such as HD has been impaired by limited access to live human disease-affected neurons. Cellular reprogramming of patient-derived somatic cells now offers an opportunity to generate live human neurons for the study of neurological conditions. We have developed a highly efficient

protocol for direct reprogramming of adult human fibroblasts (HDFs) to induced neural precursor cells (iNPs) within 21 days by co-transfection of chemically-modified mRNA (cmRNA) encoding the pro-neural transcription factors *SOX2* and *PAX6* in a defined reprogramming medium. Directly reprogrammed iNPs express the neural transcription factors GSX2, ASCL1, DLX2, and MEIS2, required for the development of MSNs. We have optimised this protocol to generate high yields of DARPP32+ neurons within 30 – 45 days of differentiation using a combination of growth factors and small molecules in a BrainPhys™ medium under physiological oxygen (5% O_2) conditions. HDFs from patients with HD (n=4; CAG repeat lengths 41 – 57) and normal subjects (n = 4; CAG repeat lengths 18 – 34) were directly reprogrammed with cmRNA *SOX2* and *PAX6*. The morphology of HD-derived neuronal cultures was compared to normal neuronal cultures at 30 and 45 days of differentiation. HD-derived neuronal cultures demonstrated significantly reduced neurite branching, smaller cell bodies, and shorter neurites compared to normal cultures. HD-derived neuronal cultures also demonstrated the presence of huntingtin protein aggregates. These results demonstrate that HD-derived neurons exhibit a distinct alternation in neuronal morphology compared to normal neurons, providing a novel *in vitro* platform for studying the pathophysiology of HD.

id #67826

Profound impact of APOE4 in human iPSC-derived microglia-like cells compared to familial Alzheimer's disease mutations

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Alzheimer's disease (AD) is a neurodegenerative disease characterised by amyloid plaques and neurofibrillary tangles that progressively leads to loss of cognition. A small percentage of cases are described as familial or early-onset AD (EOAD) and are caused by mutations in genes, including amyloid precursor protein (*APP*) gene duplication or loss of function mutations in proteins that process APP, presenilin 1 or 2 (*PSEN1, PSEN2*). The vast majority of cases are late-onset (LOAD) and are affected by multiple genetic and environmental risk factors. A major genetic risk factor for LOAD is the APOE4 genotype but the precise role of APOE in AD pathogenesis remains hotly debated [1]. For decades models of AD have focused on EOAD gene mutations, even though the disease mechanisms for EOAD and LOAD are likely distinct. *APOE* is highly expressed in microglia and it is hypothesised may play a role in AD pathogenesis via altering microglial function. However it is unclear whether the EOAD mutations also affect microglial function. We developed a high-yield protocol for producing human microglia-like cells (iMGs) from induced pluripotent stem cells (iPSCs) that express microglial signature genes and functional responses. We generated iPSC lines from LOAD and EOAD patients, healthy individuals and isogenic controls to assess EOAD mutations and APOE genotype on microglial gene expression and function. We identified that the APOE4 genotype has a profound impact on several aspects of microglial functionality whereas EOAD mutations led to only minor alterations. Our data shows that EOAD mutations and the APOE4 phenotype confer disparate phenotypes in human iPSC-derived microglia and suggest that the role of microglia in disease pathogenesis may be distinct in EOAD versus LOAD.

id #67833

Paracrine effects of MSC-like cells from gingival tissue on wound healing

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Stem cell-based therapies have been shown to have considerable potential for improving wound healing. Accumulating evidence indicates that stem cells promote tissue repair by secreting paracrine signals that improve responses to injury by resident skin cells. Human gingival derived mesenchymal stem cell-like populations (GMSC) are post-natal stem cells that express surface markers associated with MSC. GMSC have also been shown to exhibit multi-differentiation capacity and immunomodulatory properties. This study aimed to examine the effects of human GMSC secretome on acute wound healing using *in vitro* and *in vivo* models.

The effect of GMSC secretome on the capacity of keratinocytes and fibroblasts to migrate and proliferate was determined *in vitro* using scratch wound closure and WST1 assay, respectively. Additionally, two excisional wounds were created on the dorsal surface of mice (n=8/group) and 100µL of 20X GMSC secretome were intradermally injected to the wound margins. The wounds were imaged daily until day 14, for macroscopic wound area determination. Wound tissues were collected at 3, 7, and 14 days post-wounding and were stained with H&E staining to microscopic analysis of wound. Immunohistochemistry staining of tissues was performed to investigate the inflammation and collagen deposition in wound area.

Cells treated with GMSC secretome showed improved rate of scratch closure and increased cellular proliferation compared to controls. Wounds intradermally injected with GMSC secretome showed a significant reduction in macroscopic wound area compared with controls. Microscopic analysis of wound tissues showed significant decrease in dermal wound width and increased rate of reepithelialisation. Collagen deposition was increased and a decrease in inflammatory cell infiltration was observed in HGF-CM treated wounds.

These findings demonstrate a therapeutic effect of GMSC secretome therapy on wound healing *in vitro* and *in vivo*. Clinical application of GMSC secretome may be a potential option for cell-free therapy to accelerate full-thickness skin wound healing.

id #67836

Towards retinal regeneration: Using CRISPR activation to promote direct reprogramming of retinal glial cells to photoreceptors

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The loss of photoreceptors is a key hallmark of many incurable blinding diseases and regenerative medicine has great potentials of alleviating blindness in patients. Previous studies showed the feasibility of using transcription factors to reprogram glial cells into retinal neurons both *in vitro* and *in vivo* in rodents (1,2), providing an innovative gene-therapy approach to stimulate retinal regeneration. However, it remained unclear whether this reprogramming approach can translate to human cells. This study aims to use CRISPR activation (CRISPRa) to reprogram human Müller glia into photoreceptors (induced photoreceptors, iPH) in vitro.

As part of the Human Cell Atlas initiative (humancellatlas.org), we recently generated a human retina transcriptome atlas at single cell levels and identified the transcriptome of the major retinal cell types (1). From this dataset, we performed network topology analysis to predict transcription factors that could reprogram Müller glia into photoreceptors. To test the shortlisted transcription factors, we developed a CRISPRa pipeline (4) which allowed us to activate up to 9 transcription factors simultaneously. Using this CRISPRa platform, we have screened and identified cocktails of transcription factors that allow reprogramming of human Müller glia into iPH *in vitro*. qPCR and immunocytochemical analysis demonstrated that iPH expressed a panel of photoreceptor markers, including RHO and PDE6B. Subsequently we performed single cell transcriptomic to profile iPH and compared with the human retina transcriptome atlas as a benchmark, we showed that iPH reprogramming promoted transcriptomic transitions from Müller glia to photoreceptors, resulting in activation of photoreceptor markers in iPH. Our study demonstrated the feasibility of using CRISPRa to promote cell reprogramming of Müller glia into photoreceptors, providing a potential cell source for tissue engineering and regenerative medicine. Future application for *in vivo* reprogramming provides an exciting innovative approach to regenerate photoreceptors and restore vision in retinal degenerative diseases.

1. Jorstad et al. (2017) Nature, 548, 103-107
2. Yao et al. (2018) Nature, 560, 484-488
3. Lukowski et al. (2019) EMBO Journal, e100811
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id #67843

Endovascular progenitors initiate and drive *de novo* vascularisation in melanoma

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The development of new vascular structures is a pre-requisite for melanoma growth and spread. We aimed to better identify and characterize the source of *de novo* endothelium in melanoma. Among Lin-CD34+ cells, expression levels of VEGFR2 and CD31 defined three distinct endothelial sub-populations. Lineage tracing of endothelial cells (*Cdh5CreER/RosaYFP*) demonstrated a maturation sequence from endovascular progenitor (EVP) via transit amplifying (TA) to fully differentiated (D) cells in B16 and HCmel12 melanoma. In lineage tracing experiments utilising *Sox18CreER/Rosa-YFP* and *Cdh5CreER/Rosa-YFP* reporter mice, EVPs activated *Sox18* expression as early as 3 days after tumour inoculation and formed mature vascular networks. EVP cells also were the only endothelial sub-population with self-renewal and engraftment capacity. Clonal analyses of multicolour lineage tracing (*Cdh5CreER/Rainbow3*) further showed different progenitors contributing to venous and arterial structures within tumours. RNA-seq demonstrated significant differences between populations and pointed to *Sox9* and *IL-6*/*JAK/STAT* signalling to be significantly upregulated in the EVP. We next sought to specifically target EVP activity. Anti-IL6Rα and Ruxolitinib blocked JAK/STAT signalling, significantly reducing EVP infiltrate into the tumour. Importantly, this caused a significant reduction of the vascular network and a reduction in tumour size. To validate the activity of Sox9 signalling within EVP, we used Sox9 conditional knockout model *Sox9$_{lox/lox}$/Cdh5CreER/Rosa-YFP*. Deletion of Sox9 signalling significantly reduced EVP infiltrate and subsequent vessel formation in tumours. Importantly, a significant reduction in size and weight of the tumour was also observed. Notably, human Endothelial Colony Forming Cells (ECFCs) upregulate Sox9 and IL-6 signalling in the presence of human melanoma co-culture and these pathways are negatively regulated after Ruxolitinib treatment. This is the first description of Sox9 in the endothelium and its subsequent regulation by IL-6/JAK-STAT signalling.

id #67878

Could exon skipping become a treatment for recessive Ullrich Congenital Muscular Dystrophy

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COL6 related congenital muscular dystrophy (CMD) is caused by mutations in either *COL6A1*, *COL6A2* or *COL6A3* genes that disrupt the normal assembly of the COL6A1, COL6A2, and COL6A3 chains into heterotrimer fibrils essential for integration into the extracellular matrix. Autosomal recessive Ullrich CMD patients carrying mutations that lead to premature termination of translation present with severe phenotypes due to extremely low levels or lack of COL6. This is a proof-of-concept study to ascertain if an exon skipping therapy we have developed for the treatment of Duchenne muscular dystrophy could be applied to bypass mutated exons and rescue defective COL6 production in Ullrich CMD patients. We explored whether: (i) small exons in *COL6* transcripts can be efficiently and specifically excised; (ii) the equivalent exons in *COL6A1*, *COL6A2,* and *COL6A3* gene transcripts can be simultaneously excised; and (iii) these modified COL6A isoforms can assemble into functional trimer fibrils.

We analyzed COL6 protein assembly and designed antisense oligonucleotides to induce skipping of the corresponding exons of *COL6A1*, *COL6A2,* and *COL6A3* transcripts, which encode part of the triple helix structure of COL6. The initial screen for the best exon skipping sequences was performed using oligomers synthesized as 2'-*O*-methyl modified bases on a phosphorothioate backbone, and the top performing sequences are then synthesized as clinically relevant, phosphorodiamidate morpholinos oligomer. Efficient exon 23 skipping of the *COL6A2* transcript in healthy fibroblasts disrupted COL6 assembly as expected, indicating that the exon skipping we observed at the RNA level was translated into protein. We are currently screening for antisense oligomers which induce exon skipping in similar size exons in *COL6A1* and *COL6A3* transcripts to rescue a proper assembly of COL6 in the cells treated with *COL6A2* exon 23 skipping oligomer.

id #67885

DISTINCT EARLY EMBRYONIC PREIMPLANTATION DEVELOPMENTAL PROGRAMS DRIVE REPROGRAMMING INTO PRIMED AND NAIVE INDUCED PLURIPOTENCY

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Reprogramming of somatic cells in defined conditions can give rise to primed and naive human induced pluripotent stem cells (hiPSCs) that recapitulate pre-implantation and post-implantation epiblasts respectively. However, the molecular events underpinning these processes are largely unexplored, impeding further rational optimisation of the reprogramming protocols. In this study, we reconstruct high-resolution molecular roadmaps of primed and naive human reprogramming at the single-cell level, and show distinct and independent cell fate transitions along each of the reprogramming trajectories. This revealed that reprogramming into primed and naive human pluripotency initially follows a shared trajectory before bifurcating into the two distinct pluripotent states, with neither states requiring a transition through the other. By extracting cell surface marker profiles of intermediate populations during the cellular transitions, we isolated and profiled reprogramming intermediates under several other naive as well as extended pluripotent conditions throughout reprogramming. We find each of them follow either of the bifurcated trajectories. Furthermore, using the same isolation strategy, we profiled genome-wide chromatin accessibility of reprogramming intermediates uncovering both individual intronic regulatory elements in core pluripotency markers, as-well-as a global association of increased chromatin accessibility with trophectoderm (TE) and epiblast (EPI) lineage-related transcription factors during the divergence into naive human pluripotency. Taken together, our comprehensive analyses of human primed and naive reprogramming reveal a remarkable and unexpected role of the TE-lineage associated regulatory program play during this process, providing novel insights to study early human lineage specification.

id #67888

Targeting adeno-associated viral AAV vectors for systemic delivery to the skeleton

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Adeno associated viruses (AAVs) can selectively transduce different cell types although their potential for specific bone targeting remains underdeveloped. Screening AAV variants in a murine fracture model, we found that AAV8 and AAV-DJ can transduce bone cells at high levels. We generated cre recombinase expressing constructs under the control of CAG constitutive promoter or bone cell specific promoters Col2.3 or Sp7, packaged into AAV variants AAV2, AAV8, and AAV-DJ. These AAVs were systemically administered to Ai9 mice via tail vein delivery at a dose of $5\times10_{10}$ and later $5\times10_{11}$ vg/mouse. AAV8-CAG-cre was able to effectively lead to high levels of tdTomato reporter expression in osteoblasts following a single tail vein injection. Bone cell-specific promoters Col2.3 and Sp7 were able to effectively restrict cre recombinase expression to bone cells, with Sp7 proving to be more specific with very little expression in non-osseous tissues (including brain, heart, lungs, liver, spleen, and kidney). AAV8-Sp7-cre showed the highest efficiency and specificity for targeting bone cells of the skeleton following systemic delivery in mice. To conclude, this viral construct has scientific utility for creating bone-specific postnatal murine knockouts, and can be combined with CRISPR/Cas9 gene editing for functional genomics and gene therapy applications.

id #67894

Establishing the ethical and regulatory frame work for a clinically compliant iPSC cell line bank; from QA to donor re-consent.

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A global network of iPSC banks would create a strong future basis for new cellular therapies, providing GMP quality clinically complaint cell lines to facilitate research studies through to clinical trials. Setting up these banks requires clear and transparent re-consent of donors along with a strong QA and QC framework. We aim to provide a clear ethical pathway towards the creation of such a resource, along with a dynamic follow up and re-consent process of donors from the public BMDI Cord Blood Bank.

Review of the literature and a round-table workshop with Ethics and Legal experts have identified the key issues to be resolved in order to obtain consent to make iPSC lines from CB donors. Regulatory challenges that would affect global acceptance of any cell lines, along with the Quality framework and the standards required to operate as part of a global network, are being met by working in collaboration with bodies such as the Global Alliance for iPSC Therapies (GAiT) and participating in an iPSC Quality Assessment Round. CB donor letters and information sheets have been developed and Ethics approvals by an Institutional HREC, and government approval obtained. New issues of whole genome sequencing and the relevant donor safeguards and protections have been considered with input from clinical genetics services, including the rights and information flow to donors about what happens to the cells after banking and commercialization aspects. Freedom to operate and GMP manufacture and oversight is currently under investigation. This work is continuing and the ongoing success of many of these processes has confirmed feasibility and utility of using banked CB to produce clinical–grade iPSC lines for potential cellular therapies. Providing a standardized and characterized resource could lead to eventual new cell therapies that will be available to Australian and international communities.

id #67897

Proteases mediate mesenchymal stem cell homing to tumours and inhibition of metastasis

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Bone marrow-derived mesenchymal stem cells (BM-MSCs) home to tumour sites and form part of the tumour stroma. While many studies show that that BM-MSCs are supportive of continuing tumour development, contradictory findings have also been published. Therefore, although these cells are a major influence on the behaviour of the tumour, their impact on the tumour is not well defined.

