| RGC Ref.: N_HKU 763/11<br>NSFC Ref. : 31161160558 |
|---|
| *(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

Investigating the role of FOXM1 in the maintenance of human embryonic stem cell pluripotency and genome stability

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

| | Hong Kong Team | Mainland Team |
|---|---|---|
| Name of Principal Investigator *(with title)* | Dr Yao Kwok Ming | Prof Tan Yongjun |
| Post | Associate Professor | Professor |
| Unit / Department / Institution | School of Biomedical Sciences/ The LKS Faculty of Medicine/ HKU | Department of Biomedical Engineering/ College of Biology/ Hunan University |
| Contact Information | LKS Fac of Med, HKU<br>L3-69, The LKS Faculty of Medicine Building<br>21 Sassoon Road, Pokfulam<br>Hong Kong SAR, China<br>Tel: 852-3917-9275, 852-3917-9240; Fax: 852-2855-1254; Email: kmyao@hku.hk | Professor and Chief Scientist of Cell and Tissue Engineering<br>College of Biology, Hunan University<br>1 Denggao Road, Yuelu District<br>Changsha 410082, China<br>Tel: (731)8882-3211 (O)<br>Cell phone: 13467615409 |
| Co-investigator(s) *(with title and institution)* | Dr Martin Cheung<br>Assistant Professor<br>HKU | |

### 3. Project Duration

| | Original | Revised | Date of RGC/ Institution Approval *(must be quoted)* |
|---|---|---|---|
| Project Start date | January 1, 2012 | | |
| Project Completion date | December 31, 2014 | | |
| Duration *(in month)* | 36 | | |
| Deadline for Submission of Completion Report | December 31, 2015 | | |

## Part B: The Completion Report

### 5. Project Objectives

5.1 Objectives as per original application

*1. To investigate the function of FOXM1 in the maintenance of pluripotency in human embryonic stem cells*
*2. To study the protective role of FOXM1 against oxidative stress-induced DNA damage and cellular senescence in human embryonic stem cells*
*3. To define the protein interaction network of FOXM1 in human embryonic stem cells*

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)*

**Highlights of major research findings:**

- FOXM1 is expressed in undifferentiated hESCs with peak levels at G2/M phase
- FOXM1 depletion causes mitotic defect and delays G2/M progression in hESCs
- FOXM1 downregulation does not induce rapid exit of undifferentiated state in hESCs but there was transient downregulation of OCT4 and signs of early differentiation.
- FOXM1 expression is sustained in hESCs driven to differentiate into mesodermal and endodermal derivatives. FOXM1 function may be required for the development of these two lineages.
- FOXM1 depletion sensitizes hESCs to oxidative stress and the regulatory effect is likely mediated via regulation of catalase expression
- Genome-wide analysis of FOXM1 binding profile reveals 1,376 regions, showing enrichment for genes involved in regulation of cell cycle progression and mitosis. Binding motifs of ZBTB33, TP53 and PRDM14 are highly enriched in these binding regions.
- Genomic binding profile of FOXM1 in hESCs differs substantially from cancer cells

**Research outcome**

Our work reveals the critical roles played by FOXM1 in the maintenance of cell proliferation and genome stability in hESCs. These novel findings have led to the publication of one peer-reviewed article and three international conference posters, and

another peer-reviewed article under revision for resubmission to Stem Cell Research (please see Part C below). Two research postgraduate students and one undergraduate final year project students were trained in the process.

Our findings echo the pivotal roles of FOXM1 discovered by the Mainland team in mice. Prof. Tan's team demonstrated that FoxM1 mediates LIF-dependent maintenance of pluripotency in mouse embryonic stem cells (mESCs) and that FoxM1 is required for generation of induced pluripotent stem cells (iPSCs) from mouse embryonic fibroblasts (MEFs) by reprogramming. There are however interesting differences in the dependence of human and mouse ESCs on FOXM1, which deserve further investigation in the future. In summary, this joint project supported by NSFC-RGC fund provides the first evidence to support FOXM1 as critical regulator of embryonic stem cell proliferation and function. The findings are prerequisite for further development of stem cells in cell transplantation and regenerative medicine.

