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(please insert ref. above)

The Research Grants Council of Hong Kong
NSFC/RGC Joint Research Scheme
Joint Completion Report

(Please attach a copy of the completion report submitted to the NSFC by the Mainland researcher)

Part A: The Project and Investigator(s)

1. Project Title

Targeted Gene Correction and Disease Modeling Using Wilson's Disease Induced Pluripotent Stem Cells
肝豆狀核變性疾病的誘導多能幹細胞用於基因靶向治療和疾病模型建立的研究

2. Investigator(s) and Academic Department/Units Involved

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Professor Tse Hung-Fat	Professor Esteban Miguel
Post	Chair Professor	Principle Investigator
Unit / Department / Institution	Division of Cardiology, Department of Medicine, University of Hong Kong	South China Institute for Stem Cell Biology and Regenerative Medicine, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences
Contact Information	Mr. Jiayin Yang	Dr. Xichen Bao
Co-investigator(s) *(with title and institution)*	Dr. Jenny CY Ho The University of Hong Kong	Dr. Xichen Bao Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted*)
Project Start date	01-01-2013	Nil	Nil
Project Completion date	31-12-2016	Nil	Nil
Duration *(in month)*	48 months	Nil	Nil
Deadline for Submission of Completion Report	31-12-2017	Nil	Nil

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1) **Specific aim 1 (SA1)** is to generate integration-free iPSCs from individuals with Wilson's disease (WD) bearing R778L and H1069Q *ATP7B* mutations.

2) **Specific aim 2 (SA2)** is to correct the *ATP7B* mutation in WD-iPSCs using ZFNs and *piggyBac* technology.

3) **Specific aim 3 (SA3)** is to evaluate the therapeutic efficacy of gene correction by comparing the subcellular distribution of ATP7B and the pattern of copper export upon copper overload of HLCs derived from genetically modified iPSCs. As part of this aim we will also perform pathway discovery using microarrays of HLCs from wild-type and ZFN-corrected WD-iPSCs treated with increasing concentrations of copper.

4) **Specific aim 4 (SA4)** is to generate an *atp7b*-/- mouse model for assessing the *in-vivo* therapeutic efficacy of corrected iPSC-derived WD HLCs.

5.2 Revised Objectives

Date of approval from the RGC: Nil

Reasons for the change: Nil

6. Research Outcome

Major findings and research outcome

(maximum 1 page; please make reference to Part C where necessary)

6.1 Generated iPSC lines from 2 more WD patients and 1 non-affected carrier

Besides the available WD iPSC harboring R778L mutation in *ATP7B*, we have screened the *ATP7B* coding region in several Chinese patients with WD, and obtained 3 more patient's biopsy with WD that harbor mutation in ATP7B, including homozygous R952K mutations, compound heterozygous T935M and R952K mutations, and a heterozygous R952K mutation. WD iHeps produced from these iPSCs thus represent an excellent cellular model that could help to delineate the relative impact of various mutations on *ATP7B* function and identify the modifying factors. **(Appendix Figure 1 & 2)**

6.2 Footprint-Free Gene Correction of ATP7B Mediated by CRISPR/Cas9 and ssODNs or Piggybac

The best strategy for gene correction shall be seamless gene targeting, as any fragments left in the genome, even in the intron or 3' downstream elements after targeting has the risk to interrupt target gene expression (*eg.* Cre-loxP system). In this study, we transfected WD patient derived hiPSCs carrying a homozygous R778L mutation with the CRISPR/Cas9-expression plasmids together with *Piggybac* or ssODNs. CRIPSR/Cas9 cleavage induced homologous recombination, which enabled the replacement of the mutated allelic with *Piggybac* or ssODNs. After several round of screening, we successfully obtained seamless gene corrected iPSCs. **(Appendix Figure 3-8)**

6.3 Genetically corrected iHeps restore ATP7B subcellular localization

These genetically corrected iPSCs retain pluripotency and normal karyotype, and can differentiated into iHeps efficiently. We further assessed the subcellular location of ATP7B in genetically corrected iHeps, and found that more than 90% of ATP7B show co-localization with P230 (vs. 30% in WD iHeps). Indicating that genetically corrected iHeps restore ATP7B subcellular localization. To further assess the function of the ATP7B in the gene-corrected iHeps, we designed a copper overload assay with luciferase reporters, and our data show that ATP7B in gene-corrected iHeps restore copper exportation capacity **(Appendix Figure 9-11)**

6.4 Generation of an in-vivo transplantation mouse model for assessing cell therapy efficacy

As a platform, we have generated an *in-vivo* transplantation mouse model (*Ldlr*$^{-/-}$/*Rag2*$^{-/-}$/*Il2rg*$^{-/-}$) of familial hypercholesterolemia for testing LDL-C lowering drugs (see Part C8, has been published in *Stem Cell Reports*, IF=7.338). With the same concept, we have generated *Atp7b*$^{-/-}$/*Rag2*$^{-/-}$/*Il2rg*$^{-/-}$ for assessing cell therapy efficacy. And in this model, our engrafted wide-type iHeps show significant efficacy in attenuate liver injury. **(Appendix Figure 12-13)**