In vitro, BM-MSC migration and invasion was increased in the presence of breast and colon cancer cells and aspartic acid protease cathepsin D is also part of the chemoattractive mechanism. The enzyme and its precursors are produced by the cancer cells and through an enzymatic mechanism, upregulate BM-MSC movement, but not proliferation or adhesion.

In our *in vitro* system, media conditioned by BM-MSCs reduced migration and invasion of breast cancer cells. Tissue inhibitor of metalloproteinases-1 and -2 (TIMP-1 and TIMP-2) accumulated around 50-fold over the conditioning period and blocking both TIMP-1 and -2 activity increased breast cancer cell movement back to untreated values. This suggests that, over time, BM-MSCs may have an overall anti-metastatic effect.

In conclusion, proteases and their inhibitors influence both the migration of BM-MSCs towards the tumour microenvironment and the overall effects of BM-MSCs on tumour behaviour.

id #67901

Combinations of Promoters and Virus Capsids May Affect Gene Transduction Efficiency in Adeno-associated Virus

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[Purpose] Adeno-associated virus (AAV) vector has been widely used to transduce gene of interest for its safety and efficacy. Although the most of the combinations of promoters and viral capsids are appropriate for focal gene transduction, much is unknown about the mechanism of infection. Here, we compare the gene expression levels using some combinations of promoters (cytomegalovirus (CMV), CMV immediate-early enhancer (CAG), and Synapsin 1 (Syn1)) and viral capsids (AAV5 and AAVrh10). [Method] After six combinations of AAV vectorsuch as AAV5-CMV, AAV5-CAG, AAV5-Syn1, AAVrh10-CMV, AAVrh10-CAG, and AAVrh10-Syn1 that expressed green fluorescent protein (GFP) were made, $1\times10_{10}$ vg of each vector was stereotaxically administered into the right hippocampus of 4-week-old male gerbils (n = 3, each). Three weeks after injection, animals were killed for histological analysis. The GFP expressing area of three coronal sections at 1.7, 2.0, and 2.3 mm caudal to the bregma was quantified. [Results] First, AAVrh10-CMV and AAVrh10-CAG showed the widest expression of GFP (AAVrh10-CMV: 7.1 ± 1.1 mm_2, AAVrh10-CAG: 6.0 ± 0.5 mm_2, $p < 0.001$, for both, compared with control). The second widest expression of GFP was found in the AAVrh10-Syn1 and AAV5-CMV groups (AAVrh10-Syn1: 4.2 ± 0.2 mm_2, AAV5-CMV: 4.2 ± 0.2 mm_2, $p < 0.05$, for both, compared with the control). The least expression was found in the AAV-Syn1 group (AAV5-Syn1: 0.2 ± 0.2 mm_2, not significant, compared with the control). [Conclusion] Although the combinations examined in this study is limited, we should note that some of the combinations of promoters and viral capsids may lead to the failure of gene transduction.

id #67903

Using isogenic stem cells to model disease mechanisms of Juvenile Batten disease

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Juvenile neuronal ceroid lipofuscinoses (JNCL, also known as Batten disease) is a lysosomal storage disorder associated with fatal neurodegeneration. There is a need for more physiologically relevant human cell-based models to better understand the cellular changes during the disease process and as platforms for drug screening. We used CRISPR/Cas9 and H9 human embryonic stem (ES) cells to reproduce the most common cause of JNCL, a homozygous 1-kb deletion encompassing exons 7 and 8 of the CLN3 gene ($CLN3^{\Delta ex7/8/\Delta ex7/8}$), with unmodified H9 ES cell line as an isogenic control cell line. We characterised the isogenic $CLN3^{\Delta ex7/8/\Delta ex7/8}$ cell lines, as well as neurons and cerebral organoids derived from them. Confocal microscopy analysis of the $CLN3^{\Delta ex7/8/\Delta ex7/8}$ ES cell line showed increased LAMP1 immunoreactivity relative to control cultures, indicating lysosomal enlargement and suggesting waste accumulation. Electron microscopy analysis of $CLN3^{\Delta ex7/8/\Delta ex7/8}$ organoids at 5-months showed electron dense inclusions characteristics of lipofuscin and increased number of altered mitochondria, compared to controls. Immunohistochemistry of these organoids also revealed higher expression of astrocytes with longer and thicker processes, indicative of reactive astrocytes. These data provide insights on the development of JNCL phenotypes at the pre-symptomatic stage, and identified cellular phenotypes that may be suitable for drug screening.

id #67920

Modelling novel retinal gene variants in patient-derived iPSC-RPE and retinal organoids

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Inherited retinal dystrophies cause degeneration of the retina and progression to severe visual impairment. For patients to benefit from new therapeutic genetic approaches, their genetic variant/s need to be classified as pathogenic or likely pathogenic. Variants in *RPGR* are one of the most frequent causes of X-linked retinal dystrophy. We identified a retinal dystrophy family with a novel intronic variant in *RPGR*, which was classified as a variant of uncertain significance (VOUS). A unique *RPGR* isoform is exclusively expressed in the retina, raising the need for development of a model system derived from human cell types other than retina, since access to retinal tissue in humans is not easily available premortem. Fibroblasts from a patient with the novel *RPGR* variant were reprogrammed into iPSCs, and two clonal lines were differentiated into retinal pigment epithelial (RPE) cells and retinal organoids for disease modelling. qRT-PCR and immunostaining confirmed aberrant splicing, and decreased gene and protein expression in RPE cells, with mislocalisation along the transitional zone of the primary cilium. This allows reclassification of the variant to likely pathogenic, creating clinical utility. This work emphasises the value of iPSC-derived RPE as a viable model for pathogenicity and therapy studies of retinal ciliopathy genes.

id #67923

Using induced pluripotent stem cells to model primary open-angle glaucoma (POAG)

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Primary open-angle glaucoma (POAG) is an optic neuropathy characterized by gradual degeneration of retinal ganglion cells (RGCs) leading to irreversible vision loss and, if untreated, blindness. Upon diagnosis treatment options are limited and often fail to halt the progression of vision loss. This is due to the incomplete understanding of the disease. The molecular profiling of human RGCs in normal and diseased tissue is hindered by the lack of non-invasive means for obtaining RGCs from living donors. This can now be circumvented by the use of induced pluripotent stem cells (iPSCs) as a source of RGCs. With the use of an automated platform, we generated 305 human iPSC lines from glaucoma patients (n = 143) and healthy controls (n = 162). All lines underwent quality control analysis consisting of virtual karyotyping and assessment of pluripotency markers OCT-4 and TRA-1-60.Subsequently, 183 lines were differentiated into RGCs via retinal organoids and then subjected to single cell RNA sequencing (scRNA-seq) to gain in-depth information about transcriptomic differences between healthy controls and glaucoma patient samples. Understanding mechanisms underlying RGC function, maintenance of homeostasis and those conferring susceptibility to POAG is crucial to discover new therapeutic targets and commence the process of drug discovery.

id #67926

Biological variation of stem/progenitor cells in menstrual blood from healthy women

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Cyclical shedding/regeneration of the endometrium is likely controlled by endometrial stem/progenitor cells and may be dysregulated in debilitating menstrual disorders (endometriosis). We aimed to determine biological variations in endometrial stem/progenitor cell concentrations and clonogenicity in menstrual fluid between cycles and between women.

Menstrual fluid (day 2) was collected from women (n=11; 30.0±2.4yrs; 22-42) in a menstrual cup over 3 cycles (cycle 27.5±0.5d). Endometrial cells were dissociated, leukocytes depleted and red blood cells lysed. Stem/progenitor cell proportions were determined by flow cytometry and clonogenicity by colony forming assays.

SUSD2+ endometrial mesenchymal stem cells (0.13–7.2%; 2.17±0.95%) and EpCAM+ (0.77–41.0%; 27.0±4.52%) cells were present in all menstrual fluids. However, epithelial progenitor cells (ePC) were not detected in every sample (NCAD+EpCAM+; 13/17 samples; 0-0.61%; 0.29±0.06%, SSEA-1+ EpCAM+; 11/17 samples; 0-0.4%; 0.12±0.05%), with no differences within and between women. Similarly, there were no differences in clonogenicity of CD45- endometrial cells (0.34-1.87%; 0.99±0.21%). Data are %CD45- cells; range; mean±SEM.

There was little variation between stem/progenitor cell shedding between cycles within the same woman or between women. However, occasional outliers may indicate normal variation or underlying pathology. Quantification of stem/progenitor cells in menstrual blood may be a non-invasive prognostic tool for fertility and endometrial disorders.

id #67931

Composite mesh design for delivering autologous MSC influences mesh integration and biocompatibility in an ovine model of pelvic organ prolapse

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The banning of synthetic transvaginal mesh for treating Pelvic Organ Prolapse (POP) in Australia, UK, USA and NZ has left millions of women with few effective treatment options. We are developing a new mesh to deliver endometrial MSC (eMSC) to improve mesh biocompatibility and restore strength to prolapsed vaginal tissue. Here we evaluated our knitted polyamide (PA) mesh in a novel ovine multiparous, transvaginal implantation model, matching ewes for the degree of POP. Gelatin-coated PA mesh stabilised with 0.5% glutaraldehyde (PA/G) used either alone or seeded with autologous ovine eMSC (eMSC/PA/G) resulted in poor tissue integration and 42% mesh exposure in this model. In contrast, a two-step insertion protocol, whereby uncoated PA mesh was inserted transvaginally followed by application of autologous eMSC in a gelatin hydrogel onto the mesh and crosslinked with blue light in situ (PA+eMSC/G), integrated with no mesh exposure. The autologous ovine eMSC persisted 30 days *in vivo* influencing the host inflammatory, extracellular matrix remodelling, neovascularisation, fibrotic and biomechanical responses, but not mesh integration, indicating the value of an autologous approach. The stiff PA/G constructs provoked greater myofibroblast and inflammatory responses in the vaginal wall, disrupted the muscularis layer and reduced elastin fibre content compared to PA+eMSC/G constructs. This study provides new biomaterials for POP research and an improved animal model, but also highlights the complexity of the problem of POP therapy. However, it identified the superiority of a two-step protocol for implanting synthetic mesh in cellular compatible composite constructs and simpler surgical application, providing additional translational value.

id #67961

Human and mouse retinal models, and investigation of adeno-associated virus delivery

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Introduction:

Heritable diseases of the retina are one of the primary causes of vision loss, with very few treatment options currently. Our gene-editing and replacement constructs require testing for efficiency and specificity of delivery for investigation of their therapeutic effects. We are assessing the delivery efficacy of adeno-associated viral (AAV) vector serotypes in our *in vivo* and *in vitro* model retinal systems.

Methods:

Wild-type ARC mice were subjected to both intravitreal and subretinal injections of AAV2 and AAV-7m8, with transduction monitored via fundoscopy, optical coherence tomography and immunofluorescence for 1 month. Retinal pigment epithelial (RPE)

cells were also differentiated from human induced pluripotent stem cells (iPSCs) and transduced with AAV1-13. An interphase culture method was used to maintain human retinal explants *ex vivo*, which were exposed to AAV4 and AAV5.

Results and Discussion:

The mouse model system was used to assess whether the AAVs exhibited topographical transduction of the different retinal cell types. The iPSC-derived RPE cells were used to assess the transducibility of retinal cells *in vitro*, for application of this knowledge to retinal explants. Our combined approach allows selection of the most suitable AAVs with tropism to cells of interest for specific genetic retinal diseases.

id #67964

Reprogramming Cardiomyocytes into Pacemaker Cells Using Gene Therapy

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Introduction

Electronic pacemakers are currently the only viable option for the treatment of pacemaker conduction issues. They are however limited due to lack of biological control, high cost of revision surgeries and risk of lead and device related complications. We therefore aimed to develop a biological alternative to electronic devices using gene therapy to demonstrate reprogramming of cardiomyocytes into pacemaker cells. This was achieved by overexpression of the transcription factor *hTBX18* using recombinant adeno-associated viral vectors.

Methods

Neonatal rat ventricular cardiomyocytes were isolated and transduced with recombinant adeno-associated virus vector 6 encoding either h*TBX18* or *GFP* and maintained for 3 weeks. At the endpoint, mRNA and protein were collected for qPCR and western blot analysis. Immunocytochemistry was used to visualize changes in markers of pacemaker cells. Changes in cell morphology were imaged via microscopy. Voltage sensing vectors (Arclight) were used to characterise functional changes of the cells.

Results

Analysis of cardiomyocytes transduced with *hTBX18* showed faithful recapitulation of the distinctive characteristics of pacemaker cells.

Comparison of *hTBX18* treatment and *GFP* control using qPCR, western blot and immunocytochemistry showed changes in markers specific to pacemaker cells: *hTBX18 and HCN4* were significantly upregulated. *Cx45* was upregulated, though not statistically significant. *Cx43* was significantly down regulated and Sarcomeric α-actinin remained unchanged.

Cardiomyocytes transduced with *hTBX18* acquired the distinctive tapered morphology of pacemaker cells, as compared to the block like, striated appearance of cardiomyocytes.

Analysis of the action potentials generated via Arclight showed phase 4 depolarisation and decrease in the APD50 of the *hTBX18* transduced cells, highlighting a functional change.

Discussion

We have successfully demonstrated that h*TBX18* gene transfer to ventricular cardiomyocytes results in morphological, molecular, physiological and functional changes, recapitulating the native pacemaker phenotype. The generation of these reprogrammed pacemaker cells opens up new prospects for biological pacemakers in a clinically translatable setting.

id #67965

Illuminating Stem Cell Differentiation Efficacy Through Single-cell Guided Transcriptional Profiling

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In vitro stem cell based models of human development and disease can be illuminating only in so much as they resemble their in vivo counterparts. As such, considerable effort is taken to establish differentiation protocols that effectively derive only the cell types of interest. In particular, consideration must be given to unwanted lineages with related developmental ontologies that often emerge as sources of contamination. At end point, differentiation protocols are typically assessed through histochemical analysis of marker gene expression. But this approach limits the scope of markers that can be tested, and requires prior knowledge of the desired and contaminating cell types. We have analyzed single-cell RNA-sequencing data from 75 thousand gastrulating mouse embryos between E6.5 and E8.5, identifying transcriptional trajectories specific to each major lineage emerging from the epiblast. We then process bulk-RNA sequencing from an array of human stem cell differentiation protocols, perform similarity comparisons against the full cohort of lineage-defining gene signatures, and infer the presence of target or contaminating lineages. Using this strategy, we are able to quickly and sensitively assess the fidelity of a given stem cell protocol, transcriptome-wide and unguided, with no requirement for prior knowledge.

id #67971

The Impact of Science Communication on Public Attitude towards Human Genome Editing.

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Allowing scientists to research human genome editing technology is an issue that needs to involve both public stakeholders and policymakers. In this study we engaged the Australian public to express their opinions on this issue by delivering a series of 2 h educational workshops to members of University of the Third Age and Probus Australia; additionally at a Q & A forum during Sydney Science Festival. A questionnaire was distributed before and after the event. Of the 209 attendees, 75% completed the questionnaire. Majority of respondents were female (77%), aged over 60 yrs (66%), but a quarter were under 30 yrs (22%). Most (75%) identified with religion but half (51%) of these did not regularly attend a religious service. A quarter (23%) completed their education in high school but most (48%) had attended university. The majority (63%) were born in Australia. A quarter worked in medical or scientific areas, and a further 17% in corporate or professional jobs; 16% were retired. Majority (56%) had limited knowledge of gene editing before the educational event, but this changed after it (12%). 37% agreed with genome editing to allow heritable changes for health purposes before, increasing to 59% afterward. Those identifying with religion and additionally those with less formal education were less likely to change their views. Before the event 34% of respondents agreed with genome editing of human embryos for research, increasing to 63% afterward. Agreement with genome editing to treat disease as a part of a clinical trial increased from 57% before to 72% afterwards. Majority (84%) of all respondents disagreed with genome editing for non-health purposes before, with no change after. However, men were more likely to change their views. Findings from this study will assist in future science communication plans and policy development on human genome editing.

id #67975

An AGM-on-a-chip model for study definitive haematopoiesis and vascular development from hPSCs.

