RGC Ref.: X-HKU708/14
(please insert ref. above)

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

(Please attach a copy of the completion report submitted to the Scottish Funding Council by the Scottish researcher)

Part A: The Project and Investigator(s)

1. Project Title

Single-cell transcriptomics in Sertoli cells and neural crest cells

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

	Hong Kong Team	Scottish Team
Name of Principal Investigator *(with title)*	Dr. Martin C.H. Cheung	Dr. Ryohei Sekido
Post	Assistant Professor	Lecturer
Unit / Department / Institution	School of Biomedical Sciences/The University of Hong Kong	School of Medical Sciences/University of Aberdeen
Contact Information	Email: mcheung9@hku.hk	Email: rsekido@abdn.ac.uk
Co-investigator(s) *(with title and Institution)*		

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	01/01/2015		
Project Completion date	31/12/2015		
Duration *(in month)*	12 months		
Deadline for Submission of Completion Report	31/12/2016		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. To elucidate the dynamic gene expression during the differentiation of Sertoli cells and neural crest cells at the single-cell level
2. To elucidate the critical gene(s) required for the differentiations.
3. ....

5.2 Revised Objectives

Date of approval from the RGC: _____

Reasons for the change: _____

_____

1.
2.
3. ....

6. Research Outcome

Major findings and research outcome
(maximum 1 page; please make reference to Part C where necessary)

Although we have difficulty in isolating fluorescence-labeled chick single NCCs for cDNA synthesis, both HK and Scotland teams have established their own protocols to derive cDNAs from single embryonic mouse Sertoli and neural crest cells. Our qPCR validation studies indicate a certain degree of heterogeneity in tissue specific maker genes expression from both embryonic single cell types.

Comparative analysis between bulk Sox9-EGFP^{+} NC RNA-seq data and existing NC datasets identified Dlc1 as a novel regulator during NC development. We found Dlc1 exhibits asymmetric localization in cytoplasm at the front of both emigrating and migratory NCCs. Gain- and loss-of-function studies demonstrate that appropriate level of Dlc1 activity is essential for the establishment of NC polarity through spatial restriction of RhoA activity between the back and front, which is prerequisite for directional delamination and migration. Asymmetric localization of Dlc1 in NCC front relies on its binding partner, Nedd9, identified by shotgun proteomics. Importantly, this association is required for the establishment of differential rather than the total level of RhoA activity to determine NCC back-front axis. Moreover, Nedd9 and Dlc1 are subject to the transcriptional regulation of NC specifiers, Sox9 and Sox10, respectively. Thus, we reveal a novel SoxE-Dlc1/Nedd9-RhoA regulatory axis to govern NC migratory polarization. This part of the work is currently under review in *Nature Communications* (Please refer to Part C for details).

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

Since the quality of our RNA/cDNAs varies between samples, we need to improve our techniques in single-cell collection by proceeding quickly through our protocol from tissue harvest to single cell lysis; using a rapid and complete, yet gentle dissociation protocol; and optimizing cell sorting parameters, such as system pressure, nozzle size, and deflection angle. The quality and characters of isolated single cells need to be validated by their levels of tissue specific marker genes expression by qRT-PCR. Afterwards, we will proceed with single-cell transcriptome sequencing using Illumna HiSeq 1500 for the next generation sequencing followed by functional categorization based on similarities of their gene expression levels.
In case we still encounter difficulty in preparing high quality of cDNAs from single cells, we will perform single cell isolation by employing laser capture microdissection technique on freshly frozen tissue, which represents the exact, native gene expression profile of a cell and maintains spatial information.

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)

During tissue development, a pool of seemingly homogenous population of progenitors contributes to the formation of distinct functional cell types within the tissue. This process is coordinately regulated by a set of genes encoding mRNAs, which are translated into functional proteins. The entire mRNA composition (transcriptome) of an individual cell underlies their physiological functions, behavior, cell fate, and role in multicellular organisms. Recent advances of single-cell technologies unravel heterogeneity in gene expression among similar cell types that could provide regulatory insight of their functional diversity at the single-cell level in developing tissues. Leveraging our expertise in studying Sertoli cell and neural crest (NC) development, Dr. Ryohei Sekido in Scotland and myself in HK have established protocols to derive cDNAs from single embryonic mouse Sertoli and NCCs, respectively. qPCR validation assays indicate certain degree of heterogeneity in maker genes expression from both embryonic single cell types. Further technical improvements in collecting more single-cells for RNA-seq are required. Our bulk NC RNA-seq data analyses identified Dlc1 as a novel regulator in governing directional migratory behavior of NCCs. Altogether this project enhances our research capability through the development of technology platform for single-cell analysis and also reveal novel factors in neural crest development.

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)*						
		√		**Jessica Aijia Liu**, **Yanxia Rao**, May Pui Lai Cheung, Man-Ning Hui, Ming-Hoi Wu, Ben Niu, Lo-Kong Chan, Irene Oi-Lin Ng, Rakesh Sharma, Kathryn S.E. Cheah, Hodgson Louis and **Martin Cheung***	Nature Communications	Dec/2016	Yes	Yes	No

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)*
Mar/2016/Taiwan	Coordinated action of Nedd9 and Dlc1 in neural crest motility	Avian Model Systems 9: A New Integrative Platform	Dec/2016	Yes	Yes	No (because the work has not been published)

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

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

Nil