6.5 Liposome-embedded curcumin attenuates liver injury and elevate lipid metabolism in Atp7b$^{-/-}$*/Rag2*$^{-/-}$*/Il2rg*$^{-/-}$ *mouse*

Our previous *in-vitro* data shows that, addition of the pharmacological chaperone curcumin partially rescued ATP7B distribution in WD iHeps. Interestingly, in this study, our liposome-embedded curcumin generates tremendous outcomes, it can not only attenuate *Atp7b*$^{-/-}$/*Rag2*$^{-/-}$/*Il2rg*$^{-/-}$ mice liver injury but also elevate mice lipid metabolism, which is competent to be a drug candidate for WD. **(Appendix Figure 14)**

Potential for further development of the research and the proposed course of action *(maximum half a page)*

1) Assessing the in-vivo therapeutic efficacy of corrected iPSC-derived WD iHeps

We will assess the outcome of the repopulation of our genetically corrected iHeps in *Atp7b*$^{-/-}$/*Rag2*$^{-/-}$/*Il2rg*$^{-/-}$ mice, then compare WD-iHeps, WT-iHeps and vehicle, to assess *in-vivo* therapeutic efficacy, in terms of liver injury, lipid metabolism, liver functions, liver copper content, urinary copper excretion, serum holoceruloplasmin level *etc.*

2) Assess the efficacy of liposome-embedded curcumin in treatment of Wilson's disease

Based on our exciting preliminary data on immunodeficient *Atp7b*$^{-/-}$ mouse, we will then employ normal *Atp7b*$^{-/-}$ mouse to assess *in-vivo* therapeutic efficacy of liposome-embedded curcumin, in terms of liver injury, lipid metabolism, liver functions, liver copper content, liver inflammatory level, urinary copper excretion, serum holoceruloplasmin level *etc.*, potential pathways are also to be investigated to address the relative mechanisms. This will benefit the society if we get successful, as curcumin is a food supplement without safety concern. Due to significant low bioavailability, the formulation and delivery approach is still under-optimized.

7. The Layman's Summary

(describe in layman's language the nature, significance and value of the research project, in no more than 200 words)

Wilson's disease (WD) is a genetic disorder in which copper builds up in the body. Symptoms are typically related to the brain and liver. WD is due to homozygous or compound heterozygous mutation in the *ATP7B* gene. In this study, we generated a disease model with stem cells derived liver cells and investigated the feasibility of using genetically corrected method to amend the disease. Our results show that, seamless gene correction could be achieved and can correct the phonotype in our cellular model. We further created an immunodeficient knock-out mouse model for testing the efficacy of cell therapies, and we find that the transplanted normal liver cell could attenuate mice liver injury. In addition, we are happy to discover that, curcumin, which is abundant our daily food, can not only protect *Atp7b*$^{-/-}$ mice from liver injury but also enhance mice lipid metabolism. Our findings provide important insights into drug development and cell therapies to WD, and also provide a novel drug development model for many other liver diseases.

Part C: Research Output

8. Peer-reviewed journal publication(s) arising directly from this research project

(Please attach a copy of each publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications				Author(s) ***(bold** the authors belonging to the project teams and denote the corresponding author with an asterisk*)*	Title and Journal/ Book *(with the volume, pages and other necessary publishing details specified)*	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of publication	Year of Acceptance *(For paper accepted but not yet published)*	Under Review	Under Preparation *(optional)*						
-	-	-	2017	**Yang, J., Hong, X., Wong L., Wei, R.,** Yang B., Liu Y., **Lai, W., Au, K., Esteban M.A.***, **Tse, H.F.***	Footprint-Free Gene Correction of *ATP7B* Mediated by CRISPR/Cas9 and ssODNs or Piggybac	Nil	Nil	Nil	Nil
-	-	-	2017	**Yang, J., Wei, R., Wong L.,** Yang B., Liu Y., **Lai, W., Au, K., Hong, X., Esteban M.A.***, **Tse, H.F.***	liposome-embedded curcumin attenuates liver injury in *Atp7b-/-* mouse	Nil	Nil	Nil	Nil
-	2017	-	-	**Yang, J.,** Wang, Y., Zhou, T., **Wong, L.**, Tian, X., **Hong, X., Lai, W., Au, K., Wei, R.,** Liu, Y., et al., **Xichen Bao, Esteban M.A.***, **Tse, H.F.***	Generation of human liver chimeric mice with hepatocytes from familial hypercholesterolemia induced pluripotent stem cells/ *Stem cell reports*	No	Yes	Yes	Yes
-	2017	-	-	Li Li, Quanjun Zhang, Huaqiang Yang, Qingjian Zou, Chengdan Lai, Fei Jiang, Ping Zhao, Zhiwei Luo, **Jiayin Yang** et al., Hung-Fat Tse, Baoming Qin, Xichen Bao, **Miguel A. Esteban*** and Liangxue Lai	Fumarylacetoacetate hydrolase knockout rabbit model for hereditary tyrosinemia type 1/*Journal of Biological Chemistry*	No	Yes	Yes	Yes