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This study aims to understand the hemodynamic control of human embryonic stem cell (hESC)-derived haematopoiesis and vasculogenesis by applying microfluidics, live cell imaging and single cell RNA sequencing. AGM-like haematopoietic cultures developed by Ng et al. were generated by manipulating WNT and TGFß signalling during mesoderm specification. The effect of pulsatile circulatory flow was studied in a microfluidic dynamic culture system, using SOX17 (Cherry)/RUNX1c(GFP) hESC line to read out arterial endothelial and haematopoietic differentiation, respectively. Microdevices generated cardiac-like, pulsatile flow in a circulatory culture system with a volume of 2–3µL. AGM-like haematopoietic development was observed by time-lapse imaging on chip for more than two weeks. We observed cells entering the circulation from the adherent layer, and the release of lightly tethered SOX17+ cells into the circulation. In parallel bulk differentiation culture using an orbital mixer to mimic the effect of wall shear stress, single cell RNA seq was performed on 8800 single cells at day 18 of culture. Control treatments were static culture and drug vehicle. Hierarchical cluster analysis identified 18 clusters belonging to erythroid-megakaryocytic, cardiovascular and myeloid differentiation pathways. We identified HOXA expressing mesenchymal cells in AGM cultures. Shear treatment promoted proliferative MYB, RUNX1, CD31 expressing blood progenitors, a reduced proportion of unipotent erythroid and megakaryocytic lineages, and increase numbers of myeloid and bipotent megakaryocyte-erythroid progenitors. The production of endocardium and cardiomyocytes was promoted by shear in control culture. This study demonstrates the feasibility of modelling human embryonic blood formation using microfluidic technology. The single cell transcriptome data provide an entry point for deeper analysis of embryonic haematopoiesis, haematopoietic and cardiac lineage development, as well as the effects of shear stress on them.

id #67977

Quantitative assessment of a human retinal explant model using adeno-associated virus

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Scientific investigations utilising human explants obtained from eye cups are uncommon due to methodological difficulties, inconsistent tissue characterisation and technical challenges in experimentation. In this study it was hypothesised that human explants containing intact retinal-pigment epithelium (RPE), choroid and sclera (RCS explants) could be used as a suitable model for AAV mediated gene therapy studies. RCS explants were dissected from donor tissue provided by the Queensland Eye Bank and transduced overnight using AAV2, AAV5, AAV6 or AAV8 with 1-3 $\times 10_{10}$ vector copy genomes expressing secreted luciferase. RCS explants were efficiently transduced using AAV2, AAV5, AAV6 and AAV8 vectors. AAV5 and AAV6 were significantly more efficient in transducing RCS explant superstructure than AAV2 and AAV8 ($P<0.05$). When a 1:2 dilution of vitreous humour was added, AAV2 and AAV6 vector transduction was significantly inhibited ($P<0.05$), however, both AAV5 and AAV6 transduction remained significantly more efficient than AAV2 and AAV8 in these conditions ($P<0.05$). RCS explants could be kept in culture

for a minimum of 21 days without significant change in luciferase expression. Transduction efficiencies from the intact explant were different to those obtained using isolated primary mixed RPE/choroidal culture from the same source, in which AAV2 was the most efficient vector in transducing cells obtained from RCS explants, suggesting cell-cell interactions and barriers are important in AAV transduction of the outer eye structures. These findings suggest human RCS explants are a robust, useful and highly externally valid model for investigating gene therapy of the RPE and choroid.

id #67980

Modulation of CNOT3 expression to treat retinitis pigmentosa 11 using antisense oligomers

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Retinitis pigmentosa 11 (RP11) is an inherited retinal dystrophy caused by heterozygous mutations in pre-mRNA processing factor 31 (*PRPF31*). CCR4-NOT transcription complex subunit 3 (CNOT3), a transcriptional inhibitor of *PRPF31*, is found at higher levels in RP11 patients compared to asymptomatic family members carrying the same mutations. In this study, we aim to upregulate functional PRPF31 to enhance pre-mRNA processing and rescue disease phenotypes by lowering CNOT3 function. We found 10% higher expression of *CNOT3* with 17% lower *PRPF31* in a patient compared to an asymptomatic relative, both carrying heterozygous *PRPF31* c.1205 C>A. Antisense oligomers were designed to target removal of *CNOT3* exon(s) encoding essential functional domains, resulting in truncated CNOT3 isoform(s). Results exhibited 2-fold increase in PRPF31 expression in antisense oligomer treated RP11 iPSC-derived retinal pigment epithelium. Consequently, treated cells showed a significant increase in cilia incidence and length, factors crucial for retinal function. Poly(A) RT-PCR also revealed increase in *PRPF31* containing poly(A)-tail in CNOT3 depleted cells. In conclusion, this study shows antisense oligomers can lower CNOT3 function and increase *PRPF31* transcription and mRNA stability to levels expected to provide therapeutic benefit. Transcriptome analysis will be used to evaluate pre-mRNA splicing after increased PRPF31 expression.

id #67982

The use of direct cell reprogramming to identify novel targets to treat Huntington's disease.

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Huntington's disease (HD) is an autosomal dominant genetic neurodegenerative disease for which there is no treatment. We have developed a unique chemically modified mRNA-based protocol to directly reprogram adult human dermal fibroblasts (aHDFs) into induced neural precursor cells and differentiate them into different neuronal subtypes. Using this technology, small molecules were combined with overexpression of the pro-neural factors *SOX2* and *PAX6* to generate human induced lateral ganglionic eminence precursors from normal and HD patient-derived aHDFs. Subsequent differentiation gave rise to high yields of DARPP32+ medium spiny neurons (MSNs). BDNF mRNA and protein expression was reduced in HD patient-derived MSNs, while expression of the BDNF receptor TRKB was maintained. This indicates impaired transcriptional regulation of BDNF in human HD MSNs. Repression element silencing factor-1 (REST) associates with the *Huntingtin* gene (*Htt).* In the presence of mutant *Htt,* REST undergoes translocation to the nucleus, repressing BDNF. Using our HD human cell model, we investigated whether lithium can be re-directed for the treatment of HD by activating Wnt and P120-catenin signalling preventing REST repression of BDNF. Autophagy is altered in HD. Using natural compounds, we targeted autophogosomes and lysosomes in our HD human cell model to better understand their dysfunction in HD.

id #67984

Generating sperm from XX individuals

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Females have two X chromosomes, while males have one X and one Y chromosome. The mismatch between sex-chromosome makeup and phenotypic sex leads to infertility. *In vivo* studies showed that sperm formation in mice is prohibited by presence of two X chromosomes, and requires two genes from the Y chromosome: *Sry*, which directs gonadal somatic cells to form a testis, and *Eif2s3y*, which functions in germ cells to promote spermatogonial development into haploid round spermatids. Sex reversed men with XX male (de la Chapelle) syndrome do not produce sperm because they have two X chromosomes and no Y chromosome. Using fibroblasts established from an XX male mouse model, we generated *Eif2s3y*-expressing induced pluripotent stem cells (iPSCs) that have lost one X chromosome. *In vitro* germline differentiation and subsequent testis transplantation of

them would potentially allow us to create round spermatids, the reproductive potential of which will be tested by oocyte injection. This research will have relevance to overcoming infertility of XX males.

id #67989

Identifying AAV integration sites in next-generation sequencing data

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Verifying the the safety of any viral vector is an important precursor to clinical applications of gene therapy. Recent evidence links integrations of AAV genomes within or near oncogenes within the liver to hepatocellular carcinoma[1], with a potential cause being a liver-specific promoter-enhancer element in the 3' UTR of the wild-type AAV2 genome[2]. Indeed, viruses are estimated to cause 10-15% of all human cancer cases with well-characterised examples including the association between human papillomavirus (HPV) and cervical cancers, as well as hepatitis B virus (HBV) and cancers of the liver (hepatocellular carcinoma, HCC)[3]. We sought to explore the pattern of viral integration in cells treated with AAV vectors. Furthermore, with a large amount of genomic data available from consortia such as The Cancer Genome Atlas (TCGA)[2] and the International Cancer Genome Consortium (ICGC)[3], we investigated whether viruses without previously established roles in cancer might be associated with particular cancer types. We therefore aimed to investigate insertional mutagenesis in next-generation sequencing (NGS) data from AAV-treated cells and publicly available data from cancer tissue and matched healthy control tissue. To analyse these data, we developed a pipeline for the identification of viral integration sites. In this approach, we aligned reads first to the human and viral genomes, and then identified potential chimeric reads as evidence for integration. We then used this pipeline to search publicly available cancer sequence data for evidence of viral insertional mutagenesis. Preliminary results identify a large number of viral integrations in vector-treated cells, and confirm the role of known cancer-associated viruses in particular cancer types. Our findings may have implications for the safety of gene therapy vectors, and for prevention of cancers caused by novel cancer-associated viruses.

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id #67990

Assessing the role of Apolipoproteins E using isogenic human induced pluripotent stem cell-derived cerebral organoids.

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Late-onset Alzheimer´s Disease (AD) is the most common type of dementia and it appears to be caused by defective clearance of amyloid β in the brain. It is known that polymorphisms in the *APOE*gene are the major genetic risk factor. Carriers of two *APOE4*alleles (*APOE4/4*) have 15 times higher risk to develop AD than homozygous carriers of the most common allele *APOE3* (*APOE3/3*). Here, we generated isogenic induced pluripotent stem cell (iPSC) lines with different *APOE*genotypes (*APOE4/4*and *APOE3/3)* using CRISPR/Cas9 editing. Following characterisation (pluripotency, genotyping and karyotyping), cells were differentiated into cerebral organoids for assessment of amyloid secretion and phosphorylation of Tau. After 6 months in culture,the pathological forms of amyloid β 1-42 and 1-40 were detected in organoid lysates and in secreted media with no statistical difference between organoids of different APOE genotypes. However, levels of amyloid β 1-42 were slightly higher in APOE4/4 than APOE3/3 in lysates (2.71±0.68 vs 1.70±0.78 pmol/L, n=5-7) and secreted media (0.86±0.19 vs 0.39±0.11 pmol/L, n=7). Total Tau protein was also detected in organoid lysates and secreted media with no statistical difference between group. Levels of phosphorylated Tau at different amino acid sites were detected in organoid lysates but not in secreted media. Our data thus provides a quantitative snapshot of levels of amyloid and Tau in isogenic APOE cerebral organoids.

id #67993

Gene-editing in human pluripotent stem cells for disease modelling and testing therapies in the retinal dystrophies

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Genetic retinal dystrophies cause progressive degeneration or abnormality of photoreceptors leading to vision loss. Current genomic studies identify disease-causing variants in approximately 65% of cases, and we wish to extend the yield of this testing through analysis of gene-edited iPSCs differentiated to retinal tissues and organoids, which also provide a platform to investigate gene-based therapies. To determine the functional impact of several retinal disease variants, CRISPR/Cas9 guides were designed along with variant-specific oligonucleotides to mediate homology directed repair (HDR) in normal iPSCs, to generate isogenic lines of the novel variants under investigation. Using this approach, for a novel variant of the *RPGRIP1* ciliopathy gene, we generated gene edited isogenic clonal iPSC lines with the variant correctly knocked-in to both alleles. We also identified a homozygous *RPGRIP1* knockout clonal line. Retinal differentiation cultures are underway to assess changes in ciliation and function, including correct trafficking of key proteins in the photoreceptors. For gene replacement, we have modified an AAV2 vector to include an eGFP reporter and a photoreceptor-specific promoter for transgene expression in retinal organoids. Overall, we have generated isogenic iPSC lines for retinal disease modelling and gene therapy testing and will utilise this approach to examine other variants and response to therapies.

id #67994

Generation of mature intestinal stem cells from pluripotent stem cells using transcription factor guided differentiation

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Intestinal organoids and the intestinal stem cells (ISCs) that give rise to them are promising tools for disease modelling and future use in autologous stem cell therapy. However, for organoid technology to reach its full potential, there is a need to generate bona fide, mature ISCs from alternative cell sources like induced pluripotent stem cells (iPSCs) and pluripotent stem cells (PSCs) from cell banks with their various genetic backgrounds and modifications. To facilitate this, we use a predictive algorithm based on RNA sequencing data (RNAseq) and supported by chromatin state data (ATACseq), to identify key transcription factors (TFs) to direct PSC differentiation towards a desired cell state. Employing this approach, we uncovered that overexpression of *Hnf4a*, *Vdr* and *Onecut2* can guide mouse embryonic stem cells differentiation towards the budding intestinal organoid state; enabling, for the first time, the generation of self-renewing ISCs from mouse PSCs. This demonstrates the feasibility of using TFs guided differentiation to generate ISCs and mature organoids from PSCs *in vitro*. In the human system (unlike in the mouse system), published, cytokine-driven PSC differentiation protocols exist that allow organoid differentiation, albeit with immature fetal/embryonic-like features, which impedes their potential for disease modelling. Reflective of this, RNAseq and ATACseq of human primary ISCs, PSCs and PSC-derived ISCs revealed that *in vitro*-derived ISCs shared some features (associated with cell cycle, metabolism and developmental state) with both PSCs and primary ISCs. Moving forward, we are testing the use of candidate TFs to shift PSC-derived human ISCs towards a fully mature state.

id #67996

Development of a splice-switching molecular therapy for SOD1 motor neurone disease

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Amyotrophic lateral sclerosis (ALS) is the most common motor neurone disease, a severe neurodegenerative disease resulting in death within 2-5 years from diagnosis. Current therapies are non-specific, prolonging survival by less than three months in some patients, highlighting the urgent need for more targeted therapies. Mutations in Cu/Zn superoxide dismutase (*SOD1*) are a common cause of ALS, inducing disease through misfolded protein aggregates, leading to neuronal toxicity. Others have shown that SOD1 protein suppression could be therapeutic. We have developed splice-switching morpholino antisense oligonucleotides (PMOs) to induce the targeted removal of frame-shifting *SOD1* exons to disrupt the reading frame, producing a premature termination codon that leads to nonsense mediated decay of the transcript. Following PMO transfection in iPSC-derived motor neurons from multiple SOD1 patient cell strains we observe up to 93% SOD1 protein suppression (P=0.001). Importantly, direct comparison of exon skipping PMOs with alternative SOD1 knockdown strategies *in vitro*, suggests that exon skipping PMOs are more effective at suppressing SOD1, and have a superior safety profile. Preliminary results of PMO evaluation in a transgenic $SOD1_{G93A}$ mouse model showed modest *SOD1* exon skipping in some mice following administration of a low dose of PMO, with high dose treated mice currently being assessed. The levels of PMO-induced SOD1 knockdown *in vitro*, together with results by others showing the *in vivo* effects of SOD1 knockdown, strongly suggest this approach could have therapeutic potential for those with SOD1-ALS.

id #67998

Transcriptomic data of human mesenchymal stem cells secretome and targets for wound healing

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Our previous studies showed human adipose-derived mesenchymal stem cells (ADSCs) produce an activity that stimulated wound healing in eardrum keratinocytes (hTMk)[1]. By regulating ADSCs in hypoxia (<0.1% O_2), this wound healing stimulus was increased. To understand the molecular mechanism behind this paracrine activity of ADSCs on wound healing, we used a bioinformatics approach to determine ADSCs and hTMk transcriptome for secreted ligands and receptors. Primary ADSCs were cultured in ambient oxygen conditions (21%) or hypoxia for 48h without serum. hTMk were established under normoxic conditions. Conditioned media were collected from ADSCs to assess paracrine activity on hTMk proliferation and migration, and to quantify specific protein secretion using ELISA. Transcriptomic analysis of ADSCs and hTMk were assessed using RNAseq and bioinformatics. Transcripts differentially expressed (P-adj <0.05) between each primary hypoxic and normoxic ADSCs were filtered through databases to identify secreted ligands, and similarly for hTMk transcriptome to identify receptors. Both subsets were then matched to a FANTOM5 curated ligand-receptor pair database[2] to establish the final list of ligand-receptor repertoires. Results show hypoxia in ADSC consistently up-regulated VEGFA more than 3 fold, with corresponding receptors expressed at mRNA level on the hTMk. This could potentially represent a previously unknown function in wound healing.