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

This project lays the groundwork for further development of research in the following directions:
a) Elucidating the lineage specific requirement of FOXM1. Inducible depletion of FOXM1 upon differentiation of hESCs into mesodermal and endodermal derivatives will reveal its role in the epigenetic and transcriptional regulation of stem cell differentiation.
b) Exploring the interplay between FOXM1 and ZBTB33, TP53 and PRDM14 in the regulation of stem cell pluripotency. Functional analysis of FOXM1 in other hESC lines including naïve hESCs should be pursued. Considering the known involvement of ZBTB33 and PRDM14 in regulation of stem cell pluripotency, it would be very interesting to test for physical and functional interaction between FOXM1 and ZBTB33 or PRDM14 and to analyze whether ZBTB33 or PRDM14 competes with FOXM1 for binding and regulation of target genes.
c) Analyzing the role of novel FOXM1 targets (revealed in our ChIP-seq analysis) e.g. PRXD1 in protection against oxidative stress
d) Study of the genome regulatory differences of FOXM1 in hESCs and cancer cells is prerequisite for further development of cancer therapeutics. It is also worth studying whether differential expression of different FOXM1 isoforms in hESCs versus cancer cells can account for the very different genomic binding profiles of FOXM1.

Building on the expertise and research platform established between the Hong Kong and Mainland teams in this project, applications will be made in NSFC-RGC and/or RGC-GRF competitive exercises to secure funds for addressing the afore-mentioned research questions.

## 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)*

Stem cells have the ability to self-renew and differentiate into different body cell types, a property referred to as pluripotency. The establishment of pluripotent human embryonic stem cell (hESC) lines has made possible their differentiation into different cell types for cellular transplantation and regenerative medicine. However, better understanding of the molecular basis of regulation of stem cell pluripotency and genome stability is essential before the full therapeutic potential of hESCs can be exploited. In this project, we showed that FOXM1

expression was enriched in undifferentiated hESCs and was regulated in a cell cycle-dependent manner. Importantly, knockdown of FOXM1 expression led to aberrant cell cycle distribution with impairment in mitotic progression but showed subtle effect on the undifferentiated state. Interestingly, FOXM1 depletion sensitized hESCs to oxidative stress. Moreover, genome-wide analysis of FOXM1 targets by chromatin immunoprecipitation-sequencing identified genes important for regulation of mitosis including CCNB1 and CDK1. Further comparison of peak set and putative gene set against published data revealed substantial difference in the genomic binding profile of FOXM1 in hESCs. Taken together, our findings provide the first evidence to support FOXM1 as important regulator of cell cycle progression and defense against oxidative stress in hESCs.

## 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)* | | | | | | |
| 2013 | | | | **AKY Lam, AWL Ngan, M-H Leung, DCT Kwok,** VWS Liu, DW Chan, **WY Leung** and **K-M Yao*** | FOXM1b, which is present at elevated levels in cancer cells, has a greater transforming potential than FOXM1c. Front. Oncol. 3:11. doi: 10.3389/fonc.2013.00011 | Yes (June 30, 2013) | No | Yes | Yes |
| 2015 | | Under revision for resubmission (please see interim decision from Associate Editor at Stem Cell Research) | | **CTD Kwok, MH Leung,** J Qin, J Wang, YL Lee and **K-M Yao*** | The Forkhead box transcription factor FOXM1 is required for the maintenance of cell proliferation and protection against oxidative stress in the human embryonic stem cell line VAL3 | | Yes | Yes | Not yet |

**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)* |
|---|---|---|---|---|---|---|
| August/2013 /The Hong University of Science and Technology | Differential Regulation of FOXM1 Isoforms by RAF/MEK/ERK Signaling | Gordon Research Conference: Posttranslational modification networks | Yes (June 30, 2013) | No | Yes | Yes |
| June/2014/ Vancouver, Canada | Investigating the role of FOXM1 in the maintenance of human embryonic stem cell pluripotency | The 12th Annual Meeting of the International Society for Stem Cell Research (ISSCR) | | Yes | Yes | Yes |
| June/2014/ Vancouver, Canada | Investigating the protective role of FOXM1 against oxidative stress and DNA damage in human embryonic stem cells | The 12th Annual Meeting of the International Society for Stem Cell Research (ISSCR) | | 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 |
|---|---|---|---|
| Ms HUNG Oi Ying, Christy | BSc | Sept, 2010 | Final Year Project thesis submitted in May 2013/graduated in June 2013 |
| Mr Leung Man Hong | MPhil | Sept, 2012 | Candidature confirmed in August 2013/graduated in January 2015 |
| Mr Kwok Chun Ting Davis | MPhil | Sept, 2012 | Candidature confirmed in August 2013/graduated in January 2015 |

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

Collaborative link with Dr Cherie Lee at the Department of Obstetrics & Gynaecology, HKU, was established.