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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

Identifying critical transitions and gene regulatory networks controlling phases of chondrocyte differentiation in the growth plate.
確定控制軟骨細胞分化的關鍵節點和基因調控網絡的研究

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

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Prof. Kathryn SE Cheah 謝賞恩教授	Prof. Michael Q Zhang 張奇偉教授
Post	Chair Professor	Professor
Unit / Department / Institution	School of Biomedical Sciences/HKU 香港大學生物醫學學院 (formerly Department of Biochemistry)	Bioinformatics Division / Center for Synthetic & Systems Biology, TNLIST Tsinghua University
Contact Information	kathycheah@hku.hk Rm L3-73, Faculty of Medicine Building, Laboratory Block	michaelzhang@tsinghua.edu.cn No.90 mailbox in School of Medicine (Room B305), Tsinghua University, Beijing 100084, China.
Co-investigator(s) *(with title and institution)*	*Dr. Junwen Wang (王俊文博士) Assistant Professor, 助理教授, Biochemistry/HKU 香港大學生物化學系 Prof. Danny Chan (陳振勝教授) Professor 教授 School of Biomedical Sciences/HKU 香港大學生物醫學學院	Dr. Jin Gu (古槿博士), Assistant Professor, 助理教授 Department of Automation/Tsinghua University Bioinformatics Division, Center for Synthetic & Systems Biology/TNLIST/Tsinghua University 清華大學自動化系 清華信息科學與技術國家實驗

	Dr. Ray Ng(吳傑博士) Assistant Professor 助理教授 School of Biomedical Sciences/HKU 香港大學生物醫學學院 *Left HKU	室（籌）生物信息學研究部/系統與合成生物學研究中心 Dr. Juntao Gao (高軍濤博士) Associate Researcher, 副研究员 Bioinformatics Division, Center for Synthetic & Systems Biology/TNLIST/Tsinghua University 清華信息科學與技術國家實驗室（籌）生物信息學研究部/系統與合成生物學研究中心

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	1/1/2014		1/1/2014
Project Completion date	31/12/2017		31/12/2017
Duration *(in month)*	48		48
Deadline for Submission of Completion Report	31/12/2018		31/12/2018

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. Obtain transcriptome data from different populations of differentiating growth plate chondrocytes.
2. Annotate the epigenetic profiles for the *Col10a1* gene in the different populations of differentiating chondrocytes in the wild-type growth plate.
3. Use and develop bioinformatics and mathematical modeling approaches to mine the transcriptome data to identify "critical transition genes".
4. Use bioinformatics approaches to identify core gene regulatory networks.

5. Validate hypotheses using a combination of cell-based assays and in vivo models

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)

1. We have created the GP-DGEL web tool to visualize the growth plate chondrocyte microarray data as well as the SOX9, GLI1 and GLI3 ChIP-seq binding regions genome-wide. From these data we have constructed a model of SOX9-GLI-FOXA phasic GRN in chondrocyte development, and validated the model using Sox9 heterozygous null and Gli2 homozygous null mutants and cell culture-based luciferase assay (Tan et al., 2018).
2. Corresponding growth plate datasets from a transgenic mouse model (13del) of human MCDS were also obtained, supporting the idea that the integrated stress reponse (ISR), specifically the PERK-eIF2a-ATF4-CHOP pathway, transactivates *Sox9* and *Fgf21* expression and plays a central role in disrupting chondrocyte differentiation program as the pathogenetic mechanism of SMCD. By inhibiting the PERK pathway with the small molecule ISRIB we achieved almost complete rescue of the dwarfism and aberrant chondrocyte differentiation. These exciting findings point to inhibition of the ISR as a potential therapeutic strategy for treating MCDS and related skeletal dysplasia (Wang et al. 2018).
3. To investigate how hypertrophic chondrocytes become osteoblasts in bone formation, we have generated single cell transcriptome data from 349 normal cells and 94 mutant cells

of this lineage using the Smart-seq2 method. At the same time we have developed new computational tools to analyze single cell transcriptome data (Li, Xiangyu et al., 2016; Liu et al, 2017). Combining with existing tools, we have identified genes associated with the transition which will be validated. We aim to publish our findings in a high quality journal. We pioneered single cell transcriptome research in HKU and in collaboration with the Center for Genomic Sciences, which is now providing routine services for single cell RNA-seq to the HKU research community.