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id #68000

Immunosuppression agent cyclosporine reduces the progenitor and function capacity of human endothelial colony forming cells (ECFC)

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Endothelial colony forming cells (ECFC) have clear self-renewal capacity in culture and can undergo neovascularisation and promote blood reperfusion *in vivo*. Co-administration of mesenchymal stem cells (MSC) with ECFC have greater potential to be used as a cell therapy in treating ischemic cardiovascular disease. However, immunosuppression is necessary to improve cells engraftment during allogeneic cells transplantation. Nevertheless, it remains unresolved the impact of the immunosuppressant cyclosporine on injected ECFC. Here we used a hind-limb ischemia model in mice given either ECFC+MSC (weekly injection) with or without cyclosporine (daily injection). Cells were injected directly into the muscle adjacent to the severed right femoral artery. Each mouse was imaged weekly using a High Resolution Laser Doppler to ascertain level of blood reperfusion. Surprisingly, mice receiving only ECFC+MSC had a significant increase in reperfusion (74.77%±2.9;**p<0.01) compared to mice receiving both ECFC+MSC and cyclosporine (46.1%±3.4) at Day22. Further validation using *in vitro* Matrigel tube and colony forming assays showed a significant reduction (***p<0.001) in tube formation and ECFC colony forming capacity (**p<0.01) following cyclosporine treatment. This is the first study to demonstrate the negative effects of cyclosporine on ECFC and has the potential to change the design of future cell therapy for patients.

id #68001

Integrin αv is a cell surface marker of human pancreatic endocrine cells

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Type 1 diabetes is an autoimmune disease driven by T-cell mediated destruction of the pancreatic beta cells, resulting in blood glucose dysregulation. Although islet transplantation is currently used in clinical practice, there is a shortage of donor tissue. Pluripotent stem cells (PSCs) may potentially fill this shortfall by providing an unlimited supply of endocrine cells for transplantation.

We have generated a human embryonic stem cell line in which sequences encoding green fluorescent protein (GFP) and mCherry fluorescent protein (mCh) were placed under the regulatory control of the INSULIN and GLUCAGON genes respectively

(INS$_{GFP/w}$ GCG$_{mCh/w}$). This cell line enables endocrine cell specification to be monitored in real-time, as well as facilitating isolation of alpha and beta cells.

We utilized this INS$_{GFP/w}$ GCG$_{mCh/w}$ PSC line to identify novel cell surface markers expressed at intermediate stages of differentiation as well as on mature endocrine cells. RNAseq was performed on cells sorted based on their GFP and/or mCherry expression and compared to pancreatic progenitor cells. We discovered that a number of integrin subunits were dynamically regulated during the course of PSC differentiation. Specifically, we found that integrin αv marked both PSC-derived and adult human pancreatic endocrine cells.

We have used antibodies to integrin αv to isolate pancreatic endocrine cells from both adult cadaveric tissue and differentiated PSC cultures by flow cytometry. This method of endocrine cell enrichment permits the isolation and analysis of clinically-relevant pancreatic cell types, and is potentially of great value for cell replacement therapies for type 1 diabetes.

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id #68002

USING HUMAN EMBRYONIC STEM CELL MODELS OF MITOCHONDRIAL DISEASE TO IDENTIFY CANDIDATE DRUG TREATMENTS

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This project aims to differentiate human embryonic stem cell (hESC) models of mitochondrial disease to a cardiac cell fate to facilitate preclinical treatment screens and investigation of the underlying cellular mechanisms of disease. With >320 genetic causes of oxidative phosphorylation (OXPHOS) disorders, proving therapeutic efficacy remains challenging despite a number of agents showing promise[1,2].

We generated a panel of 15 knockout models of nuclear-encoded OXPHOS genes in hESCs, allowing us to study tissue-specific effects via differentiation in an isogenic background. In general, we focussed on generating cell lines for disease genes where patients have presented with biallelic Loss-of-Function mutations that cover a range of primary and secondary roles in OXPHOS biogenesis.

Our studies focussed on *SURF1*$^{-/-}$ *and COA6*$^{Del80/p.W59C}$ hESC clones that display OXPHOS complex IV (CIV) deficiency[3,4]. To overcome any blocks in maturation potentially arising as a result of the mutation, we also generated inducible correction lines for the *SURF1* and *COA6* clones. *SURF1*$^{-/-}$ clones form beating cardiomyocytes that show abnormal calcium handling and a significant decrease in contraction force in an organoid system[5,6]. *SURF1* is a CIV assembly factor and quantitative proteomic analysis of contractile cardiomyocytes shows downregulation of CIV subunit proteins while most other mitochondrial proteins – including other CIV assembly factors – trend toward upregulation.

Although *COA6* is also a CIV assembly factor, the *COA6*$^{Del80/p.W59C}$ clones fail to differentiate to cardiomyocytes under standard conditions. However, adding drugs targeting specific mitochondrial pathways in the differentiation medium has shown some capacity to support the cells getting further through the differentiation.

Data from these cell lines are promising for our other hESC models of mitochondrial disorders. The *SURF1* proteomic data demonstrate compensatory mitochondrial proliferation in response to decreased CIV assembly. Currently, these hESC models are being used for preliminary investigations of disease-specific treatment options.

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id #68003

Multipotent *RAG1+* progenitors emerge directly from hemogenic endothelium in human PSC derived hematopoietic organoids.

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Defining the ontogeny of the human adaptive immune system during embryogenesis has implications for understanding childhood diseases including leukemias and autoimmune conditions. Using RAG1:GFP human pluripotent stem cell (PSC) reporter lines, we examined human T-cell genesis from PSC derived hematopoietic organoids. Under conditions favouring T-cell development, RAG1+ cells progressively up regulated a cohort of recognised T-cell associated genes, arresting development at the CD4+CD8+ stage. Sort and re-culture experiments showed that early RAG1+ cells also possessed B-cell, myeloid and erythroid potential, with B-cell potential correlating with co-expression of the progenitor marker VECAD. Consistent with this, RNAseq analysis of the early RAG1+ cells indicated that, in addition to T-lineage genes, this population expressed genes associated with erythroid and myeloid lineages. In addition, flow cytometry and single cell RNA sequencing data showed that early RAG1+ cells co-expressed the endothelial/hematopoietic progenitor markers CD34, VECAD and CD90 whilst imaging studies identified RAG1+ cells within CD31+ endothelial structures that co-expressed SOX17+ or the endothelial marker, CAV1. Collectively, these observations provide evidence for a wave of human T-cell development that originates directly from haemogenic endothelium via a RAG1+ intermediate with multi-lineage potential.

id #68004

Comparative analysis of the heterogeneity of iPSC-derived and tissue-derived MSCs

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Multipotent mesenchymal stem/stromal cells (MSCs) are currently the subject of over 1000 clinical trials. However, they are primarily sourced from tissue donations, meaning their supply is subject to lack of available donors, donor-donor variation and low cell yields. To address this, MSCs have recently been derived from induced pluripotent stem cells (iPSCs). While the use of iPSC-derived MSCs in the clinic has produced very favourable results, questions still exist about comparability of iPSC-MSCs to their tissue-derived counterparts. This is complicated by the fact that the MSC phenotype is not well defined and MSCs vary between donors, tissue sources, and within individual cell populations.

Here we have compared iPSC and tissue-derived MSCs from a variety of donors and tissue sources. We show differences in proliferation, morphology and, not only differentiation capacity, but also the resulting cell phenotype. We also detect variation in individual surface-antigen profiles. iPSC-derived MSCs were found to compare favourably to their tissue-derived counterparts, and our data also suggests varying levels of heterogeneity between iPSC and tissue-derived MSCs. Our results help to elucidate the sources and extent of heterogeneity amongst both tissue-derived and iPSC-MSCs and support iPSC-MSCs as a promising alternative in the clinic.

id #68005

Antigen-encoding bone marrow transfer deletes mature antigen-specific B cells in recipients and inhibits antigen-specific antibody production

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Unwanted antibody responses arising from pathological B-cell responses underlie many diseases. Non-specific immunosuppression remains the frontline therapy, and induction of antigen-specific tolerance remains elusive. Immunotherapies typically manipulate the T-cell component of pathogenic immune responses but few directly target B cells. Transfer of gene-engineered hematopoietic stem cells (HSC) is an approach endowed with features that engender great promise for antigen-specific immunotherapy, particularly the potential to deliver antigen in a form directly tolerogenic to B cells. However this approach must be used in a way that preserves bystander immunity.

Gene-engineered bone marrow encoding ubiquitous ovalbumin (OVA) expression was transferred after low-dose (300cGy) immune-preserving irradiation. Homeostasis of pre-existing OVA-specific B cells and those arising after BM transfer was monitored using flow cytometry and responsiveness to immunisation was tested. OVA-specific B cells were purged from the pre-exiting mature B-cell population in recipients following transfer of OVA-encoding BM as well as from newly-developed B cells that arose after BM transfer. OVA-specific antibody production was largely prevented after OVA-encoding BM transfer and this was consequent to inhibition of B-cell activation, development of germinal centres and plasmablast differentiation. Low levels of gene-engineered bone marrow chimerism (~20%) were sufficient to limit antigen-specific antibody production. These data show that antigen-specific B cells within an established B cell repertoire are susceptible to *de novo* tolerance induction and this can be mediated by transfer of gene-engineered bone marrow.

This study provides an important proof-of-principle that HSC-mediated gene therapy has the capacity to modulate a mature developed B-cell repertoire under conditions where bystander immunity is preserved.

id #68006

Atherogenic ox-LDL results in human endothelial progenitor depletion and dysfunction through pathological endothelial-to-mesenchymal transition

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Background: The potent regenerative capability of the vasculature is attributed to populations of resident progenitors, termed endothelial colony-forming cells (ECFCs). ECFCs maintain endothelial function and is capable neovascularisation *in vivo*, implicating their role in tissue vascular regeneration. However, ECFCs can often acquire mesenchymal phenotype, via a process termed endothelial-to-mesenchymal transition (EndMT), which is thought to contribute to the development of atherosclerosis. Our study aims to address the pathological catalyst that drives EndMT and delineate its role in the progression of atherosclerosis. **Methods and Results:** In the presence of excessive oxidised low lipoprotein (ox-LDL), cultured ECFCs demonstrated relative mRNA and protein increase in mesenchymal markers such as *Fsp-1*, and transcription factors such as *Sox9* and *Runx2*. Long-term treatment of ECFCs with ox-LDL directly contributed to accelerated de-differentiation towards a mesenchymal phenotype and subsequent depletion of the ECFC progenitor pool, observed under flow cytometry. The self-renewal capacity of ECFCs, assessed through single-cell colony formation assay, was also significantly reduced following 5-days of ox-LDL treatment. **Conclusion:** The finding of this study supports the idea that atherosclerosis is a consequence of endothelial progenitor depletion and fate choice, representing a paradigm shift from the current understanding and open new avenues for preventive therapies.

id #68007

AAV vector gene delivery of DNase I in lupus-prone mice doesn't improve renal function but does decrease C3/IgG renal deposition

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Systemic Lupus Erythematosus (SLE) is an autoimmune disorder that can result in end stage renal failure if left untreated but current treatments are non-specific contributing to significant morbidity and mortality. A characteristic feature of this multisystem disease is plasma anti-nuclear antibodies and their co-deposition with complement C3 in diseased kidneys. Additionally, kidneys from lupus patients also exhibit neutrophil extracellular traps (NETs), which comprise extracellular DNA (ecDNA) and proteins released from dead and dying cells. Based on these and other observations there is compelling incentive to develop the enzyme DNase I as a non-toxic therapeutic to clear DNA both as an antigenic source of anti-nuclear antibodies and to disrupt NETs in diseased kidneys. To test therapeutic potential of DNase I, an AAV vector encoding murine DNase I under the transcriptional control of a powerful liver-specific promoter was packaged into serotype 8 capsid to transduce the murine liver and induce high levels of plasma DNase I . Vector performance was confirmed in HuH7 *in vitro* and in mice *in vivo*, the latter showing a 150-fold increase in plasma DNase I levels over control treated animals. Vector was also injected into lupus prone NZBWF1 mice and animals were monitored long term (>8 months). While there was no improvement in endpoints such as anti-nuclear antibody levels, kidney function or life span, there was a significant decrease in renal IgG and C3 co-deposition in treated mice. Further studies are in progress to also investigate the incidence of NETS in diseased kidneys but the current outcomes demonstrate the vector system requires further development to confer therapeutic efficacy in the NZBWF1 mouse model.

id #68008

Proline induces neural differentiation and promotes embryo development in a growth factor-like manner

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L-Proline added to mouse ES cells (mESCs) promotes their differentiation to mature neural cells via a series of embryologically relevant cell types, including pluripotent primitive ectoderm, the germ layer definitive ectoderm, and neurectoderm. Confirmation of the progression through these intermediate stages to mature neurons comes from marker analysis using flow cytometry and qPCR, and morphological analysis using microscopy and video microscopy. Our results indicate L-Proline is acting as an instructive signal, rather than by a default mechanism, by (i) acutely activating a range of signalling pathways (such as mTOR and MAPK/ERK) and (ii) stimulating anaerobic metabolism with involvement of the Pentose Phosphate Pathway. Thus, a combination of L-Proline-mediated cell signalling and metabolic changes are required to induce lineage commitment in this *in vitro* model of embryo development. The involvement of metabolic flux in lineage commitment is consistent with the recently emerging paradigm that changes in metabolism can be an effector of differentiation rather than just a passenger to it.

In embryos themselves, L-Proline stimulates development of pre-implantation mouse embryos from the 2-cell stage to the blastocyst stage. The effect is observed in isosmotic medium in low-density culture (1 embryo/10 µL), where autocrine support is absent, and is not observed in high-density culture, where autocrine support is available. L-Proline also stimulates hatching independent of embryo culture density.

