Childhood Eye Cancer Trust (CHECT) Research Grant Final Report (end of grant report)

Please complete all sections of this report and return to petra.maxwell@chect.org.uk.

Project title:	Modelling retinoblastoma using human induced pluripotent stem cells		
Project reference:	(To be completed by CHECT)		
Total award:	£34,103		
Details of any additional funding:	N/A		
Lead investigator:	Sandy Hung & Sandra Staffieri	Administering institution:	Centre for Eye Research Australia
Start date of award:	1st July 2015	End date of award:	31st December 2016

1. Final Report

Please structure your report as follows:

a. Summary of findings/results/outcomes of this project (with reference to the aims and objectives stated in your original application).
b. If your aims and objectives changed during the course of the project, please explain why and in what way.
c. Any problems or challenges impacting on the findings / results / outcomes of this project.
d. How will these findings or outcomes impact patients or the public, and in what timescale? Do you foresee any obstacles / barriers to patients benefitting from the research findings?

Summary:

In this project, we have successfully derived induced pluripotent stem cell (iPSC) lines for each of the three groups: control, retinoblastoma (RB) survivors, as well as generated RB1 knockout samples using CRISPR. Characterisation of these iPSC lines confirmed the quality of the cells. We showed that the iPSCs were pluripotent and retained potential to differentiate into all three germ layers, and using CRISPR we have successfully generate RB1 knockout iPSCs.

Subsequently, we differentiated these iPSC lines into retinal organoids and performed transcriptome analysis, which allow us to study the effect of RB1 mutation on human retinal cells. We were able to detect expression of retinal marker genes in the organoids, including rod photoreceptors, cone photoreceptors, Muller glia, astrocytes, microglia, bipolar cells, retinal ganglion cells, amacrine cells and horizontal cells. These results showed that the retinal organoid samples contained all major neural retinal cell types and supported the quality of the derived organoids. We performed differential gene expression analysis to analyse the molecular differences between iPSC from the different groups. Overall, we detected 189 differentially expressed genes between control and RB survivor derived organoids, and 4937 differentially expressed genes between control and RB CRISPR knockout organoids. These results showed that RB1

CRISPR knockout organoids show more transcriptome abnormalities compared to those derived from RB survivors, which is consistent with data showing that RB survivors exhibited mutations which may cause a knockdown and reduction in RB1 gene function, rather than a knockout of RB1 in the CRISPR knockout lines. Next, we performed gene cluster analysis to identified gene groups with consistent trajectory, including Group 1 genes (1100 genes) which are up-regulated in RB survivor/RB1 CRISPR knockout, and Group 2 genes (1761 genes) which are down-regulated in RB survivor/RB1 CRISPR knockout. Interestingly, subsequent gene ontology analysis showed that the group 1 genes are involved in processes related to oncogenesis, including mitotic cell cycle, damaged DNA binding, DNA replication. On the other hand, group 2 genes are implicated in cell attachment and morphological changes, including processes in extracellular matrix organization, collagen binding and PDGF binding. As a result, we have identified the gene targets involved in these processes, providing a list of downstream effectors of RB mutations in retinal organoids and potential gene targets to develop novel treatment for RB.

Aims and objective changes/ problems and challenges:
A few of the objectives had to be changed during the course of the experiment due to difficulties in maintaining long term cultures for up to 1 year, due to maternity leave and unforeseen laboratory moves. RNA samples were harvested for transcriptomic analysis instead of taking cells for immuno-staining and FACS experiments, due to the low cell number and insufficient numbers of optic cups available. It is also for this reason that the time points as outlined in the proposal could not be performed.

Finding outcome and impact on patients:
In summary, our results supported that iPSC-derived retinal organoids can be used as a disease model to recapitulate the pathological effects of RB dysfunction. The RB iPSC model generated in this project would enable further studies to understand the mechanism underlying RB pathology, as well as a useful in vitro platform for drug discovery to improve treatment options for RB.

2. Plain English summary (appendix 1).

Please provide a brief plain English summary of your final report above, including any findings or outcomes, and their potential impact on patients or the public. CHECT (and funding partners) will publish this summary in the public domain to demonstrate how we support research, therefore please do not include any confidential or commercially sensitive information.

In this project we set out to determine if we can generate a stem cell model in the laboratory to study the retinoblastoma disease. To do this we collected skin cells from survivors of retinoblastoma with known retinoblastoma gene mutations. Using these skin cells, we made stem cells using a technique called cellular reprogramming, and subsequently turned the stem cells into retinal cells by providing the cells with different growth factors. This strategy allows us to study what's happens in retinal cells derived from the patient without asking the patient to donate their retina, and understand the process of how gene mutations can affect the retinal cells in retinoblastoma. Using our stem cell model, we found that retinal cells from retinoblastoma survivors possess abnormal gene expressions with a cancer signature. The results from our findings validates the use of using this stem cell model to study and test potential treatments for retinoblastoma.

