RGC Ref.: A-HKUST604/14
(please insert ref. above)

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

(Please attach a copy of the completion report submitted to the ANR by the French researcher)

Part A: The Project and Investigator(s)

1. Project Title (ANR Acronym)

Molecular regulation of muscle stem cell aging by canonical Wnt signaling (MyoAge)

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

	Hong Kong Team	French Team
Name of Principal Investigator *(with title)*	Dr. Tom Hiu Tung Cheung	Dr. Fabien Le Grand
Post	Associate Professor	CNRS Research Director (DR2, tenured)
Unit / Department / Institution	Division of Life Science, The Hong Kong University of Science and Technology	Institut Cochin. France (now Institut NeuroMyoGène, Lyon)
Contact Information	tcheung@ust.hk	fabien.le-grand@inserm.fr
Co-investigator(s) *(with title and institution)*		

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted*)
Project Start date	01/04/2015	01/04/2015	06/12/2017
Project Completion date	31/03/2018	30/09/2018	06/12/2017
Duration *(in month)*	36	42	06/12/2017
Deadline for Submission of Completion Report	31/03/2019	30/09/2019	06/12/2017

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. Genetic analysis of ß-catenin function in age-related decline of MuSC functionality.
2. To study the epigenetic profiles and determinants of Wnt-mediated young and old SC function.
3. To analyze the effect of Wnt/ß-catenin signaling in the control of asymmetric MuSC division with age.

5.2 Revised Objectives

Objectives were carried out as planned.

6. Research Outcome

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

To assess roles of ß-catenin on MuSCs in vivo, We have generated mouse lines with MuSC-specific ß-catenin knockout and activation. Using these mice, we monitored the impact on MuSCs during homeostasis and muscle regeneration. Under both conditions, our findings suggest that ß-catenin levels do not affect MuSC maintenance during aging. However, we found that ß-catenin is important for MuSC regeneration function as ß-catenin knockout delayed muscle regeneration and decreased the size of regenerated muscle fibers.

We have delineated the transcription program regulated by Wnt-ß-catenin signaling through RNA-seq. We identified that ERBB3 and small GTP binding protein RAC1 signaling pathways are essential targets of ß-catenin, transcripts involved in these pathways are downregulated in the absence of ß-catenin. ß-catenin is also a regulator of energy metabolism. In ß-catenin knockout MuSCs, transcripts related to AMPK signaling, sphingolipid metabolism and ketone bodies synthesis and degradation are upregulated.

Regarding the roles of Wnt/ß-catenin signaling in epigenetic regulation in MuSC aging, we realized ChIP-seq using a reduced cell number input. Using our optimized ChIP-seq pipeline, we have demonstrated substantial changes in histone marks H3K4me3 and H3K27me3 during aging of MuSCs. These findings lay the foundation for our subsequent investigations about the relevance of ß-catenin to these epigenetic changes. Part of the data has been presented on Gordon Conference on Myogenesis 2015 (Abstract attached).

To elucidate transcriptional changes underlying asymmetrically MuSC division modulated by Wnt/ß-catenin signaling, we have optimized a scRNA-seq platform and built a cell atlas of skeletal muscle, containing 10 resident cell populations. We also discovered a novel myogenic cell population. These data have been presented on Victorian Muscle Network Symposium 2018 and

FASEB Summer Research Conference, "Skeletal Muscle Satellite Cells and Regeneration" 2018 (Conference program attached). Findings are published in "Giordani L, He GJ, Negroni E, Sakai H, Law JY, Siu MM, et al. High-dimensional single-cell cartography reveals novel skeletal muscle resident cell populations. Molecular Cell. 2019;304683" (PDF attached).

We also present a few technical advancements, which are critical for the succeed of the project. Among which, we devised a method to visualize DNA segregation in live cells using Stimulated Raman Scattering (SRS). We provide solid data to prove that during asymmetrical division of stem cells, replicated DNA undergoes non-random segregation into the two daughter cells: the more "stem" daughter cell inherits the parental copy of DNA while the newly synthesized DNA are co-segregated into the more "committed" daughter cell. Such findings allow identification of symmetrical and asymmetrically divisions as well as distinguishing asymmetrically daughter cells with distinct fates.