Collectively, these data show L-Proline acts in a growth-factor-like manner by activating a range of molecular mechanisms – including signalling, metabolic, and epigenetic – which presumably integrate in complex fashion to drive development. Consistent with being a growth factor, L-Proline acts at various developmental stages including blastocyst formation, hatching, and at various

points along the pre-neural to neural lineage pathway. To our knowledge, L-Proline is the first of any amino acid to be shown to act in a growth-factor-like manner in any biological system.

id #68009

Generation of a molecular atlas to define age-related changes to the transcription factor network

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Ageing is the greatest risk factor for virtually all degenerative diseases. Importantly, on a cellular level, ageing appears to be a largely epigenetic phenomenon that can be manipulated by pluripotency induction or transient expression of iPSC reprogramming factors. Although reprogramming can interconvert different cell types, what would be most exciting is to directly transform a given cell type from an “aged” to a “healthy young” state without a need for oncogenic pluripotency factors. Towards this goal we have developed & validated a network-based method to predict the TFs necessary for desired cellular transitions that we are integrating with chromatin state data to understand how ageing reshapes the cellular identity network. We have compelling data that youthful regenerative capacity and metabolism can be restored in aged mouse intestinal stem cells in vitro by directly correcting the age-altered transcriptional circuitry with appropriate TFs. To help build a foundation for reprogramming based rejuvenation strategies we are in the progress of creating a molecular atlas of 40 mouse and 4 human cell types to reveal the TFs (using RNAseq and ATACseq) that drive ageing in different cell types and lineages and whether there is conservation across species. In addition to bulk based approaches we are also profiling three somatic stem cell populations and their differentiation products via single RNAseq and single cell ATACseq to understand how homo- or heterogeneous the age-altered TF network is on a single cell level. Ultimately, we hope that our molecular atlas, once complete, will help build knowledge towards therapeutic control over the ageing process by advancing our understanding of cellular ageing.

id #68011

Mutation characterisation in patients with Usher syndrome type 2A and application of therapeutic alternative splicing

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Mutations in the *USH2A* gene are responsible for Usher syndrome type 2A, a combined deaf blindness disease. *USH2A* encodes usherin, a basement membrane protein highly expressed in photoreceptors of the retina and hair cells of the cochlea. On screening in different cell types, the early part of the *USH2A* transcript was amplified from Huh7 cells, the ARPE-19 cell line and primary human myotubes, but not from healthy or patient fibroblasts. Therefore, mutations in the early and later parts of the *USH2A* transcripts were analysed in induced myogenic cells from fibroblast and primary fibroblast cultures, respectively. We found that an intronic mutation in intron 46 of *USH2A* weakened the natural splice donor site and activated a cryptic splice site, resulting in deletion of the last 153 nucleotides of exon 46 from the mature transcript. Using patient fibroblast-derived induced myogenic cells, we also confirmed a previous report that the synonymous mutation, c.949C>A, caused the deletion of the last 93 nucleotides of exon 6. In future studies, patient fibroblasts will be reprogrammed to induced pluripotent stem cells and differentiated into retinal organoids that will be treated with antisense oligonucleotides to induce alternative splicing as a molecular therapy for Usher syndrome type 2A.

id #68012

Delivery of microRNAs to manipulate mesenchymal stem cell mechanotransduction and improve osteogenesis of cells encapsulated in a hydrogel

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Physical cues from the cellular microenvironment have a strong influence on the properties and fate specification of mesenchymal stem cells (MSCs) in which factors including the extracellular matrix specificity, presentation and mechanical properties all affect MSC fate via mechanotransductive signalling pathways. This has implications for the development of MSC-based tissue-

engineering strategies where the challenge of efficiently directing MSC differentiation into the desired tissue type still remains. For example, although the encapsulation and subsequent differentiation of MSCs in hydrogels has many advantages, the inherent softness of hydrogels means that the mechanical properties are poorly matched to the mechanical cues that drive efficient osteogenesis. We hypothesise that by understanding and manipulating mechanotransductive signalling it will be possible to override the physical signals that MSCs receive from their environment and drive efficient differentiation.

We have previously identified a mechanosensitive miRNA signalling axis in which substrate stiffness alters levels of miR-100-5p and miR-143-3p which then act via the mTOR pathway to regulate MSC osteogenesis. Here we develop and characterise a simple system for *in situ* delivery of these miRNAs to hydrogel-encapsulated MSCs and test their ability to enhance osteogenic differentiation. Comparing the influence of different transfection agents and hydrogel compositions, we show that it is possible to transfect MSCs with miRNAs *in situ* and further determine the factors affecting transfection agent release and MSC transfection. We then compare the efficacy of both pre-transfection and *in situ* transfection on the osteogenic capacity of hydrogel-encapsulated MSCs, showing via increased mineralisation and osteogenic gene expression, that *in situ* transfected samples actually outperform their pre-transfected counterparts. Overall this system shows the potential to modulate mechanotransduction in MSCs to improve differentiation outcomes whilst developing a system that is simpler and more effective than previous protocols. We therefore believe that this strategy shows significant future promise for MSC-based tissue-engineering.

id #68013

Antisense oligonucleotide mediated knockdown of RNA-binding proteins SAM68 and hnRNP-A1 to modulate expression of the survival motor neuron 2 gene.

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Spinal muscular atrophy (SMA), is a severe childhood disease most commonly caused by the homozygous loss of the survival motor neuron 1 (*SMN1*) gene that encodes an essential protein, SMN. A homologous gene, *SMN2* provides insufficient levels of SMN and is unable to compensate for the loss of *SMN1*. A single nucleotide change in *SMN2* exon 7 creates a splice silencer, leading to the predominant production of truncated transcripts missing exon 7 that encode a non-functional SMN protein. Pre-mRNA splicing of *SMN2* exon 7 is modulated by RNA-binding proteins SAM68 and hnRNP-A1 that promote exon 7 exclusion from the mature *SMN2* transcript. Targeted knockdown of SAM68 and/or hnRNP-A1 has potential to increase the amount of functional SMN produced by the sub-optimal *SMN2* gene. Antisense oligonucleotides designed to knockdown SAM68 and hnRNP-A1 were delivered individually and in combination with *Anti ISS-N1*, an antisense compound targeting *SMN2* directly, into SMA patient fibroblasts. Gene transcript and functional protein analysis showed SAM68 and hnRNP-A1 knockdown subsequently increased functional SMN production in an additive manner when combined with *Anti ISS-N1*. There is thus potential for antisense therapeutics targeting SAM68 and/or hnRNP-A1 to be used to augment the action of *Anti ISS-N1* as a combinatorial treatment for SMA.

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id #68014

A novel *pixel-clique* statistical model for probing subcellular FRET efficiency of Rho-GTPases using live cell imaging

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The complex and dynamic nature of cell migration is due to the rapid and continuous remodelling of cytoarchitecture. During this process, spatiotemporal regulation of signalling networks bridges external stimuli to cellular responses. Rho-family GTPases are key components of these signalling networks and most members of this protein family act as molecular switches that cycle between a GDP-loaded form and GTP-loaded form. By applying Raichu FRET probes (with a CFP/YFP fluorescent protein pair) in live endothelial cells, this study develops a novel *pixel-clique* statistical methodology to quantify the subcellular distribution of Rho-GTPases activity. This new imaging tool is used to observe the Rho GTPase activities during HUVEC cell membrane protrusion. The Matlab statistical toolbox™ was used to implement a linear mixed effect model using the function *fitlme*[1]. The function 'getFRET[1]' performs the analysis with the input images of Raichu-probes expressing cells and single-colour probe expressing cells. In this study, multiple protrusions and retracting membrane segments co-exist in one given cell, which agrees with Yamao and colleagues' tug-of-war model and Insall's pseudopod-centred view of cell migration. With this statistical tool, we can begin to examine more in-depth as to how periodic changes in membrane motion is correlated with Rho GTPase activity. The methodology can thus be extended to other (especially non-canonical) Rho GTPase members. An example will be to apply

the *pixel-clique* model to dissect the distinctive and functional overlapping roles of canonical Rac1 and the haematopoietic cell specific Rac2. With the advance in probe design to distinguish highly homologous Rac2 from Rac1 and the *pixel-clique* model for data interpretation, we can improve our understanding of the diverse roles of Rho GTPases whether in model cell lines, primary cells, stem cells, or in whole small animals.

1. [1] Software for analysis of FRET data developed by Dr Robert Nordon.
2. Raichu probes are encoded by Raichu Plasmids (pRaichu) which were imported from the Matsuda lab in Kyoto University, Japan.

id #68016

HOPX regulation of cardiomyocyte proliferation and heart regeneration is metabolically dependent

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Billions of cardiomyocytes die after myocardial infarction and fail to be replaced, reducing pumping function and causing heart failure - the leading global cause of death. Understanding how cardiomyocyte proliferation is regulated remains central for determining mechanisms of heart regeneration and effectively targeting therapies to address heart failure. Previous studies have demonstrated the non-DNA binding homeodomain protein, HOPX, governs cardiomyocyte proliferation during heart development. We aimed to determine the role of HOPX in adult heart regeneration. Utilizing zebrafish, a model organism with innate heart regeneration, we tested the role of *hopx* in regulating cardiomyocyte proliferation at the organ-level *in vivo*. We generated cardiomyocyte-specific *hopx*-overexpressing zebrafish and assessed their regenerative response seven days after ventricular injury. Wound-adjacent cardiomyocytes from *hopx*-overexpressing zebrafish proliferated significantly less compared to clutchmate controls, demonstrating ectopic *hopx* expression is sufficient to blunt acute cardiac regeneration. To determine if *HOPX* deficiency promotes proliferation in human cardiomyocytes *in vitro*, we generated cardiac organoids using CRISPRi *HOPX* conditional loss-of-function human induced pluripotent stem cells (hiPSCs). *HOPX* knockdown (KD) tissues reflecting the metabolic environment of early heart development produced significantly increased force attributable to increased cardiomyocyte proliferation relative to control tissues. To investigate the transcriptomic signature of *HOPX* loss-of-function, we performed genome-wide CAGE-sequencing. *HOPX* KD resulted in significant enrichment for cholesterol biosynthesis, an essential regulatory pathway, among others, underlying cardiomyocyte proliferation. To test the mitogenic potential of *HOPX* deficiency in post-mitotic cardiomyocytes, we metabolically-matured hiPSC-derived cardiac tissues. We show metabolic maturation is sufficient to block HOPX-dependent cardiomyocyte proliferation, likewise resulting in no significant difference in force generation compared to controls. Collectively, this study provides critical insights into regulatory mechanisms underlying heart development and regeneration by demonstrating that *HOPX* directly regulates cardiomyocyte proliferation after organ-level injury and establishing that the mitogenic potency of *HOPX* is dependent on the cardiomyocyte metabolic state.

id #68017

L-proline acts like a growth factor to drive the differentiation of mESCs to neurectoderm via embryologically relevant cell populations

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During the early stages of mammalian development, cells of the embryo transition through the inner cell mass, primitive-ectoderm, definitive-ectoderm and neurectoderm before giving rise to cells that make up the nervous system. Due in part to the complexity of differentiation, the small size of the embryo and its relative inaccessibility *in utero*, the molecular mechanisms underlying the formation of these cell populations, and thus development of the neural lineage, are relatively poorly understood. We developed a protocol using mESCs to recapitulate mammalian nervous system development *in vitro* using L-proline. D3 and 46C-*Sox1*-GFP mESC cell lines were cultured as embryoid bodies (EBs) with time-dependent additions of 400 µM L-proline and 10 µM SB431542 over 9 days. On Day 9, EBs were seeded in serum-free conditions and allowed to differentiate for a further 6 days, after which they were assessed for neural cells (D3: 61±14% and 46C: 68±9% (n≥3)). Using qPCR (data expressed as peak $\log_2$ fold change, n≥3), mESCs differentiated to *Dnmt3b*$^+$ (3±0.5) and *Fgf5*$^+$ (5±0.3) primitive-ectoderm between Days 3–5, followed by *Penk1*$^+$ (1±0.6) and *Pard6b*$^+$ (2±0.1) definitive-ectoderm between Days 5–7. From Days 5–9, a *Sox1*$^+$ population of neurectoderm (2.5±0.3) was produced, at the expense of *Mixl1*$^+$ mesendoderm (-2.1±1.2). By Day 9, flow cytometry analysis showed that 68±2% cells cultured in L-proline were *Sox1*-GFP$^+$, and immunofluorescence imaging indicated that this expression was maintained in some cells at the core of EBs cultured until Day 15. Taken together, neurectoderm production from mESCs was achieved prior to the addition of SB431542, thus providing evidence for an instructive model of neurectoderm induction in the presence of L-proline. This is the first study, to our knowledge, that shows that a simple amino acid acts like a typical growth factor to induce differentiation at multiple stages of mammalian development.

id #68018

High-throughput selection of novel AAV capsids in a murine xenograft liver model: towards next generation of human liver-tropic AAV variants

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rAAV vectors are the technological foundation underpinning numerous recent successes in human gene therapy trials, with high-profile examples targeting diseases of the eye, liver and central neuron system. AAV is the only *in vivo* viral gene delivery tool to achieve regulatory approval by the US FDA or the European Medicines Agency. These successes hinge on continuing progress in the development of AAV-based gene delivery systems, driven primarily by advances in *capsid development*.

To this end, the Children's Medical Research Institute (CMRI) and Boston, US-based LogicBio Therapeutics have established a joint program, with the goal to develop the next generation of AAV bioengineered vectors for liver-targeted therapy. This unique combination of academic and industrial expertise has allowed the team to set up a AAV development pipeline, which combines selection of the most functional AAV variants with an early focus on improving manufacturability and immunological profiles.

Here we present the vector selection and validation pipeline, as well as novel AAV variants optimised for highly efficient functional transduction of primary human hepatocytes and high-yield manufacturability. This pipeline includes generation of highly variable AAV capsid libraries based on DNA-shuffling and peptide-display, selection and validation in a biologically-predictive xenograft model of human liver, and evaluation of vector manufacturability and seroreactivity. The pipeline takes advantage of our proprietary selection and evaluation platform which allows to screen capsids based on the physical (DNA) and functional (RNA) transduction of target cells. We will present functional data for several novel AAV variants that transduce primary human hepatocytes with significantly higher efficiency than the three clinically-tested AAV capsids: AAV2/2, AAV2/8 and bioengineered AAV-LK03. Importantly, the technology developed can be directly applied to perform selection on other target tissues, significantly enhancing the impact of the project and paving the way to the development of next-generation of AAV vectors for clinical applications.

id #68019

Making the inactive active through changes in antisense oligomer chemistries

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Antisense oligomers (AOs) are short, single-stranded nucleic acid sequences that may anneal to a mRNA or pre-mRNA by complementary base pairing and, depending on the base and backbone chemistries, can induce a variety of mechanisms to alter the gene expression. We have designed AOs to redirect the splicing process and have used this strategy to by-pass disease-causing mutations in the dystrophin gene (*DMD*) by inducing a specific exon-skipping to restore the reading frame. When developing a panel of AOs to skip all dystrophin exons, remarkably we found that one in three AOs appeared to be completely ineffective. In this study, we modified the composition of some ineffective AOs and increased their annealing potential by incorporating locked nucleic acid residues into the sequences. AOs targeting the skipping of exon 16, 23 and 51 of human *DMD* transcripts were synthesized as two different chemistries, 2'-O-methyl (2'-OMe) oligomers and locked nucleic acid (LNA)/2'-OMe mixmers. Primary human myoblasts were treated with 2'-OMe or LNA/2'-OMe AOs, and the *DMD* transcripts were analysed for exon skipping. The 2'-OMe AOs showed no significant exon skipping while all the LNA/2'-OMe mixmers exhibited skipping of the targeted exons. Interestingly, the LNA/2'-OMe mixmer targeting exon 51 induced two additional transcripts, representing incomplete skipping of exon 51 with retention of 95 or 188 bases from the beginning of exon 51. These results indicate that LNA/2'-OMe mixmers may be more efficient at exon skipping, but this improvement may come at the cost of activating alternate cryptic splice sites.

id #68021

In vivo genome editing of primary human hepatocytes: towards a universal, mutation-independent, therapy for metabolic liver disease