3. Publications

Please list all published or accepted papers and abstracts from the work of this grant (journal style) (attach copies where available)

Hung SSC*, McCaughey T*, Swann O, Pébay A, Hewitt AW (2016) Genome Engineering in Ophthalmology: Application of CRISPR/Cas to the Treatment of Eye Disease. Progress in Retinal and Eye Research 53:1-20

Hung SSC, Chrysostomou V, Li AF, Lim JKH, Wang JH, Powell JE, Tu L, Daniszewski M, Lo C, Wong RCB, Crowston JG, Pebay A, King AE, Bui BV, Liu GS*, Hewitt AW*(2016) AAV-mediated CRISPR/Cas gene editing of retinal cells in vivo. Investigative Ophthalmology & Visual Science 57(7):3470-3476

Hung SSC, Khan S, Lo C, Hewitt A, Wong R. (2017) Drug discovery using induced pluripotent stem cell models of neurodegenerative and ocular diseases. Pharmacology and Therapeutics, 177:32-43

Hung SSC, Li F, Wang J, King AE, Bui BV, Liu G, Hewitt AW (2018) Methods for in vivo CRISPR/Cas editing of the adult murine retina. Methods in Molecular Biology (Retinal Gene Therapy) 1715:113-133

4. Dissemination of results

Please list where and by whom any results/findings have been disseminated (e.g. conferences, workshops, public engagement events)

The following presentation were given by Dr Sandy Hung

Invited oral presentation:
- Asia ARVO (The Association for Research in Vision and Ophthalmology) conference (2017, Brisbane, Australia)
- Therapeutic Technologies Research Initiative symposium- Industry meets Academia (2016, Melbourne Australia)

Poster presentation at conference:
- Therapeutic Technologies Research Initiative symposium- Industry meets Academia (2016, Melbourne Australia)

5. Intellectual property (IP)

Please list any IP arising from the research, and whether it is wholly owned by the researcher.

N/A

6. Collaborations

Please list any collaborations which have arisen during or as a result of this research.

N/A

7. Future research and funding

Please provide details of any further research/ideas planned and where potential funding will be sourced from as a result of this project

- Awards received as continuation to this project:

- (2016) Therapeutic Technologies Research Seed Funding (AU$8,000) CIs: Raymond Wong, Sandy Hung, Sandra Staffieri, Alice Pébay, Alex Hewitt, David Mackey. Modelling retinoblastoma using human induced pluripotent stem cells (iPSC)
- (2017) The Ophthalmic Research Institute of Australia- Brenda Mitchell Grant (AU$50,000) CIs: Sandy Hung, Sandra Staffieri. Using patient-derived stem cells to model retinoblastoma.
- (2021) Global Ophthalmology Awards Program (GOAP) from Bayer (US$49,650) CI: Sandy Hung. Constructing a disease retina gene atlas for retinoblastoma using spatial transcriptomics

8. Any further comments

N/A

Report completed by:	Sandy Hung	Date of report:	30/10/2020

Appendix 1: Writing a plain English summary in your Childhood Eye Cancer Trust (CHECT) final report [i]

A plain English summary is a clear explanation of your research that should be accessible by an interested audience.

Your final report will be reviewed by experts on the CHECT Scientific Advisory Committee but also by lay members of the SAC and CHECT Board members who are not scientific experts. It will also be accessible through the CHECT website.

A good quality plain English summary providing an easy to read overview of your whole study will help:

- those carrying out the review (reviewers and Board and panel members) to have a better understanding of your research
- inform others about your research such as those affected by retinoblastoma (Rb), members of the public, health professionals, policy makers and the media
- research funders to publicise the research that they fund.

The summary is important. If it is felt that your plain English summary is not clear and of a good quality then you may be required to amend your summary prior to the final closure of the project.

What to include in your plain English summary

Your plain English summary should be 300 words or less. When writing the summary consider including the following information:

- Aim(s) of the research
- Findings or outcomes
- Potential impact on Rb individuals and families

How to write a plain English summary

The people who will read your summary will be an interested audience, but are not necessarily specialists. Therefore write your summary with this audience in mind, for example at the same level as an article in a newspaper.

There are a few simple rules for writing in plain English. In summary these are:

- Avoid wherever possible using jargon, abbreviations and technical terms. If you have to use them provide a clear explanation
- Avoid complicated English or uncommon words
- Use active not passive phrases: for example say ‘we will do it’ rather than ‘it will be done by us’
- Keep sentences short
- Think about the order and structure
- Break up the text. For example use bullet lists
- Ask patients / carers / colleagues to read a draft to find out if anything is unclear.

The plain English summary is not the same as a scientific abstract. Please do not cut and paste this or other sections of your final report to create the plain English summary. Further guidance on plain English summaries for research is available on https://www.invo.org.uk

[i] Based on the NIHR guidance available at https://www.nihr.ac.uk/about-us/CCF/PPI/Plain_English_summaries_in_National_Institute_for_Health_Research_funded_research.pdf