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

Wnt-ß-catenin signaling is one the various pathways that exhibit aging-related changes. It is interesting to understand the crosstalk between ß-catenin signaling and other perturbed in aged niche and MuSCs. For example, Notch signaling, a quiescence maintaining signaling, is diminished in aged MuSCs, shifting MuSC fate from self-renewal to differentiation and depleting MuSC reserve. Research can be designed to examine if these pathways would act synergistically or antagonistically to regulate MuSC aging. Moreover, Notch ligand Delta-like 1 (Dll1) is a ß-catenin target. It is therefore interesting to test if Notch signaling acts downstream to ß-catenin signaling to regulate MuSC aging.

Understand the involvement of the Wnt/ß-catenin signaling in aging-related declines of MuSC functionality deems translational importance. In addition to modulation of MuSC functionality and cell fate using Wnt/ß-catenin activators and inhibitors, normalization of ß-catenin level/activity is a possible means of reversing the aging phenotypes of MuSCs. We previously demonstrated that cognate inhibitors of Wnt signaling Dkk1 and Srfp2 can reduce asymmetrical MuSC division. Otherwise, pharmacological drugs can be developed for clinical application as more specific and controllable Wnt/ß-catenin signaling modulation.

7. The Layman's Summary

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

Muscle stem cell (MuSCs) are the cells that responsible for muscle regeneration. They stay in a quiescent state in resting muscle and could quickly activate to regenerate muscle. Therefore, the state of quiescence and the process of activation and regeneration should be strictly controlled so as to preserve the MuSC pool and functionality. Such control is orchestrated through various pathways. However, some of these pathways become malfunction when the organism aging. It is known that changes in the environment causes a hyperactive ß-catenin signaling in old MuSCs. Our work aims to understand how ß-catenin contribute to the decline in MuSC pool and functionality. Using elegantly designed mouse models mimicking ß-catenin malfunctioning, we will first address the impact on MuSC functionality and number. Then, we will answer the question that how does ß-catenin cause such influences in MuSCs. We will focus on molecular changes inside MuSCs, namely at chromatin and gene expression levels. Succeed of our work will not only advance our understand about MuSC aging, it also open new opportunities to slow down or prevent aging of MuSCs and treat aging-related muscle wasting.

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)*						
2019				Lorenzo Giordani, Gary J. He, Elisa Negroni, Hiroshi Sakai, Justin Y.C. Law, M. Mona Siu, Raymond Wan, Aurélien Corneau, Shahragim Tajbakhsh, Tom H. Cheung* and Fabien Le Grand*	High-Dimensional Single-Cell Cartography Reveals Novel Skeletal Muscle-Resident Cell Populations. Molecular cell 74.3 (2019): 609-621.	2019	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)*
June/ 2015/ Lucca	Non-coding RNAs as regulators of myogenic lineage progression.	Gordon Conference on Myogenesis	30 Aug 2019	Yes	Yes	No

June/ 2018/ Melbourne	Molecular regulation of muscle stem cell quiescence and activation (Invited talk)	Victorian Muscle Network Symposium	2019	No	Yes	No
July/ 2018/ Steamboat Springs	Differential mRNA processing during Satellite Cell Activation	FASEB Summer Research Conference, "Skeletal Muscle Satellite Cells and Regeneration"	2019	Yes	Yes	No

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
Ran YI	PhD	2016-09-01	2020-03-31
Jiawei HE	PhD	2017-09-01	2021-08-31

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

We are currently collaborating with Jianan Qu from Department of Electronic & Computer Engineering, HKUST to establish the optical tweezer platform to facilitate bringing MuSCs and Wnt ligand-bead to close proximity and achieve localized Wnt signaling on MuSCs. The platform is valuable to our investigation about the influence of asymmetrically Wnt/ß-catenin signaling on MuSC fate. Such platform is also applicable to investigations of effects of other ligands on MuSCs.