4. To address the pressing need to develop more versatile and powerful computational methods to analyze epigenetic and chromatin conformation data, we have published advance computational tools to perform genome-wide analysis of interactions between DNA fragments bound by a specific protein using ChIA-PET data (Li, Guipen et al., 2017; He et al., 2016; He et al., 2015; Djekidel et al., 2015; He et al., 2014; Gao et al., 2016; Du et al., 2017)
5. In addition, we have created a variety of computational/interactive tools to meet with the need of the frontiers of biomedical research: Web3DMol for interactive protein structure visualization (Shi et al, 2017); fast dimension reduction and integrative clustering of multi-omics data for cancer molecular classification (Wu et al., 2015); non-coding RNA characterization (Hu et al, 2014); nuclear genome super-resolution imaging of non-repetitive DNA molecular beacon probes (Ni et al, 2017) and Super-resolution dipole orientation mapping via polarization demodulation (Zhanghao et al., 2016).

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

Single cell omics has recently received tremendous attention in terms of technology development because of its potential power in elucidating developmental process and disease progression at unprecedented cellular resolution for epigenome, transcriptome and proteome profiling. Our pioneering work in the field has been well acknowledged and also led to publications. Further work is guaranteed to generate data for different projects and to develop new experimental and in silico approaches to expand the scope of single cell analyses, for example by coupling the acquisition of single cell biophysical information with transcriptome/epigenome profile.

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)

We have profiled gene expression of different phases of growth plate chondrocyte differentiation and identified a central gene regulatory network that control the differentiation program. This is critical for longitudinal bone growth, since disruption may need to disorder such as dwarfism. We have also profiled gene expression in a skeletal disorder (MCDS) mouse model (13del) which displayed shortened limbs, and found out that cellular stress response is responsible for disease progression and a small molecule (ISRIB) can target the stress response to improve the bone shortening phenotype.

We previously showed that chondrocytes can become osteoblasts. By employing cutting edge single cell transcriptome profiling, we discovered that the chondrocyte to bone transition involves cell cycle re-entry and that hypertrophic chondrocytes are plastic and can also become mesenchymal-like cells, and at low frequency, adipocytes. This discovery implicates chondrocytes as a source of abnormal marrow fat composition and has important implications for metabolic disorders. We have also developed many

bioinformatic tools to analyse sophisticated, high-throughput data epigenetic, transcriptomic and super-resolution imaging data to meet the increasing demand of the research community to handle big data.

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)*						
2018				Wang, Cheng, Tan, Zhijia, Niu, Ben, Tsang, Kwok Yeung, Tai, Andrew, Chan, Wilson C.W., Lo, Rebecca L.K., Leung, Keith K.H., Dung, Nelson W.F., Itoh, Nobuyuki, **Zhang, Michael M.Q., Chan, Danny, Cheah, Kathryn Song Eng***	Inhibiting the integrated stress response pathway prevents aberrant chondrocyte differentiation thereby alleviating chondrodysplasia. Elife. 2018 Jul 19;7. pii: e37673.	2018	Yes	Yes	Yes

2018				Tan, Zhijia, Niu, Ben, Tsang, Kwok Yeung, Melhado, Ian G., Ohba, Shinsuke, He, Xinjun, Huang, Yongheng, Wang, Cheng, McMahon, Andrew P., Jauch, Ralf, Chan, Danny, **Zhang, Michael M.Q., Cheah, Kathryn Song Eng***	Synergistic co-regulation and competition by a SOX9-GLI-FOXA phasic transcriptional network coordinate chondrocyte differentiation transitions. PLoS Genet. 2018 Apr 16;14(4):e1007346.	2018	Yes	Yes	Yes
2017				Li, Guipeng, Chen, Yang, Snyder, Michael P, **Zhang, Michael Q***	ChIA-PET2: a versatile and flexible pipeline for ChIA-PET data analysis.Nucleic Acids Res. 2017.1.9, 45(1):e4	2018	Yes	Yes	Yes
2017				Shi, Maoxiang, **Gao, Juntao*, Zhang, Michael Q***	Web3DMol: interactive protein structure visualization based on WebGL. Nucleic Acids Res. 2017.5.8, 45, W523~W527	2018	Yes	Yes	Yes
2017				Ni, Yanxiang, Cao, Bo, Ma, Tszshan, Niu, Gang, Huo, Yingdong, Huang, Jiandong, Chen, Danni, Liu, Yi, Yu, Bin, **Zhang, Michael Q***, Niu, Hanben*	Super-resolution imaging of a 2.5 kb non-repetitive DNA in situ in the nuclear genome using molecular beacon probes. Elife. 2017.5.9, 6(e21660)	2018	Yes	Yes	Yes

