PICK 'N' MIX I

TIME: 15.25 – 16.05

LOCATION: PARSONS ROOM

DEVELOPMENT OF IPSC DERIVED BASAL CELLS AND DIFFERENTIATION INTO LUNG AIRWAY ON AIR LIQUID INTERFACE

Ivo Djidrovski, Institute of Genetic Medicine, Newcastle University

The conducting airways of the lungs are constantly exposed to the damaging effects of pollutants, gasses and nanoparticles present in the atmosphere. It is of particular interest to understand and model those interactions as part of risk assessment evaluations in the process of drug discovery. Current *in vitro* models rely on the availability of primary lung epithelial cells or the use of immortalized cell lines which poorly represent human biology, highlighting the need for a better model.

We developed a new strategy to isolate iPSC derived basal like cells of high purity from a mixed population of lung progenitors by integrating several approaches used in primary bronchiolar airway cells. Those cells are all positive for lung basal cell markers *cytokeratin 14*, *NGFR*, *Integrin alpha 6* and *ΔNp63* and can be expanded for several passages while maintaining their multi-potency. When those cells were differentiated on an air-liquid interface, they formed tight junctions and a structured pseudostratified epithelium, strikingly similar to *in vivo* like airway. The iPSC derived airway-contained functional basal cells, goblet cells, club cells and ciliated cells confirmed by immunohistochemistry. A mucus layer was formed on the apical side and beating cilia could be observed on BF microscopy. Preliminary testing in collaboration with PHE gave us encouraging results.

This is the first functional iPSC derived lung airway model on an air liquid interface, which offers many advantages to the alternative existing models which is exciting, however further characterisation will be needed to understand the full potential of the model.

OPTIMISED *IN VITRO* TESTING FOR RETINAL DISEASE: GENERATING 3D RETINAL ORGANOIDS FROM RAT AND PRIMATE PLURIPOTENT STEM CELLS

Madeleine Carter, Institute of Genetic Medicine, Newcastle University

Degenerative retinal diseases, such as retinitis pigmentosa (RP) and age-related macular degeneration (AMD), are the leading cause of irreversible blindness worldwide. Currently there are no available treatments to effectively restore vision for affected patients and many suffer complete vision loss. Retinal organoids (ROs) generated from pluripotent stem cells (PSCs) offer a platform to study the development and response of retinal cells to potential therapeutic agents in an *in vitro* model system. This project aims to expand on existing research by developing protocols for the differentiation of ROs from toxicologically relevant animal species and thereby provide a novel model system to reduce or replace *in vivo* testing in pre-clinical studies.

Using a 96-well plate format and by optimising protocols published for mouse PSCs we have successfully differentiated rat induced PSC (iPSCs) and embryonic stem cell (ESC) lines into organoids, with an early neural-retinal identity as characterised by gene expression and immunohistochemistry. Organoids showed genetic upregulation and cells positive for the early retinal markers *Chx10, Pax6 and Rax*. Similarly, by adapting protocols published for human PSCs we have generated organoids from cynomolgus macaque iPSCs, which have retinal identity. This is determined by *Crx, Vsx2* and *γ-synuclein* positive cells and a phase-bright morphology highly characteristic of neuroepithelium in human ROs.

Further method optimisation will improve organoid maturity and generate functional photoreceptor cells.
This represents a significant transition away from the traditional use of animals in drug development studies towards an innovative, scalable and faster approach to treating retinal disease.

IDENTIFICATION, OPTIMIZATION AND EVALUATION OF IPCS INHIBITORS AS NEW ANTI-LEISHMANIALS

Courtney Covington, Durham University

Leishmaniasis is a vector-borne neglected tropical disease caused by protozoan of the genus Leishmania. With an annual incidence of 1.3 million new cases across 98 countries and 350 million of the world's most vulnerable populations considered at risk, this is global health challenge. Current chemotherapies are insufficient, have variable efficacies, and face growing drug resistance. For these reasons, is imperative to find new lead compounds that can be translated into safe, cheap, and easy to administer drugs. Inositol phosphorylceramide synthase (IPCS) is a membrane bound enzyme involved in sphingolipid biosynthesis. This enzyme is important for the pathogenesis and survival of Leishmania. As the parasitic IPCS (LmjIPCS) has no direct mammalian orthologue, our group has identified it as a potential drug target which would allow access to highly selective drugs. We have developed a simple biochemical assay of LmjIPCS activity to assess potential inhibitors. In collaboration with GSK-DDW, further development enabled a high throughput screen of a 1.8 million compound library against LmjIPCS. Two of the six most promising compounds identified contained a benzazepine core as potential pharmacophore. These compounds demonstrated high in vitro activity coupled with low mammalian cytotoxicity. This lecture will present the details of this screening campaign together with ongoing synthetic studies towards additional benzazepine analogues with more favourable physicochemical properties, the development of structure activity relationships, and confirmation of on target effects.

USING WHOLE EXOME SEQUENCING TO IDENTIFY DE NOVO MUTATIONS IN CASES OF SEVERE IDIOPATHIC MALE INFERTILITY

Hannah Smith, Biosciences Institute, Newcastle University

Infertility affects around 1 in 6 couples worldwide and in around 50% of these cases, the infertility can be attributed to a male factor. Whilst causes such as Klinefelter's and Y chromosome microdeletions have been well distinguished, the genetic causes behind severe spermatogenic failure are largely unknown, with around 40% of all male infertility cases remaining idiopathic. *De novo* mutations (DNMs) arise spontaneously in the germline or post-zygotically and are known to be associated with early onset disorders. These mutations however, are rarely studied due to the difficulty in obtaining parental samples. In our study, we have examined and sequenced 160 patients suffering from non-obstructive azoospermia or severe oligozoospermia and their fertile parents. Initially we have identified and validated 90 protein-altering DNMs, which show an enrichment of loss-of-function (LoF) variants in extremely LoF intolerant genes. Of all these DNMs, 52 are likely to disrupt normal gene function and lie in genes expressed in the testis. Of those, 22 lie in genes involved in sperm production such as *TOPAZ1* and *ODF1*. Currently, none of these genes are widely recognised as human male infertility genes. Our data provides the first indications that DNMs may play an important role in severe male infertility however, further replication studies in larger cohorts are required in order to further validate the findings of our initial results and to identify any novel disease-causing mutations and the genes involved. In the future, this research could help us to move towards more successful diagnoses for male infertility patients.