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Genome editing technology has immense potential for treating inherited diseases and is likely to supplant contemporary gene addition approaches already delivering exciting clinical success in multiple organ systems. Such progress has been underpinned by advances in adeno-associated virus (AAV) vector technology and the use of humanised preclinical models, however, challenges such as achieving therapeutically effective levels of editing *in vivo* remain to be resolved. In a previous study, we used the human ornithine transcarbamylase (*OTC*) gene as a therapeutic target and developed functionally validated dual AAV vectors for precise CRISPR/saCas9 cleavage and single nucleotide editing using AAV-mediated homology-directed repair (HDR). These reagents were evaluated *in vivo* on the native *OTC* locus in patient-derived primary human hepatocytes xenografted into the FRG ($Fah^{-/-}Rag2^{-/-}IL2rg^{-/-}$) mouse liver. We observed an unprecedented level of *in vivo* editing, with up to 29% of alleles showing the expected single nucleotide change back to wild-type. These data demonstrated that targeted repair can be achieved in primary human hepatocytes *in vivo* at levels that would provide benefit for even the most therapeutically challenging liver disorders. Notably, however, HDR is inherently dependent on cell division which limits possible therapeutic targets. Here we describe a universal, homology-independent, approach designed to correct cells in both a dividing or non-dividing state. As initial proof-of-concept, we will focus on correcting the human *OTC* locus *in vivo* in a panel of OTC-deficient primary human hepatocytes engrafted into the FRG mouse.

id #68024

Generation of novel cardiac specific AAV vectors by directed evolution in human iPSC derived cardiomyocytes

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Recombinant adeno-associated viral (rAAV) vectors have emerged as one of the most promising gene therapy vectors. However, recent evidence has indicated that successful rAAV-mediated gene therapy in animal models may not translate to the same therapeutic benefit in humans. This is mainly due to the species difference in rAAV transduction efficiency, thus leading to the search for new capsids which exhibit high efficiency in human cells. This may be achieved through capsid shuffling, in which rAAV capsid gene sequences can be reconstituted from fragments of naturally occurring rAAV capsids to generate a library comprised of novel rAAVs with unique capsid protein sequences. This library could be screened using Directed Evolution (DE) in human cardiomyoctyes to select for cardiotropic rAAVs. In the development of strategies for personalised medicine, patient-specific iPSC-CMs could be used to evolve patent specific rAAVs. To test this strategy, we have used an rAAV library (rAAV1-12) to perform DE in human iPSC-CMs. The resulting rAAVs were then recovered from cell lysates and used for subsequent rounds of selection in fresh iPSC-CMs. After six rounds of selection, the recovered rAAVs were analysed for enrichment of cardiotropic candidates. Five candidates emerged. Functional analysis using rAAV-GFP indicated that while rAAV6 was the most efficient at transducing iPSC-CM among natural rAAVs, two of our novel variants led to higher GFP expression at the same vector dose. Our results validate the use of DE to develop novel, highly functional rAAVs for use in clinical studies.

id #68042

Characterisation of fibroblasts from an Usher syndrome patient with *USH2A* mutations and subsequent generation of induced pluripotent stem cell lines

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Background: Mutations in *USH2A* can cause Usher syndrome type 2A, characterised by congenital hearing loss and progressive vision loss due to retinitis pigmentosa.

Aims: Characterise fibroblast lines derived from patient samples containing *USH2A* c.949C>A and c.1256 G>T mutations, and healthy controls. Next, generate and characterise iPSC lines.

Methods: Fibroblasts were characterised using viability assays and immunocytochemistry; and reprogrammed to pluripotency using Oct4, Sox2, Lin28, Klf4, and L-Myc with episomal vectors. On Day 25, colonies were selected for clonal expansion and *USH2A* mutations confirmed using Sanger sequencing. iPSC gene expression was measured using qRT-PCR; protein expression was analysed using immunocytochemistry.

Results: Fibroblast lines had typical elongated morphology, and normal cells had faster growth than patient cells. All iPSC displayed typical growth characteristics and morphologies of pluripotent stem cell colonies. Pluripotency proteins (OCT4,

NANOG, SOX2 and SSEA-4) and genes (*OCT4*, *NANOG*, *SOX2* and *KLF4*) were expressed similarly in all lines. Trilineage genes (*PAX6*, *DCX*, *TBXT*, *AFP*, and *SOX7*) had minimal expression in undifferentiated iPSC and increased expression in embryoid bodies derived from these iPSC.

Conclusion: Development and characterisation of iPSC lines from patients with Usher syndrome represents a unique opportunity to study differences in inner ear development from those of healthy controls.

id #68047

Rapid administration of third-party cytomegalovirus (CMV), Epstein-Barr virus (EBV) or adenovirus (Adv) specific T-cells (VSTs) post-haemopoietic stem cell transplant (HSCT)

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Introduction

Partially-HLA matched VSTs are safe and effective after failure of at least two weeks standard antiviral therapy to resolve viral infection. In this phase I trial, we assessed their safety when administered earlier in the course of viral infection post-HSCT.

Methods

A cell bank of VSTs was established from G-CSF mobilised peripheral blood from healthy donors. After stimulation with peptide mixes, VSTs were selected by expression of CD137 and cultured with cytokines. HSCT recipients were treated with up to 4 doses of $2x10_7$of VST/$m_2$commencing within 7 days of initial treatment for viral reactivation.

Results

A total of 188 doses of VST were manufactured from 7 donors with 12 product manufacturing runs (CMV n=3, EBV n=4 and Adv n=5). Median virus specificity was 75% for CMV, 83% for EBV and 37% for Adv. Thirty HSCT recipients were treated with VSTs within 7 days of initial antiviral treatment for viral reactivation at a median of 55 days post-transplant. Data from 25 patients were available for analysis (CMV n=22, EBV n=2, Adv n=1). Median age was 58 (0-71). 18 patients received a single infusion, 6 received 2 and 1 received 4 infusions. There were no immediate infusion-related toxicities. 23/25 patients (92%) had complete viral clearance (CR), 2 had a partial response (PR). Median time to best viral response was 20 days. There were 5 deaths (refractory aGVHD in 2 patients, pulmonary veno-occlusive disease/CMV pneumonitis, relapse of haematological malignancy, and sepsis/aspiration pneumonia). Overall survival at a median 348 days follow up post-infusion (56-668) was 80%.

Conclusion

Early infusion of third-party donor partially HLA-matched VSTs for viral reactivation after HSCT is associated with no immediate toxicity, a low rate of graft-versus-host disease and complete viral clearance in 92% of recipients.

id #68059

Clinical Development - Cell and Gene Therapies

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Gene and cell therapies promise game changing therapeutic outcomes but they also present unique challenges both before the clinic and during the clinical development phase. Before the clinic it is important to establish an appropriate manufacturing capability and manufacturing process for the investigational product. It is equally important to develop a nonclinical toxicology strategy that will not only satisfy regulatory expectations but is also practical in terms of time and resources required.

Drawing on the experience of the Novotech group in bringing gene and cell-based therapies into and through clinical trials, a range of clinical development case studies will be presented focusing on development performed in Australia.

id #68062

Modelling the mitochondrial disease Sengers syndrome using human embryonic stem cells

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Sengers syndrome is a potentially fatal mitochondrial disease characterised by hypertrophic cardiomyopathy, congenital cataracts, lactic acidosis, and exercise intolerance[1]. This disease is caused by mutations in the acylglycerol kinase *(AGK)* gene[2,3]. The enzymatic activity of AGK functions in mitochondrial lipid metabolism[4,5].More recently, AGK was discovered to be a subunit of the mitochondrial TIM22 protein import complex. AGK stabilises the TIM22 complex and facilitate mitochondrial carrier protein biogenesis independently to its kinase function[6,7].

This project aims to model and investigate the molecular and cellular pathomechanisms underlying Sengers syndrome using human embryonic stem cells (hESCs) differentiated to clinically relevant cardiomyocytes.

AGK$^{-/-}$ hESCs were generated using CRISPR/Cas9 gene editing technology, and validated for pluripotency and karyotype. Mutants were characterised by genetic (DNA and RNA) and immunoblot experiments. Selected clones were differentiated into cardiomyocytes and further functional analyses performed including calcium imaging and electron microscopy experiments.

DNA sequencing and cDNA studies identified multiple hESC clones with *AGK* mutations causing a frameshift and premature stop codons, or splicing defects. In hESCs with bi-allelic *AGK* mutations, the TIM22 complex, and subsequently the ATP carrier protein, were not detected by BN-PAGE. Disrupted ATP carrier biogenesis had a negative impact on ATP production in *AGK*$^{-/-}$ hESCs. Additionally, preliminary results indicate that cardiomyocytes derived from *AGK*$^{-/-}$ hESCs display an irregular beating pattern and abnormal calcium handling compared to controls. Furthermore, samples analysed by electron microscopy suggest that mutant cardiomyocytes possess disorganised myofibrils.

The *AGK*$^{-/-}$ hESCs have the potential to provide clinically relevant tissue samples for further investigations of disease pathomechanisms, with future experiments including proteomic and lipidomic analyses of mutants. Ultimately, these cells could be used to facilitate pre-clinical studies testing potential treatments for Sengers syndrome.

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id #68104

Progressing human lens regeneration via lens cell transplantation as an improved childhood cataract treatment

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Cataracts cause interrupted light transmission through the ocular lens, and as such are a leading cause of low vision and blindness in children worldwide. Surgical removal of childhood cataract often leads to suboptimal visual outcomes – due in part to the loss of focussing ability at key stages of a child's motor, educational and social development. As a result, childhood cataract patients self-report similar quality of life to childhood cancer patients. Our research aims to regenerate lenses – via transplantation of stem cell-derived lens epithelial cells – to improve childhood cataract treatment. To do this we have developed complementary lens cell transplantation models, using live rabbits and *ex vivo* bovine lenses. Preliminary *in vivo* data shows that: i) lens material grows in rabbit capsular bags after transplantation of human pluripotent stem cell-derived lens epithelial cells; and ii) human-specific lens crystallin protein sequences can be detected 3 months after transplantation. To further optimise the lens cell

transplantation surgery, we have created a 3D-printed bovine lens holder that enables lens cell transplantation into bovine lens capsular bags. Together, these complementary *in vivo* and *in vitro* lens regeneration models are progressing development of lens cell transplantation as an improved treatment for childhood cataract.

id #68105

Organoids as a model for colorectal cancer: the first 50 patient lines

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Colorectal organoids are three-dimensional cultures of cells established from healthy patient tissues and tumours that remain biologically representative of the tissue from which they are derived. Commencing recruitment in August 2015, we have established organoids from healthy and tumour tissue for more than 50 patients who have undergone surgical resection for the treatment of CRC at Cabrini Hospital. Each of the patients consented to this study are included in the prospectively maintained, clinician-led Cabrini Monash Colorectal Neoplasia Database. This provides access to comprehensive clinical records and treatment outcomes that can be correlated to the characteristics of their own tissue-derived organoid lines. The median patient age is 72 years with a range from 27 to 92 years, in equal proportions of male to female. We have successfully established lines from primary and metastatic tumours, adenocarcinoma and adenocarcinoma mucinous histological subtypes and both proficient- and deficient- mismatch repair systems. Patient-derived tumour organoids are a powerful tool for cancer research and as such we are utilising them for the development of drug response assays, testing routine cancer therapies including radiotherapy and chemotherapy with the aim of developing a pre-clinical test that can predict treatment outcomes for CRC patients, before they undergo therapy.

id #68106

Next generation of gene therapy vectors for clinical applications: unlocking the hepatotropic potential of prototypical AAV2

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Publish consent withheld

id #68107

Resistance to the chemotherapeutic drug 5-FU is associated with a stem cell signature in patient-derived colorectal cancer organoids

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Colorectal cancer (CRC) is the second leading cause of cancer death in Australia. One of the key challenges with therapeutic strategies is defining inter-individual differences that impact a patients' response to therapy. A personalised cancer therapy approach would transform the management of CRC. As organoids recapitulate many of the *in vivo* characteristics of the original tumour tissue, we aim to develop preclinical models for predicting patient-specific responses to treatment using patient-derived organoids (PDOs). In this study, we examined the histological, molecular and phenotypic characteristics including the stem cell

signature in 11 PDOs and patient-matched primary tumours. The PDOs recapitulate the histopathological characteristics of their primary tumours displaying distinct morphological differences between patients. Immunohistochemistry and gene expression analyses for markers of different categories of stem cell populations including the LGR5+, reserve and revival stem cells revealed similar expression patterns between PDOs and primary tumours. In response to chemotherapeutic treatment with 5-Fluorouracil (5-FU), PDOs displayed a 3-fold difference in sensitivity, and interestingly more resistant organoid lines were associated with higher expression of stem cell markers. This study provides insight into the inter-tumoural heterogeneity in stem cell signatures and the clinical relevance of PDOs as preclinical models to predict drug sensitivity.

id #68110

Defining altered Cl^- conductance and phagocytosis in retinal pigment epithelium containing an F305S BEST1 mutation associated with Best disease

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Best disease is a rare form of macular dystrophy caused by mutations in the human BEST1 gene. BEST1 protein is highly expressed in the retinal pigment epithelium (RPE). Thus the loss of high acuity vision seen with Best disease – that involves RPE degeneration within the macula – is thought to arise from BEST1 mutations disrupting RPE functions critical for maintenance of a healthy retinal micro-environment. For example, the ability of the RPE to phagocytose and degrade damaged photoreceptor outer segments is thought to involve RPE responses to light-induced variations in osmotic pressure within the subretinal space. We therefore hypothesised that analysis of RPE containing mutant BEST1 protein would reveal impaired RPE cell volume regulation, as well as impaired phagocytosis and/or processing of photoreceptor outer segments. To test these hypotheses, we investigated RPE containing the F305S BEST1 mutation responsible for Best disease in some patients. This mutant RPE demonstrated an inability to respond to osmotic challenges, and showed reduced ability to process photoreceptor outer segments. Partial rescue of these functions in the diseased RPE was observed on application of a synthetic small molecule. Further investigations are underway to define the mechanisms by which this molecule restores these key RPE functions.

id #68111

Defining protective responses of human micro-lenses to cataract initiating factors

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Cataract continues to be a leading cause of low vision and blindness worldwide, despite the fact that cataract surgery routinely restores vision immediately for many cataract patients. Accordingly, defining risk factor-specific mechanisms of cataract formation, and associated protective responses within the lens, holds promise for identifying drugs that can delay cataract formation or progression. As heat is a known cataract risk factor, we hypothesised that exposing human lens tissue to increased temperature would initiate protective responses. To begin defining the initial responses of human lens tissue to increased temperature, we used human pluripotent stem cell-derived, ROR1-expressing (ROR1e) human lens epithelial cells and micro-lenses. Our results show that exposing human micro-lenses to 39°C for 15 minutes did not affect transparency or focusing ability. In contrast, exposing micro-lenses to 46°C or 55°C for 15-minutes significantly decreased both micro-lens light focusing and transparency. Mass spectrometry analyses of ROR1e cells and micro-lenses exposed to 39°C identified 2 proteins – HSPA1A and HSPA1L – that were quickly and significantly upregulated in ROR1e lens epithelial cells in response to the heat treatment. These results provide new information on lens protection mechanisms that may be relevant to future attempts at delaying cataract formation.

id #68112

Using intestinal organoids to analyse stem cell function in inflammatory bowel disease

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Inflammatory Bowel Disease (IBD) currently affects more than 75,000 individuals in Australia with a total estimated cost of care exceeding $3billion. This multifactorial disease is characterised by inflammation occurring through the gastrointestinal tract. However, the underlying mechanisms of disease are yet to be elucidated and no cures are currently available. We aim to identify differences in intestinal stem cell populations by utilising the organoid culturing system to grow intestinal cells combined with anaerobic culturing to characterise the bacteria from human paediatric intestinal biopsies obtained from patients with or without IBD. We are currently recruiting and have established 39 human paediatric small intestinal organoid lines. Transcriptional analysis will allow us to define the stem cell signature of IBD-derived organoids, compared to controls. Staining of organoid sections for intestinal stem cell markers will allow us to study the localisation of these markers further. We will also examine the impact of

bacterial populations on stem cell function. By doing so, this study will provide insights into the effects of IBD on intestinal epithelium and allow us to further interrogate whether stem cell function is compromised in IBD.

id #68118

Using gastric organoids to investigate how NF-kB1 prevents the development of gastric cancer.