2017				Li, Xiangyu , Chen, Weizheng, Chen, Yang, Zhang, Xuegong, **Gu, Jin*, Zhang, Michael Q***	Network embedding-based representation learning for single cell RNA-seq data. Nucleic Acids Res, 2017.8.28, 45(19), e166	2018	Yes	Yes	Yes
2016				He, Chao, Li, Guipeng, Nadhir, Diekidel M., **Chen, Yang*, Wang, Xiaowo*, Zhang, Michael Q.***	Advances in computational ChIA-PET data analysis. Quantitative Biology. 2016.9, 4(3), 217~225	2018	Yes	Yes	Yes
2016				**Juntao Gao***, Xusan Yang, Mohamed Nadhir Djekidel, Yang Wang, Peng Xi, **Michael Q. Zhang**	Developing bioimaging and quantitative methods to study 3D genome. Quantitative Biology. 2016.6. 4(2), 129~147	2018	Yes	Yes	Yes
2016				Zhanghao, Karl, Chen, Long, Yang, Xu-San, Wang, Miao-Yan, Jing, Zhen-Li, Han, Hong-Bin, **Zhang, Michael Q., Jin, Dayong , Gao, Jun-Tao* ,** Xi, Peng*	Super-resolution dipole orientation mapping via polarization demodulation. Light: Science & Applications .2016.10.2, 5.	2018	Yes	Yes	Yes
2015				Djekidel, Mohamed Nadhir, Liang, Zhengyu, Wang, Qi Hu, Zhirui Li, Guipeng, Chen, Yan*, ***Zhang, Michael Q.***	3CPET: finding co-factor complexes from ChIA-PET data using a hierarchical Dirichlet process. Genome Biology (2015) 16:288	2015	No	Yes	Yes

2015				He, Chao, **Zhang, Michael Q.**, Wang, Xiaowo*	MICC: an R package for identifying chromatin interactions from ChIA-PET data. Bioinformatics (2015) 1–3	2015	No	Yes	Yes
2015				Wu, Dingming, Wang, Dongfang, **Zhang, Michael Q.*** 、**Gu, Jin***	Fast dimension reduction and integrative clustering of multi-omics data using low-rank approximation: application to cancer molecular classification. BMC Genomics (2015) 16:1022	2015	No	Yes	Yes
2014				Sheng MiaoMiao, Zhong Ying, Chen Yang, Du JianChao, Ju XiangWu, Zhao Chen, Zhang GuiGen, Zhang LiFang, Liu KangTai, Yang Ning, Xie Peng, Li DangSheng, **Zhang, Michael Q.***, Jiang ChengYu*	Hsa-miR-1246, hsa-miR-320a and hsa-miR-196b-5p inhibitors can reduce the cytotoxicity of Ebola virus glycoprotein in vitro. Science China Life Sciences, 2014.10, 57(10), 959~972	2018	Yes	Yes	Yes
2014				Chao He, Xiaowo Wang*, **Michael Q. Zhang***	Nucleosome eviction and multiple co-factor binding predict estrogen-receptor-alpha-associated long-range interactions. Nucleic Acids Research. 2014.4.29, 42(11), 6935~6944	2018	Yes	Yes	Yes