9. Recognized international conference(s) in which paper(s) related to this research project was/were delivered

Please attach a copy of each delivered paper. All listed papers must acknowledge RGC's funding support by quoting the specific grant reference.)

Month/Year/ Place	Title	Conference Name	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
06/2017	Familial Hypercholesterolemia iPSC-Derived Hepatocytes Enhance Ldl-C Clearance and Respond to Ldl-C Lowering Drugs in *Ldlr$^{-/-}$/Rag2$^{-/-}$/Il2rg$^{-/-}$* Mice (Poster presentation)	The International Society for Stem Cell Research (ISSCR) 2017 Annual Meeting	Nil	Yes	Yes	No
10/2017	Footprint-Free Correction of ATP7B in Wilson Disease Human Induced Pluripotent Stem Cells	Keystone Symposia on Molecular and Cellular Biology T3: Regenerative Biology and Applications: Cell Differentiation, Tissue Organization and Biomedical Engineering.	Nil	Yes	Yes	Not sure

10. Student(s) trained *(Please attach a copy of the title page of the thesis.)*

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
Yang Jiayin	PhD	01-07-2014	13-07-2018
Wei Rui	MPhil	01-09-2016	31-08-2018

11. Other impact *(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, etc.)*

Award/Prize directly derived from this project

Year	Title of Award	Awarding Organization
2017.12	"**Best Presentation Award**" in 22nd Research Postgraduate Symposium.	LKS Faculty of Medicine, HKU
2017.10	"**Locals award registration subsidy**" in Keystone Symposia on Molecular and Cellular Biology T3: Regenerative Biology and Applications: Cell Differentiation, Tissue Organization and Biomedical Engineering.	The Croucher Foundation and HKU
2017.6	YS and Christabel Lung Postgraduate Scholarship 2016/2017	YS and Christabel Lung fund; LKS Faculty of Medicine, HKU
2017.3	"Young Investigator Award (Oral category)" in 12nd International Symposium on Healthy Aging	LKS Faculty of Medicine, HKU

Collaboration with other research institutions

This grand helps us further develop the collaboration with GIBH, especially with Dr. Miguel Esteban's lab, we have obtained 2 more grants and several decent publications:

1) Grants

1. CAS - Croucher Funding Scheme for Joint Laboratories (2016-2019), Croucher Foundation

Title: Discovery of New Approaches for Treating Hyperlipidemia Using an Induced Pluripotent Stem Cell (iPSC)-Based Platform.

2. Innovation and Technology Support Programme GD-ITF (GHP/046/17GD, 2017-2019)
Title: Generation of Familial Hypercholesterolemia Human Liver Chimeric Rabbit Model.

2) Publications

1. Yang, Jiayin, et al. "Generation of Human Liver Chimeric Mice with Hepatocytes from Familial Hypercholesterolemia Induced Pluripotent Stem Cells." *Stem cell reports* 8.3 (2017): 605-618.
2. Li, Li, et al. "Fumarylacetoacetate Hydrolase Knock-out Rabbit Model for Hereditary Tyrosinemia Type 1." *Journal of Biological Chemistry* 292.11 (2017): 4755-4763.
3. Xu, Jian-Yong, et al. "Generation of induced cardiospheres via reprogramming of skin fibroblasts for myocardial regeneration." *Stem cells* 34.11 (2016): 2693-2706.
4. Ng, Kwong-Man, et al. "Amelioration of X-Linked Related Autophagy Failure in Danon Disease With DNA Methylation Inhibitor." *Circulation* (2016): CIRCULATIONAHA-115.
5. Bao, Xichen, et al. "The p53-induced lincRNA-p21 derails somatic cell reprogramming by sustaining H3K9me3 and CpG methylation at pluripotency gene promoters." Cell research 25.1 (2015): 80-92.
6. Liu, Longqi, et al. "Transcriptional pause release is a rate-limiting step for somatic cell reprogramming." *Cell Stem Cell*15.5 (2014): 574-588.
7. Tse, Hung-Fat, et al. "Patient-specific induced-pluripotent stem cells-derived cardiomyocytes recapitulate the pathogenic phenotypes of dilated cardiomyopathy due to a novel DES mutation identified by whole exome sequencing." *Human molecular genetics* 22.7 (2013): 1395-1403.