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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

Epigenetic Mechanism of Mitoflashes Facilitating Early Phase of Reprogramming

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

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Prof. Chan Wai-yee	Prof. Liu Xingguo
Post	Chair Professor	Professor
Unit / Department / Institution	School of Biomedical Sciences, CUHK	Guangzhou Institutes of Biomedicine and Health (GIBH), CAS
Contact Information	+852-39431383 chanwy@cuhk.edu.hk	liu_xingguo@gibh.ac.cn
Co-investigator(s) *(with title and institution)*	Prof. Miu Kai Kei, CUHK	Prof. Qin Dajiang, GIBH Dr. Ying Zhongfu, GIBH Dr. Chen Keshi, GIBH

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	01/01/2017	N/A	N/A
Project Completion date	31/12/2020	N/A	N/A
Duration *(in month)*	48	N/A	N/A
Deadline for Submission of Completion Report	31/12/12021	N/A	N/A

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. to study how mitoflash affects global DNA methylation pattern during the early phase of reprogramming.
2. to study the molecular mechanisms of mitoflash in regulating the demethylation of DNA.
3. to identify the signaling pathway regulated by DNA demethylation under mitoflash changes during reprogramming.

5.2 Revised Objectives

Date of approval from the RGC: N/A

Reasons for the change: N/A

1.
2.
3. ....

6. Research Outcome

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

Through the activation of mitoflash by chemical modulators mastoparan and paraquat/CypD overexpression, we discovered that mPTP opening and the subsequent generated ROS may signal to regulate the expression of both DNA demethylase TET2 and histone demethylase PHF8, indicative of a positive-feedforward process for mitoflash activation; somatic cells rely on ROS as a secondary messenger to directly activate oxidative stress-related NRF2 pathway through elimination of the repressive marks on pluripotent genes. Moreover, PHF8 levels also finetuned the action of miRNAs as illustrated by global hmC and mC sites by RRHP profiling and nano-hmC-seal methods to profile the genome-wide distribution of 5-hmC during early stage of fibroblasts reprograming into stem cells. In particular, we observed miR-101c along mitoflash modulation of DNA demethylation changes compared to control.

Indeed, miR-101c mimics effectively promoted PHF8 expression whereas specific miRNA inhibitor shall prevent the elevation of PHF8 under CypD overexpression. Furthermore, we showed that the Pten-inhibitor SF1670 contributed to sustaining mouse ESCs while Pten activation suppressed pluripotency. Indeed, the embryoid bodies derived from Pten-deficient ESCs or SF1670-treated wild-type ESCs showed greater expression of ectoderm and pluripotency markers, a clear demonstration that the functional expression of the tumour suppressor PTEN may interrupt stem cell pluripotency to favour differentiation along different germ layers. With that, we profiled both DNA methylation pattern by whole genome bisulphite sequencing and active demethylation sites through whole genome 5-hydroxymethylcytosine (5-hmC) nano-seal profiling in these lines representative of either the naïve or primed pluripotent states.

Our collaboration extended to study not just the key aspects of epigenetic control by stem cell reprogramming to gain pluripotency, but also the reverse control in how stem cells resisted differentiation by maintaining pluripotency. In fact, it was understood that both mice and humans embryonic stem cells indeed existed in both naïve and primed pluripotent states, where unique molecular and cellular features staged their inter-conversion. Recent discovery into the use of small pathway inhibitors for the maintenance of pluripotency had implicated the Wnt signaling; especially when Wnt modulator Gsk3β where its pharmacological intervention shall block stem cell differentiation. Interestingly, our group were the first to discover that $Pten^{-/-}$ ESCs could maintain naïve pluripotency by blocking Gsk3β activity.

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)

Our joint funding aimed to reveal the epigenetic mechanisms underlying enhancement of stem cell reprogramming upon induction of mitoflash frequency. We had verified that mitoflashes would serve as a proxy marker for mitochondrial reactive oxygen species (mtROS) released upon the opening of mitochondrial permeability transition pores (mPTPs). Such opening of mPTPs instigated epigenetic changes in nuclear DNA, where both active DNA demethylase TET2 and histone demethylase PHF8 would be induced, leading to subsequent reduction of the repressive mark H3K27me3 and H3K9me2 on pluripotent genes to enhance stem cell reprogramming. In the contrary, the Wnt modulator Gsk3β, where its pharmacological intervention shall block stem cell differentiation, drives an epigenetic mechanism that governs inter-conversion between both naïve and primed pluripotent state. Such reversed process also required similar epigenetic mechanisms that dictate how stem cells resisted differentiation to maintain pluripotency. To sum up, collective research from our joint collaboration had described how modulating mitochondrial activity by either chemical intervention or gene overexpression shall instigate subsequent nuclear DNA changes that would affect both stem cell reprogramming and differentiation.

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)*						
1	2018	N/A	N/A	N/A	**Ying Z**, Xiang G, Zheng L, Tang H, Duan L, Lin X, Zhao Q, **Chen K**, Wu Y, Xing G, Lv Y, Li L, Yang L, Bao F, **Long Q**, Zhou Y, He X, Wang Y, Gao M, Pei D, **Chan WY**, **Liu X***.	Short-Term Mitochondrial Permeability Transition Pore Opening Modulates Histone Lysine Methylation at the Early Phase of Somatic Cell Reprogramming. Cell metabolism, 28(6), 935–945.	Yes (2018)	Yes	No	Yes

