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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

Interaction study of PinX1 and nucleophosmin and the effect of inhibiting this interaction on tumor growth ((PinX1与Nucleophosmin (NPM)相互作用研究及抑制这相互作用对肿瘤生长的效果)

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

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Shaw, Pang-Chui (Professor) 邵鹏柱(教授)	Huang, Jun-Jian (Professor) 黄君健(教授)
Post	Professor	Professor
Unit / Department / Institution	School of Life Sciences, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong (香港中文大学生命科学学院)	Laboratory of Tumor and Molecular Biology, Institute of Biotechnology, Academy of Military Medical Science of the PLA, Beijing China (中国人民解放军军事医学科学院)
Contact Information	pcshaw@cuhk.edu.hk	junjianhuangbit@163.com
Co-investigator(s) *(with title and institution)*	Kung, Hsiang Fu (Professor and Academician, CAS) (孔祥复,教授及中国科学院士)	Jin, Rui (Associate Professor)(金蕊,副教授)

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*

Project Start date	1/1/2013		
Project Completion date	31/12/2016		
Duration *(in month)*	48		
Deadline for Submission of Completion Report	31/12/2017		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. To determine the importance of PinX1/NPM interaction on telomere length maintenance by telomerase.
2. To examine the importance of PinX1/NPM interaction on tumor growth.
3. To test the anti-tumor growth effect by targeting PinX1/NPM interaction

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)

The study has revealed the novel interaction between nucleophosmin and PinX1 and confirmed the interaction with *in vivo* co-immunoprecipitation assays and *in vitro* pull-down assays. Endogenous co-immunoprecipitation assay and immunofluorescence assay were used to demonstrate the interaction between PinX1 and NPM in mammalian cells. Critical amino acids on the N-terminal of NPM for association with PinX1 were identified using systematic charged-to-alanine mutagenesis and co-immunoprecipitation assays. NPM mutant with reduced PinX1 association was also found to have decreased hTERT binding, suggesting the role of PinX1 as a linker between NPM and hTERT.

Furthermore, the effects of expression level manipulation were investigated by myc-and FLAG-tagged proteins co-immunoprecipitation assays. It was found that over-expression and knock-down by siRNA of NPM only minimally affected PinX1/hTERT association, while NPM/hTERT association was affected positively by PinX1 expression levels. At the same time it was shown that NPM and PinX1 have highly similar hTERT binding pattern. These confirm that NPM associates with hTERT through the binding with PinX1.

Moreover, with TRAP assay it was found that NPM could partially attenuate PinX1 inhibition on telomerase activity, and this attenuation ability was concomitant to the ability of NPM to interact with PinX1. Since the overexpression of NPM did not disrupt the interaction between PinX1-C and hTERT, this result suggests that NPM does not attenuate the PinX1 inhibition by displacing PinX1-C from telomerase. The aforementioned research findings have been published in an article in *Scientific Reports* (please refer to Part C).

Immunofluorescence studies further revealed that NPM and PinX1 co-localize with hTERT throughout early- to mid-S phase. After mid-S phase a large proportion of hTERT began to return to their scattered loci within the nucleus. An immunofluorescence study on tagged NPM variants suggested that a NPM four-point mutant with preserved PinX1 binding translocated to more scattered loci within the nucleus and the mutations increased protein instability as well (Fig. 3). Long-term knockdown experiments were also carried out using NPM and PinX1 siRNA. It was suggested that NPM long-term knockdown would decrease telomere length gradually. The concurrent PinX1 and NPM long-term knockdown however did not further reduce telomere length compared to each of the single knockdown. Transient expression of wtNPM would rescue reduced telomere length in long-term NPM knocked down cell, while expression of NPM mutant with abolished PinX1 interaction would not. This suggested that NPM's effect on telomere length is likely exerted through

PinX1. At the same time, NPM knockdown would increase the sensitivity of several anti-cancer drugs in HeLa cells, confirming NPM's role in telomere maintenance.

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

During the course of the research, efforts were made to overcome complications arose from the multi-functionality of nucleophosmin in order to address the effects of perturbing NPM/PinX1 interaction on telomere length and tumor growth. We believe an appropriate direction in the future would be genome-editing using techniques like CRISPR to generate viable cells that express mutant NPM proteins with reduced PinX1 binding, followed with biochemical and cellular assays.

Preliminary data from our collaborator suggested that NPM participates in the modulation of Pot1/PinX1 interaction and affects telomere length through this interaction. Future efforts may be directed to studying the effects of perturbing NPM and the Pot1/PinX1 interaction.

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)

Telomere maintenance and telomerase activation have significant implication in cancer. PinX1 is a telomerase regulator. In this study we employed various biochemical techniques to identify nucleophosmin (NPM) as a novel interacting partner of PinX1. NPM is a protein that has been shown to positively correlate with telomerase activity. We further showed that PinX1 acts as a linker in the association between NPM and hTERT, the catalytic subunit of telomerase. Additionally, the recruitment of NPM by PinX1 to the telomerase complex could partially attenuate the PinX1-mediated inhibition on telomerase activity. Taken together, our data reveal a novel mechanism that regulates telomerase activation through the interaction between NPM, PinX1 and the telomerase complex, and the research as a whole will provide a possible new direction on the investigation of telomerase regulation.

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)	Title and Journal/ Book	Submitted to RGC	Attached to this report	Acknowledged the support of this Joint Research Scheme	Accessible from the institutional repository
Year of publication	Year of Acceptance *(For paper accepted but not yet published)*	Under Review	Under Preparation *(optional)*	***(bold** the authors belonging to the project teams and denote the corresponding author with an asterisk*)*	*(with the volume, pages and other necessary publishing details specified)*	*(indicate the year ending of the relevant progress report)*	*(Yes or No)*	*(Yes or No)*	*(Yes or No)*

2017				Derek Hang-Cheong Cheung, **Sai-Tim Ho,** Kwok-Fai Lau, **Rui Jin,** Ya-Nan Wang, **Hsiang-Fu Kung, Jun-Jian Huang*, Pang-Chui Shaw***	Nucleophosmin Interacts with PIN2/TERF1-interacting Telomerase Inhibitor 1 (PinX1) and Attenuates the PinX1 Inhibition on Telomerase Activity, *Sci. Rep.* 7, 43650; doi: 10.1038/srep43650 (2017)	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/2014/ Greece	Nucleophosmin interacts with PinX1, which results in the reduction of the inhibitory effect of PinX1 on the telomerase activity	The 19th World Congress on Advances in Oncology and 17th International Symposium on Molecular Medicine	No	Yes	Yes	Yes
July/2017/ Canada	Nucleophosmin Interacts with PIN2/TERF1-interacting Telomerase Inhibitor 1 (PinX1) and Attenuates the PinX1 Inhibition on Telomerase Activity	The 31st Annual Symposium, The Protein Society	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
Sai-Tim Ho	Ph.D.	August, 2013	On-going
Yeuk-Fei Tam	M.Phil.	August, 2011	July, 2013

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