2017				Du, Zhenhai , Zheng, Hui Huang, Bo, Ma, Rui, Wu, Jingyi, Zhang, Xianglin, He, Jing, Xiang, Yunlong, Wang, Qiujun, Li, Yuanyuan, Ma, Jing, Zhang, Xu, Zhang, Ke, Wang, Yang, **Zhang, Michael Q, Gao, Juntao,** Dixon, Jesse R, Wang, Xiaowo, Zeng, Jianyang, Xie, Wei*	Allelic reprogramming of 3D chromatin architecture during early mammalian development. Nature. 2017.7.13, 547(7662), 232~235	2018	Yes	Yes	Yes
2017				Liu, Zehua, Lou, Huazhe, Xie, Kaikun, Wang, Hao, Chen, Ning, Aparicio, Oscar M., **Zhang, Michael Q.**, Jiang, Rui*, Chen, Ting*	Reconstructing cell cycle pseudo time-series via single-cell transcriptome data. Nature Communications. 2017.6.19, 8(22)	2018	Yes	Yes	Yes
2016				Wang, Jingyu , Chen, Fengling, Liu, Longwei, Qi, Chunxiao, Wang, Bingjie, Yan, Xiaojun, Huang, Chenyu, Hou, Wei, **Zhang, Michael Q.**, Chen, Yang*, Du, Yanan*	Engineering EMT using 3D micro-scaffold to promote hepatic functions for drug hepatotoxicity evaluation. Biomaterials. 2016.6, 91, 11~22	2018	Yes	Yes	Yes
2014				Hu, Long , Di, Chao, Kai, Mingxuan, Yang, Yu-Cheng T, Li, Yang, Qiu, Yunjiang, Hu, Xihao, Yip, Kevin, Y, **Zhang, Michael Q,** Lu, Zhi John*	A common set of distinct features that characterize noncoding RNAs across multiple species. Nucleic Acids Research. 2014.12.12, 43(1), 104~114	2018	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)*
2018	Molecular control of the trans-differentiation of hypertrophic chondrocyte to osteoblast in skeletal development and growth.	Gordon Conference on Bones and Teeth, Galveston, TX, USA. January 28, 2018.	No	No published abstract (meeting program attached)	Yes (Acknowledged at the presentation)	Yes
2017	Chondrocyte Plasticity and Fate Determination in Development and Disease. (Invited speaker)	Gordon Research Conference on Cartilage Biology & pathology. Gordon Research Conference on Cartilage Biology & Pathology. Lucca (Barga), Italy. April 2-7, 2017.	No	No published abstract (meeting program attached)	Yes (Acknowledged at the presentation)	Yes
2017	Single cell transcriptomes reveal a mesenchymal state during chondrocyte to osteoblast transition.,	Keystone Symposium Single Cell Omics, May 26-30, 2017, Stockholm	No	Yes	Yes (Acknowledged at the presentation)	Yes
2016	An integrative bioinformatics approach for establishing transcriptomic identities of single-cell populations	Single Cell Biology, 8-10 March 2016, Wellcome Genome Campus, Hinxton, Cambridge, UK	No	Yes	Yes (Acknowledged at the presentation)	Yes
2016	Dissecting the transition from hypertrophic chondrocyte to osteoblast in skeletal development and growth	Gordon Research Conference – Bones & Teeth, Galveston, TX, USA, February 14-19, 2016	No	No published abstract (meeting program attached)	Yes (Acknowledged at the presentation)	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

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

Prof. K. Cheah was speaker, invited to present the work at the Gordon Research Conference on *Cartilage Biology & Pathology. Gordon Research Conference on Cartilage Biology & Pathology* . April 2-7, 2017.

Dr. Tan, postdoctoral fellow received awards at the 2017 GRC Seminar and Conference.

1. Tan Zhijia, Best Poster Presentation Award "Synergistic co-regulation and competition underlies a SOX9-GLI- FOXA phasic transcriptional network that coordinates growth plate chondrocyte differentiation " in *Gordon Research Seminar (Cartilage Biology and Pathology) Comprehending Cartilage Formation, Function and Failure for Improving Joint Health*, April 1 - 2, 2017 Lucca (Barga), Italy.

2. Tan Zhijia, Best Poster Presentation Award "Synergistic co-regulation and competition underlies a SOX9-GLI- FOXA phasic transcriptional network that coordinates growth plate chondrocyte differentiation " in *Gordon Research Conference on Cartilage Biology & Pathology. Gordon Research Conference on Cartilage Biology & Pathology. Lucca (Barga), Italy*. April 2-7, 2017.