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Gastric cancer (GC) is the third highest cause of cancer-related death worldwide and is often only diagnosed when the genetic alterations are already irreversible.

NFkB1 is a transcription factor implicated in regulating cellular proliferation, inflammation and senescence. NFkB1 polymorphisms have been associated with the inflammation process preceding GC establishment and phenotypes observed in a NFkB1$^{-/-}$mouse model clearly demonstrate the role of NFkB1 in modulating inflammation.

The gastric epithelium has a high turnover which is fuelled and controlled by gastric stem cell (GSC) populations found in the isthmus and in the base of gastric glands.

In order to analyse the effect of NFkB1 loss in GSCs, gastric organoids are employed in this project. Analysis of the effects of NFkB1 loss in the mouse model suggest interactions between the immune system and the epithelium are crucial for tumorigenic evolution. Organoid cultures provide an opportunity to investigate epithelial cell-specific phenotypes and analyse specific effects on GSCs through flow cytometry, immunofluorescence and functional assays. Gaining insight into the mechanisms of NFkB1 action may lead to the development of intervention options to interrupt the progression of inflammation to gastric cancer.

id #68119

Identification and Expression of Islet-enriched microRNAs

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Several studies have demonstrated that Dicer-null mouse islet beta cells show up to 90% reduction in insulin gene transcription [1,2,3]. Similar mechanisms are believed to exist in human islet beta cells, but no specific micro(mi)RNAs are yet identified (in humans or mice) to explain the observations made using Dicer-null mouse beta cells. We profiled 758 known and validated miRNAs in ~700 different human tissues (including over 220 human islet preparations) and used machine-learning algorithms to identify a signature of 22 miRNAs that are predictive and associated with insulin gene expression. We generated three sources of Doxycycline-regulated cell lines: human islet-derived progenitor cells (hIPCs), pancreatic ductal cells (PANC-1) and liver cells (HUH7) (not shown), to express each of the 22 different miRNAs. We are assessing the expression of 50 different gene transcripts (islet hormones, transcription factors, key ligands/receptors, glucose transporters) and the selected miRNAs. We aim to generate these data characterising the impact of forced expression of key islet-associated miRNAs on the expression of pancreatic gene transcripts, so as to identify a select set of miRNAs that are necessary and essential for insulin gene transcription. The major aim of this study is to understand if regulated overexpression of key islet miRNAs drives the expression of pancreatic islet transcription factors, key islet-associated genes and major islet hormones. Further studies are planned to identify miRNAs that can be used in a stage-specific regulated overexpression protocol to assess if forced insulin-associated miRNA overexpression could kick-start/boot-up endogenous islet-enriched miRNA expression and if it improves the differentiation potential of hIPCs. If successful, *in vitro* and *in vivo* functional studies would be needed to assess their potential for cell replacement therapy in diabetes.

1. (Lynn F et al 2007 Diabetes)
2. (Kalius M et al 2011 PLos One)
3. (Melkam-Zehavi T et al 2011 Embo J)

id #68120

Liver-specific Delivery of GalNac-siRNA Targeting TMPRSS6 Ameliorates Ineffective Erythropoiesis and Anaemia in β-Thalassaemia mice

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β-Thalassaemia is an inherited red blood cell disorder characterised by diminished or absent β-globin chain synthesis leading to ineffective erythropoiesis and anaemia. The failures of erythropoiesis and chronic anaemia lead to abnormal iron metabolism and systemic iron overload, contributing to cardiac, hepatic and endocrine dysfunction. Several studies have identified the hepatic peptide hepcidin (the master regulator of iron homeostasis) to be suppressed in β-thalassaemia contributing to increased gastrointestinal iron absorption and iron retention by the reticular endothelia system. Hepcidin is under the negative control of the transmembrane serine protease 6 (TMPRSS6) via the cleavage of haemojuvelin (HJV), a co-receptor for the BMP-SMAD signalling pathway. Considering the central role of the hepcidin/HJV/TMPRSS6 axis in iron homeostasis, inhibition of TMPRSS6 expression increases hepcidin production and ameliorate anaemia in β-thalassaemia in mice. In this study, we investigated RNAi-mediated reduction of TMPRSS6 in β-thalassaemia (Hbbth3/+) mice using a liver-specific delivery with an optimised GalNac-conjugated siRNA targeting TMPRSS6 (SLN124). Two s.c doses of SLN124 (3mg/kg) in Hbbth3/+ mice over 35 days, was sufficient to normalise hepcidin expression and significantly improve red cell indices, as shown by an increase in haematocrit level, and reduction in reticulocyte counts, red cell distribution width and reactive oxygen species (ROS). We also observed a significant improvement in erythroid maturation and erythrocyte production, which was associated with a reduction in spleen size and regression of cardiac hypertrophy. Collectively, we show that SLN124 is able to ameliorate multiple clinical symptoms associated with chronic anaemia in a mouse model for β-thalassaemia intermediate. The liver-specific reduction of TMPRSS6 expression using SLN124 represents a promising pharmacologic modality for the treatment of β-thalassaemia, and potentially other disorders associated with ineffective erythropoiesis and iron overload.

id #68124

EVALUATING THE EFFECT OF MONOMERIC AMYLOID β ON HEALTH OF FOREBRAIN NEURONS DERIVED FROM HUMAN EMBRYONIC STEM CELLS

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There is an increase in effort and interest around the world for developing appropriate disease models for Alzheimer's disease (AD). Despite such awareness, most of the *in vitro* AD models do not resemble the AD patient brain environment. To address this critical issue, we treated human embryonic stem cells (hESCs)-derived neurons with monomeric Aβ (1-42) at pathophysiologically more relevant concentrations for longer period compared to other conventional AD studies. To generate forebrain neurons we used a combination of small molecules for the first 9 days of differentiation. The small molecules include inhibitors of SMAD signalling and a Wnt signalling pathway inhibitor to derive central nervous system lineages, followed by other small molecules to derive forebrain neurons. The Aβ (1-42) monomer treatment initiated on day 50 of differentiation for 15 days and the monomer concentrations ranged from 0 -10 μM. Immunocytochemistry demonstrated that there is a reduction in both thickness and length of axons with low concentrations of Aβ (1-42) monomer. Currently, inflammatory cytokine level changes during the Aβ treatment are under investigation by using cytometric bead array (CBA) assay. Overall, the combination of current differentiation protocol and extended Aβ (1-42) treatment successfully produces a more disease relevant model for AD.

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id #68126

Understanding the role of W*NT* signalling in generating midbrain dopaminergic neurons of the substantia nigra pars compacta /A9 subtype neurons

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Cell replacement therapy is one of the most promising strategies for the treatment of Parkinson's disease (PD). The degeneration of A9 sub-type midbrain dopaminergic (mDA) neurons leads to the movement disorders characteristic of PD. Consequently, there has been considerable interest in the potential of hPSCs to provide a source of A9 neurons for transplantation. Protocols for directed differentiation of hESCs into mDA neurons have been developed, based on knowledge of mouse brain development. Several challenges remain, including the need to develop protocols that can enrich for A9 sub-type neurons, and the need to modulate the maturation of progenitors and survival of mature neurons. Wnt signalling has been reported to influence mDA neuron specification and phenotype. We studied the phenotype of cells during differentiation using a genetic reporter targeted downstream of the Lmx1a promoter in hESCs. We exposed our cultures to Wnt mimetics in the early (Day 3 -11) and maturation stages (Day 40 – 65). We observed that concentration-dependent activation of Wnt signalling altered the phenotype of mDA neurons both in early patterning and at the maturation stage. This study provides insight into the development of appropriate cell therapies for PD.

id #68127

Midbrain dopaminergic phenotype and function are regulated by maturation media

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Human embryonic stem cell-derived midbrain dopaminergic (mDA) neurons could be the next generation of *in vitro* models for neuropsychiatric disorders like schizophrenia. A hallmark feature of this disorder is a dysregulation of dopaminergic neurotransmission in the midbrain. Present *in vitro* models of schizophrenia are confounded by the fact that the influence of system bias and observational bias are excluded from the experimental design and analysis. Our system may be an improved model for studying schizophrenia since mDA neurons are likely to express receptors and contain intracellular signal transduction mechanisms that are relevant to neurons *in vivo*; thus reducing system bias. In order to reduce the impact of observational bias, we developed a liquid chromatography-tandem mass spectroscopy method to quantify changes in dopamine concentration in the culture media of mDA neurons. We found that the state of maturity of developing cultures correlated strongly to the concentration of dopamine in the culture media. We then showed that changes in the maturation media have a profound influence on the phenotype of neurons even during late stages of maturation. Finally, mDA cultures were used to study the effect of chronic haloperidol administration on gene expression in order to probe its effect on cells.

id #68128

SCF Promotes Proliferation during Definitive Endoderm Differentiation of Human Pluripotent Stem Cells

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Publish consent withheld

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2. 2. Yasunaga, M. et al. Induction and monitoring of definitive and visceral endoderm differentiation of mouse ES cells. Nature Biotechnology 23, 1542-1550 (2005).

id #68129

AAV Testing Kit: fast-track method to identify highly functional AAV variants

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Adeno-associated virus (AAV) vectors are quickly becoming the “poster child” for therapeutic gene delivery. To this date, hundreds of naturally occurring isolates and synthetically engineered variants have been reported. While factors such as high production titre and low immunoreactivity are important to consider, the ability to deliver the genetic payload (physical transduction) and drive high transgene expression (functional transduction) remain the most important features when selecting AAV variants for (pre)clinical applications. Reporter expression assays are the most commonly used methods of determining vector fitness. However, such approaches are very time consuming and become impractical when evaluating large numbers of vector variants. Limited access to primary human tissues or challenging model systems, such as 3D organoids, further complicates vector testing. A convenient high-throughput method to determine AAVs’ transduction efficiencies would be beneficial.

We build a next-generation sequencing (NGS)-based AAV Testing Kit compatible with *in vitro*, *ex vivo* and *in vivo* applications. The AAV Testing Kit consists of a mix of 30 AAV variants that encode a CMV-eGFP cassette, that is identical between them apart from a unique 6-N barcode in the 3’ untranslated region of eGFP, allowing NGS barcode analysis at the DNA and RNA/cDNA levels. To validate the Kit, individually packaged barcoded variants were mixed at a 1:1:1 ratio and used to transduce cells/tissues of interest. DNA and RNA (cDNA) were extracted and subsequently analysed by NGS to determine the physical/ functional transduction efficiencies.

We were able to reliably assess the transduction efficiencies of immortalised cells, primary cells and iPSCs *in vitro* as well as *in vivo* tests in naïve mice and a xenograft liver mode. Our data is in agreement with previously reported transduction characteristics of individual capsids, validating our testing approach. More importantly, novel previously unknown tropisms were identified for some AAV variants.

id #68130

Developing safe and allo-tolerated pluripotent stem cells

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The development of stem cell-based regenerative medicine is rapidly advancing and has great potential to treat human diseases intractable to conventional medicine. Major hurdles, however, still hinder the widespread clinical application of cell-based therapeutics, including immune rejection of allogeneic cells and the risk of teratoma formation and malignant growths. Employinggenome-editing strategies, weaddressed the issues of safetyand immune-tolerabilitythrough developing the SafeCell(CS) and induced-allograft tolerance (iACT) systems, respectively.The SC system inserts a suicide gene into a cell division essential locus, allowing selective elimination of proliferative cells through the administration of a pro-drug, whilst also protecting the suicide gene from inactivation. The iACT system incorporates combined overexpression of immunomodulatory genes involved in inducing immune-tolerance in allogeneic transplantation settings. Here, we sought to demonstrate the application of our SC and iACT systems for treatment of neurological diseases. By incorporating expression of enhanced firefly luciferase, we used bioluminescence imaging to monitor the survival and proliferation of gene-edited mESCs after stereotaxic injection into the mouse brain. Here we show that SC mESCs canengraft, proliferate and differentiate in the mouse brain following injection. The proliferative component of the graft can be eliminated followingadministration of pro-drug. When injected subcutaneously in allogeneic mice, theiACTsystem has been proven to prevent rejection and allow long-term survival of allografts. We provide additional evidence that after stereotaxic injection into the brain, the iACT system protects the allograft against rejection during neuroinflammation.If validated, our approach harnessing the SC and iACT technologies would contribute to the development of safe, immune tolerant and more effective cell therapies for treating neurological disease.

id #68132

Meise System Autologous Serum Eye Drops Validation

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Introduction

Autologous Serum Eye Drops (ASED) is used as therapy for the treatment of a number of conditions that result in dry eye syndrome. There are numerous eye drop dispensing systems, including bottles, tubes and pouches.

In this study, we evaluated the safety and suitability of the Meise dispensing system for the production and transport of ASED in order to replace the discontinued tubing system manufactured by Macopharma.

Methods

A volume of 200-300mL of whole blood was collected from three donors and allowed to clot for a minimum of 2hrs. The serum was separated from the clot into a new transfer bag and centrifuged at 3000g for 15mins and 10°C. The serum was separated from erythrocytes using the Optipress and diluted with 0.9% Sodium chloride to a final 20% serum concentration. The bag containing ASED was sterile connected to a Meise system and filled. The vials were packaged, labelled and stored in a -80°C freezer. Temperature conditions during transport were evaluated in a simulated hot environment.

Results

A negative bioburden of the final product and a transport time at -20°C for 2hrs were the acceptance criteria. Serum yield was also evaluated.

- bioburden tests of the final ASED product were negative
- transport time at -20°C was maintained for up to 2hrs 30min.
- Average serum yield was 32% (range 18% to 46%)

Discussion

This validation demonstrated a better separation of serum from RBC using the Meise protocol, good serum yield and a transport time at -20°C of 2hrs, and no impact on serum eye drops bioburden results with the omission of the filtration step. Therefore the Meise eye drop system is a suitable replacement for the Macopharma tubing system.

id #68134

Vector stability in stored blood samples

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Vector persistence in blood is commonly examined during the development and use of gene transfer medicine. Reliable and standardised analysis of transgenes in blood also forms the basis of detecting the misuse of gene transfer technology in sport, called gene doping. The current study tested the effect on vector detection of blood samples stored at different temperatures for different time periods. As a model system, we used nonviral and AAV vectors carrying the human erythropoietin transgene spiked at 1500 copies per millilitre of blood from eight donors. Using previously optimised methods for DNA extraction and real-time PCR transgene detection, we followed the developed protocols for sample processing prior to storage and for sample freezing, and showed that storage of blood samples at -80°C for at least six months did not affect the reliability of transgene analysis. Storing samples for 3 months at -80°C or -20°C is equally acceptable, while storage for up to 30 days can be done in a fridge or

at -80°C or -20°C without affecting transgene detection. More informative and reliable results from vector clearance studies in gene therapy and consistent inter-laboratory comparisons may be attained by the adoption of these described protocols.

id #68136

AAV-mediated gene therapy for OTC deficiency: The trajectory to the clinic

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Summary: An adeno-associated viral (AAV) vector-mediated gene addition approach to treat paediatric patients with OTC deficiency has been optimised for highly efficient gene expression and delivery to human hepatocytes. Pre-clinical biodistribution, toxicology and vector stability studies have been carried out and funding obtained for a Phase I/II open label multicentre clinical trial.