2	2018	N/A	N/A	N/A	Bao F, Shi H, Gao M, Yang L, Zhou L, Zhao Q, Wu Y, **Chen K**, Xiang G, **Long Q**, Guo J, Zhang J, **Liu X***.	Polybrene induces neural degeneration by bidirectional Ca2+ influx-dependent mitochondrial and ER–mitochondrial dynamics. Cell Death Dis 9, 966.	Yes (2018)	Yes	No	Yes
3	2019	N/A	N/A	N/A	Wu Y, **Chen K**, Xing G, Li L, Ma B, Hu Z, Duan L, **Liu X***.	Phospholipid remodeling is critical for stem cell pluripotency by facilitating mesenchymal-to-epithelial transition. Science advances, 5(11), eaax7525.	No	Yes	No	Yes
4	2020	N/A	N/A	N/A	Li L, **Chen K**, Wang T, Wu Y, Xing G, Chen M, Hao, Z, Zhang C, Zhang J, Ma B, Liu Z, Yuan H, Liu Z, **Long Q**, Zhou Y, Qi J, Zhao D, Gao M, Pei D, Nie J, Ye D, Pan G, **Liu X***.	Glis1 facilitates induction of pluripotency via an epigenome–metabolome–epigenome signalling cascade. Nat Metab 2, 882–892.	No	Yes	No	Yes
5	2020	N/A	N/A	N/A	Zhao Y, **Long Q**, Wu H, Li W, Qi J, Wu Y, Xiang G, Tang H, Yang L, **Chen K**, Li l, Bao F, Li H, Wang Y, Li M, **Liu X***.	Topology-dependent, bifurcated mitochondrial quality control under starvation. Autophagy, 16(3), 562–574.	No	Yes	No	Yes
6	2020	N/A	N/A	N/A	**Chen K, Long Q,** Xing G, Wang T, Wu Y, Li L, Qi J, Zhou Y, Ma B, Han RS, Nie J, Pei D*, **Liu X***	Heterochromatin loosening by the Oct4 linker region facilitates Klf4 binding and iPSC reprogramming. The EMBO journal, 39(1), e99165.	No	Yes	No	Yes
7	2020	N/A	N/A	N/A	Yang L, Lin X, Tang H, Zeng S, Jia L, Li Y, Shi Y, He S, Wang H, Hu Z, Gao X, Liang X, Yang Y, **Liu X***	Mitochondrial DNA mutation exacerbates female reproductive aging via impairment of the NADH/NAD+ redox. Aging cell, 19(9), e13206.	No	Yes	No	Yes
8	2020	N/A	N/A	N/A	Yang L, Tang H, Lin X, Wu Y, Zeng S, Pan Y, Li Y, Xiang G, Zhuang S, Song Z, Jiang Y, **Liu X***	OPA1-Exon4b Binds to mtDNA D-Loop for Transcriptional and Metabolic Modulation, Independent of Mitochondrial Fusion. Frontiers in cell and developmental biology, 8, 180.	No	Yes	No	Yes
9	2020	N/A	N/A	N/A	Wang W, Lu G, Su X, Tang C, Li H, Xiong Z, Leng C, Wong M, Liu H, Ma J, Cheung H, Kung H, Chen Z, **Chan WY***	Pten-mediated Gsk3β modulates the naïve pluripotency maintenance in embryonic stem cells. Cell Death Dis 11, 107.	No	Yes	Yes	Yes

10	2020	N/A	N/A	N/A	Guo J, Duan L, He X, Li S, Wu Y, Xiang G, Bao F, Yang L, Shi H, Zheng L, Hu H, **Liu X***	A Combined Model of Human iPSC-Derived Liver Organoids and Hepatocytes Reveals Ferroptosis in DGUOK Mutant mtDNA Depletion Syndrome. Adv. Sci. 2021, 8, 2004680.	No	Yes	Yes	Yes
11	2021	N/A	N/A	N/A	Wang W, Lu G, Xiong Z, Leung HD, Cao R, Pang AL, Su X, Law PWN, Zhao Z, Chen ZJ, **Chan WY***	Pten Regulates Cardiomyocyte Differentiation by Modulating Non-CG Methylation via Dnmt3. Adv. Sci. 2021, 8, 2100849.	No	Yes	Yes	Yes
12	N/A	2021	N/A	N/A	Wang Z, Fung S, Yu C, Law P, Hao S, **Miu K*, Chan WY***	Dynamic epigenetic regulation of miR-10 family governs the caudalization of floor plate neural progenitors. Re:GEN OPEN, 2021.(Accepted)	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)*
Oct 2020, San Diego, CA	Dynamic epigenetic regulation of miR-10 family governs the caudalization of floor plate neural progenitors	American Society for Human Genetics 2020 Annual Meeting	No	Yes	Yes	Yes
Oct 2021, Virtual	PTEN disrupt dynamics of 5-hydroxymethylcytosine in mESC-derived neuronal differentiation	American Society for Human Genetics 2021 Virtual Meeting	No	Yes	Yes	Yes

Oct 2021, Virtual	Explore the dynamic spatial and temporal regulation mechanism during embryonic sex development	American Society for Human Genetics 2021 Virtual Meeting	No	Yes	Yes	Yes

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
HAO, Suyu	MPhil-PhD	01/08/2018	31/07/2022

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

Our past encounters during the research conferences fostered collaboration between the PIs of the School of Biomedical Sciences, CUHK and GIBH. Together, the two institutions, initiated by our PIs, had established a research collaboration agreement with another sister institution Max Planck Institute, with an intention to collaborate with each other on research topics related to both tissue engineering and stem cell. Recently, our collaboration also fostered project collaborations at the GIBH CAS INNOCentre.

12. Statistics on Research Outputs *(Please ensure the summary statistics below are consistent with the information presented in other parts of this report.)*

	Peer-reviewed journal publications	Conference papers	Scholarly books, monographs and chapters	Patents awarded	Other research outputs (Please specify)
No. of outputs arising directly from this research project [or conference]	4	3	0	0	0