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RACP Foundation Research Awards

YEAR 1 PROGRESS REPORT

Project / Program Title		Characterising the molecular basis of cystic kidney diseases using kidney organoids derived by directed differentiation of induced pluripotent stem cells
Name		Dr Thomas Forbes
Award Received		2016 Jacquot NHMRC Award for Excellence
Report Date		1 December 2016
Chief Investigator / Supervisor		Thomas Forbes / Melissa Little & Catherine Quinlan
Administering Institution		Murdoch Children's Research Institute
Funding Period	Start Date:	8 February 2016
	Finish Date:	8 February 2019

PROJECT SUMMARY

Nephronophthisis (neff — ron — off— thee — sis; NPHP) is the most common form of genetic kidney disease affecting children and young adults and inevitably leads to the need for dialysis and kidney transplantation. There is a large and ever expanding list of genes shown to associate with NPHP, however, for 50-70% of patients undergoing genetic testing, no gene mutation is found. Additionally, we don't completely understand how these abnormal genes lead to this form of cystic kidney disease.

It has been established that all the genes associated with NPHP (as well as those responsible for other cystic kidney diseases in children and adults) are found in the primary cilium. The primary cilium is part of the cell that resembles and acts like an antenna which detects what is going on around the cell and guides specific pathways inside it, controlling important cell functions (for example: maintaining the correct cell orientation and controlling the sequence of cell division.)

Recent advances in stem cell research allow us to create stem cells from patient skin cells (called induced pluripotent stem cells or iPSC). These stem cells can be instructed to become living kidney tissue in a dish. The opportunity to research living kidney tissue carrying the exact DNA of a patient is unprecedented. Additionally, new gene editing techniques allow us to correct suspected DNA abnormalities and examine the effect of that correction on cell function.

In this PhD project, I aim to examine the DNA messaging of stem cell derived kidney tissue in patients with NPHP and compare this to the same patient's gene-corrected kidney tissue to discover the ways that these mutations cause NPHP. This research may also help discover new genes for patients where modern genetic testing hasn't found a recognised gene mutation. By learning the disease mechanisms of NPHP, we hope to guide the development of treatments to slow the progression of this disease.

PROJECT AIMS / OBJECTIVES

The aims of the project are:

1. to apply, optimise and validate the existing kidney differentiation protocol to patient derived iPSC lines.

2. to characterise dysfunctional molecular pathways in iPSC kidney organoids from patients with nephronophthisis due to recognised mutations.

3. to apply the same approach to nephronophthisis patients where whole exome or genome sequencing has found novel mutations or no mutations.

Suitable patients have been recruited to the KIDGEN consortium which operates a translational clinical and research program at multiple paediatric and adult hospitals and laboratories in Australian state capitals.

iPSC lines are derived using episomal-based transfection strategies integrating CRISPR/Cas9 gene editing technology to correct known mutations, currently the gold standard control in this field.

Following directed differentiation to renal endpoint, organoids will be dissociated and magnetically sorted to isolate the epithelial (EpCAM positive) fraction for further analyses.

RNA extracted from this epithelial fraction will be subject to RNA sequencing using Illunnina NextSeq sequencer (Institute of Molecular Biology in Brisbance). Following bioinformatic analysis of the sequencing output and prioritisation of differentially expressed genes, validation of the findings using qPCR, innmunofluoresence, cyst culture and other techniques as applicable will validate sequencing results.

SIGNIFICANCE AND OUTCOMES

There is increasing recognition of the health and economic burden of genetic renal disease, with up to 20% of adults and 47% of children on dialysis or post-kidney transplantation having an underlying genetic aetiology for their primary disease. Advances in genomic diagnostics is likely to see these figures rise.

End stage renal disease (ESRD), including dialysis and kidney transplantation, is predicted to cost the Australian healthcare system as much as $12.3 billion annually by 2020. The opportunity to develop a greater molecular understanding of genetic renal disease and guide the development of treatments to delay the onset of ESRD represents a significant opportunity to improve health related quality of life, reduce healthcare expenditure and patients waiting for kidney transplants. Kidney organoids may well prove useful in testing new drug treatments for personalised therapeutics.

During this PhD, we plan to extend this research to patients recruited by the KIDGEN consortium where novel mutations have been identified or where no mutation has been identified on massive parallel sequencing.

PUBLICATIONS / PRESENTATIONS

I have contributed to an invited textbook chapter entitled 'Recapitulating development to generate kidney organoid cultures' authoring subsections on (i) Comparisons between different renal differentiation protocols and (ii) Disease modelling using kidney organoids (currently under editorial review)

This year I have presented this project at the Australian and New Zealand Paediatric Nephrology Association AGM (Sept) and the KIDGEN Renal Genetic Symposium (Nov).