Background: We have a long-standing interest in developing AAV vector-based therapies for paediatric metabolic liver disease with a focus on X-linked inherited OTC deficiency. OTC is a key enzyme of the urea cycle which functions primarily to detoxify ammonia, a by-product of protein catabolism. Excess ammonia is highly neurotoxic and failure to maintain normal levels leads to recurrent life-threatening hyperammonaemic decompensations and adverse neurological development. Severe presentation during the neonatal period can be fatal if treatment is delayed. Life-long medical supervision is required, with conventional interventions rarely achieving a normal quality of life. While liver transplantation is curative, clinical demand outweighs supply and patients risk disease progression while waiting for a donor liver. Furthermore, transplantation necessitates life-long immunosuppressive therapy accompanied by increased risk of malignancy. Alternative treatment options are desperately required.

Methods and Results: An AAV vector expressing human codon-optimised OTC successfully normalised urinary orotic acid (a sensitive marker) and conferred supraphysiological activity in a mouse model of OTC deficiency when pseudo-serotyped with the murine-tropic AAV8 capsid. The vector was re-packaged with a novel human-specific liver-tropic capsid, LK03, and tested in OTC patient hepatocytes engrafted in immune-deficient FRG mice. At ~30% human liver chimerism, urinary orotic acid is elevated. Following treatment with AAV-LK03.hOTC, orotates were normalised, remaining elevated in mock injected and control vector groups. Long-term toxicology studies in wildtype mice show no significant levels of tumorigenesis. Results from safety and biodistribution studies in NHPs are currently being analysed and stability studies on vector stored long-term are ongoing.

id #68140

Similar pluripotency, immunomodulatory, osteogenic and chondrogenic characteristics of canine induced pluripotent stem cell-derived mesenchymal stem cells and harvested mesenchymal cells

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With a view towards harnessing the therapeutic potential of canine mesenchymal stem cells (cMSCs) we have examined the immunomodulatory and anti-inflammatory properties of cMSCs produced from canine induced pluripotent stem cells (ciPSC.MSCs) in comparison with MSCs harvested from canine adipose tissue (cAT.MSC) and bone marrow (cBM.MSC). Deep sequencing of the ciPSC.MSC transcriptome confirmed that ciPSC.MSCs share a greater similarity with cBM.MSCs than ciPSCs. ciPSC.MSCs and cBM.MSCs express a range of pluripotency factors in common with ciPSCs; however, *ESRRB* and *PRDM-14*, factors associated with naïve, pluripotency were expressed only in the ciPSCs. *LOXL-2*, involved in epithelial to mesenchymal transition (EMT), is upregulated in both types of cMSCs while *CDH-1*, which is repressed during EMT, is downregulated in the MSCs compared to the ciPSCs. ciPSC.MSCs constitutively express immunomodulatory factors *iNOS, GAL-9, TGF-β1, PTGER-2α* and *VEGF*, and pro-inflammatory mediators *COX-2, IL-1β* and *IL-8*. When stimulated with pro-inflammatory cytokines canine tumor necrosis factor-α (cTNF-α), canine interferon-γ (cIFN-γ), or combination of both, ciPSC.MSCs upregulated expression of *IDO, iNOS*, *GAL-9, HGF, TGF-β1, PTGER-2α, VEGF, COX-2, IL-1β* and *IL-8.* To examine osteogenic and chondrogenic capabilities of ciPSC.MSCs in comparison to cAT.MSCs and cBM.MSCs, MSCs were cultured with chondrogenic and osteogenic differentiation media. Culture of ciPSC.MSCs and harvested MSCs with chondrogenic medium showed expression of cartilage-specific markers *SOX-9*, *COLII*, *aggrecan,* and *COMP*. When cultured in osteogenic medium all MSCs expressed *COLI* and immunostained positively for hydroxyapatite. All 3 types of MSCs were also seeded into decellularised extracellular matrix (DECM) scaffolds. DECM scaffolds seeded with iPSC.MSCs and cultured in chondrogenic medium showed deposition of new cartilage matrix confirmed by glycosaminoglycan (GAG) staining. DECM scaffolds seeded with iPSC.MSCs and cultured in osteogenic medium showed some staining with Alizarin red for calcium compounds. These findings support further investigation into the use of ciPSC.MSCs for management of canine immune-mediated and inflammatory disorders.

id #68141

Developing a methodological infrastructure for the qualitative and quantitative evaluation of unstable genomic elements in rAAV vectors

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In the adeno-associated viral vector (rAAV) system, single-stranded transgenic cargo is flanked by two 145nt T-shaped structures consisting of three internal, self-annealed inverted repeats. These structures, termed *inverted terminal repeats* (ITRs), are the only genomic element of the parental virus retained in the recombinant vector as they are required for vector genome replication, translocation into pre-assembled vector particles, and enhance extrachromosomal stability of the exogenous DNA in transduced cells. In spite of these important roles, the underlying mechanisms and their relation to ITR sequence and structure remain poorly understood. As capsid bioengineering continues to improve the efficacy of specific applications and tissue targets, elucidating the nature of the ITRs stands to benefit the platform as a whole, including both manufacturing and (pre)clinical applications. Unfortunately, although they are presumably stable in their physiological context, plasmid-encoded ITRs are susceptible to mutations and rearrangements, likely due to their high guanine/cytosine content and proclivity for secondary structure. Furthermore, these same features hinder our ability to quantitatively assess the nature and extent of ITR disrepair. The lack of analytical insight into ITR integrity negatively affects basic vectorological research as well as clinical and preclinical studies. We will present our progress towards establishing novel analytical techniques for robust and accurate analysis of ITR integrity in rAAV plasmid constructs. This work will greatly facilitate ITR research and form the foundation of novel quality control (QC) standards that will help enhance the clinical impact of rAAV vectors in gene therapy applications.

id #68142

Neuregulin 1 promotes intestinal stem cell proliferation and improves tissue regeneration following damage

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Identifying signals that regulate intestinal stem cell (ISCs) proliferation may enable stem cell pools to be manipulated in degenerative diseases and intestinal pathologies. A molecule which holds promise to support ISC-mediated tissue regeneration is the growth factor Neuregulin 1 (Nrg1).

We examined the expression of Nrg1 and its receptors in the small intestine using immunofluorescence and qRT-PCR. We observed that supporting niche cells express Nrg1, while ISCs express ErbB receptors, supporting a model where Nrg1/ErbB signalling directly regulates ISCs.

We investigated the role of Nrg1 *in vivo* using both an inducible gene knockout approach and a model where activation of Nrg1 signalling was achieved by injecting mice with 15ug Nrg1 for 5 days. Elevation of signalling increased intestinal cell proliferation and altered cellular differentiation. The molecular changes induced by Nrg1 treatment were examined using RNA sequencing which defined a strong impact on the cell cycle and conversion of progenitor cells to an intestinal stem cell identity. Loss of Nrg1 resulted in a significant decrease in cell proliferation in both stem and progenitor cells.

Using two mouse models of injury/regeneration (irradiation and 5-FU-induced damage), we observed that the expression of Nrg1 was 5-10 fold increased during the intestinal regenerative phase. Importantly, loss of Nrg1 dramatically impaired intestinal tissue regeneration following injury. In contrast, the regenerative response was significantly improved when mice were treated with Nrg1. This was reinforced by using an in vitro model of regeneration; Nrg1 significantly promoted organoid growth and the formation of colonies from single ISCs.

Overall, our results demonstrate Nrg1 is a potent niche signal that drives proliferation and regulates ISC identity.

id #68143

Precise AAV-mediated genome editing in primary hepatocytes: outcomes and consequences.

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Recombinant adeno-associated viral (rAAV) vectors are the current system of choice for *in vivo* delivery of reagents for liver gene therapy. The recent development of novel synthetic capsids has markedly improved the efficiency with which human hepatocytes can be transduced, bringing an increasing number of disease phenotypes within reach by conventional gene addition and enhancing the prospects for more challenging strategies such as precise gene repair. Precise gene repair involves the correction of the genetic defect directly at the mutant locus, allowing retention of physiological expression under endogenous control elements. In this study, we sought to correct a disease-causing mutation in the ornithine transcarbamylase (*OTC*) gene in patient-derived hepatocytes. Using rAAV vectors packaged in a highly hepatotropic capsid, we delivered CRISPR/Cas9 editing reagents to the livers of FRG (Fah-/-Rag2-/-IL2rg-/-) mice bearing xenografted primary human hepatocytes at three at clinically-relevant doses. Following delivery, we detected efficient cutting at the target locus, with up to 71.9% of *OTC* alleles modified. In mice treated at an intermediate dose the desired precise single nucleotide change was present in 12.9±1.9% of alleles with resultant restoration of OTC enzymatic activity. At the highest dose, the number of corrected alleles increased to 29%. On-target analysis revealed that a wide variety of modifications, including indels and vector integrations, could also be detected at the target site. Importantly, no modifications were detected by deep sequencing at predicted off-target sites. This study is the first demonstration of successful correction of a disease-causing mutation in patient-derived hepatocytes *in vivo*. Moreover, we were able to show that editing at therapeutic levels can be achieved in primary human hepatocytes despite activation of the DNA Damage Response and a transient inhibition of hepatocellular proliferation caused by the vectors.

id #68144

The abundance of adeno-associated viral (AAV) element cap, rather than rep, is the limiting factor in AAV vector packaging.

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Recombinant vectors based on adeno-associated virus (rAAV) are showing immense therapeutic promise in human trials, resulting in greater demand for clinical-grade rAAV vector production capacity.

rAAVs are traditionally produced via a transient transfection of three plasmids encoding:

1) a transgene flanked by inverted terminal repeats,

2) adenoviral helper proteins, and

3) two AAV genes, *rep* and *cap*,

into HEK293 cells. We hypothesised that the equimolar ratio of *rep*-to-*cap* genes, while evolutionarily optimised for packaging of the wild type virus, may be suboptimal for packaging rAAV vector. By separating *rep/cap* onto individual plasmids we were able to test the impact of different *rep*-to-*cap* ratios on the yield of natural and bioengineered AAV variants.

As the current AAV packaging model indicates that Rep proteins are involved in loading replicated AAV genomes into preassembled AAV particles, we hypothesised that increasing the amount of *rep* gene would enhance rAAV packaging. Additionally, since current vector production methods produce more empty than full capsids when quantified prior to purification, we hypothesised that the *cap* gene was provided in excess. In contrast, the data indicates that increasing the amount of *cap* was associated with improved packaging whilst increasing *rep* actually resulted in a reduction in packaging efficiency. The effect was more significant for variants that are known to be difficult to package, such as AAV2 and LK03, for which up to 3-fold improvements were achieved. Interestingly, vectors that were efficient at packaging using the canonical *rep/cap* plasmid such as AAV8 were found to be already optimal at a 1:1 *rep*-to-*cap* ratio.

The observed improvements in vector yields using our novel packaging system have the potential to revolutionise not only the lab-scale preclinical AAV manufacturing, but also large-scale clinical manufacturing, enhancing preclinical research and making AAV-based therapies more affordable for patients suffering from genetic disorders.

id #68145

The immunosuppressive capacity of mesenchymal stromal cells is conferred by their apoptosis in vivo

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Mesenchymal stromal cells (MSCs) have been extensively utilised in clinical trials to treat a wide range of diseases. However, their mechanism of action remains poorly understood, as there is little evidence that these cells survive *in vivo* administration. Therefore we sought to understand how MSCs mediate therapeutic effects that persist beyond their survival. We showed that MSCs localised to the lungs immediately after intravenous administration, where they underwent caspase 3-dependent apoptosis within 1 hour. Apoptotic MSCs were efferocytosed by lung phagocytes, which then shut down inflammation. Immunosuppression did not require MSCs to remain viable, as chemically induced apoptotic MSCs elicited immunosuppressive effects to a similar

extent as viable MSCs. Treatment of MSCs with pharmacological compounds that selectively inhibit regulators of apoptosis revealed that human and mouse MSCs utilised different pro-survival proteins. Having identified the molecular players involved in MSC apoptosis and survival, we generated apoptosis-resistant MSCs via CRISPR/Cas9 technology. Utilising disease models commonly used to define MSC potency, we were able to assay the therapeutic efficacy of these apoptosis-resistant MSCs to answer the outstanding question of how short-lived MSCs are immunosuppressive. Our data have significant implications for the development of MSC potency assays and biomanufacturing for clinical translation.

id #68148

Comparison of Aerobic and Anaerobic bacteria, yeast and mould detection using the BacT/Alert® 3D™and Virtuo™ systems for Haematopoietic Progenitor Cells

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The St George Hospital Microbiology department perform bioburden testing of blood products collected from HPCs, received from the Cell and Molecular Therapies (CMT) laboratory at RPAH. The Microbiology lab is introducing the new BacT/Alert® Virtuo™ to replace the BacT/Alert® 3D™ system. The Virtuo™ instrument is an automated microbial test system capable of incubating, agitating and continuous monitoring for reduced time-to-detection of aerobic, facultative, and anaerobic microorganism growth from blood and other body fluids.

Bact/ALERT® FAN Plus aerobic, anaerobic and paediatric bottles manufactured by BioMerieux are used with both blood culture instruments and the adsorbent polymeric beads and media volume remained unchanged.

AIM:

The testing will compare BacT/Alert® 3D™ and BacT/Alert® Virtuo™ monitoring systems and establish that the Virtuo™ is fit for purpose to detect contamination of blood products.

METHOD:

A group of 13 fungi, aerobic and anaerobic bacteria were used during validation. The organisms were inoculated into FAN Plus aerobic, anaerobic and paediatric bottles in a seeded bottle trial. The bottles contained human plasma and processed cells collected and processed from patients at at RPAH.

RESULTS

In the seeded trial and in the bottles containing patient plasma, all 13 organisms were isolated within the incubation period from at least one of the FAN Plus aerobic, anaerobic or paediatric bottles loaded after inoculation. All expected organisms were isolated from the FAN Plus paediatric bottles containing processed cells with DMSO.

CONCULSIONS

This validation confirms that the new method using the new BacT/Alert® Virtuo™ instrument and FAN Plus bottles range is able to detect a range of organisms from patients undergoing treatment regimes that include chemotherapy and/or mobilisation regimes (e.g. granulocyte colony stimulating factor), and from processed cells for cryopreservation with the addition of DMSO. It also demonstrates that the 5-day incubation period is sufficient to recover fungi, aerobic and anaerobic bacteria.

id #68250

In vitro modelling of SCN2A genetic epilepsy using human pluripotent stem cells

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SCN2A, encoding a brain sodium channel responsible for regulation of excitability, has emerged as a major single gene implicated in neurogenetic disorders. The spectrum of SCN2A conditions includes mild and severe forms of early or childhood-onset epilepsy, autism, intellectual disability and schizophrenia. Severe epilepsy is often combined with cognitive and behavioural impairments and these highly severe conditions are called developmental and epileptic encephalopathies (DEEs). Analyses of SCN2A variants suggests a correlation between the clinical presentation, the pharmacosensitivity and the functional impact measured in different disease models. Given the high phenotypic heterogeneity, the mechanisms underlying the SCN2A pathophysiology have not been fully unravelled.

In this study, we interrogated two of the most recurrent SCN2A variants detected in early (<2 weeks) and late (>3 months) onset DEE, respectively: R1882Q and R853Q. Patient-derived iPSC lines and the corresponding isogenic controls were differentiated using the NGN2 overexpression protocol to obtain cortical glutamatergic neurons. Electrophysiological properties and expression profile were assessed to identify any functional abnormality. The results suggest increased activity of R1882Q neurons and no significant changes for R853Q compared to the controls.

Overall, our data show the validity of a iPSC-derived *in vitro* model to investigate genetic epilepsies and subsequently to test/screen innovative therapies.