RGC Ref.: N_HKBU435/12 NSFC Ref. : 81261160503
(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

Skeletal Role of CK2-interating Protein-1 in Regulating Osteoblastic Bone Formation: Molecular Mechanism and Reversing Osteoporosis

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

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Prof. Zhang Ge (M.D.)	Prof. He Fuchu (Ph.D.)
Post	Professor	Professor, Academician of Chinese Academy of Sciences
Unit / Department / Institution	School of Chinese Medicine, Hong Kong Baptist University	State Key Laboratory of Proteomics, Beijing Institute of Radiation Medicine, The Academy of Military Medical Sciences
Contact Information	zhangge@hkbu.edu.hk	hefc@bmi.ac.cn
Co-investigator(s) *(with title and institution)*	Dr. Guo Baosheng (Ph.D.) School of Chinese Medicine, Hong Kong Baptist University	Mr. Liang Chao (M.Ph.) State Key Laboratory of Proteomics, Beijing Institute of Radiation Medicine, The Academy of Military Medical Sciences

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	January 1, 2013	NA	
Project Completion date	December 31, 2016	NA	
Duration *(in month)*	48	NA	
Deadline for Submission of Completion Report	September 30, 2017	NA	

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. To determine the molecular mechanism by which CKIP-1 enhances the ubiquitin ligase activity of Smurf1 in osteoblast.
2. To examine the effect of osteoblast-specific CKIP-1 deletion on bone formation with a conditional knockout mice model.
3. To validate the organ- and osteoblast-like-cell-specific delivery of CKIP-1 siRNA by $(DSS)_6$-liposome and the subsequent cell-specific gene knockdown in mouse models of male and female primary osteoporosis.
4. To evaluate the bone anabolic action of $(DSS)_6$-liposome-CKIP-1 siRNA in mouse models of male and female primary osteoporosis.

5.2 Revised Objectives

Date of approval from the RGC: NA

Reasons for the change: NA

6. Research Outcome

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

1. We demonstrated that PH domain of CKIP-1 could interact with ubiquitin and mutations of key residues in PH domain could reduce the binding ability. This finding will help to further reveal the mechanism of CKIP-1 PH domain in Smurf1-ubiquitin-proteasome pathway. Both of N-terminal PH domain and C-terminal LZ domain of CKIP-1 may be potential drug target for bone anabolic strategy.
2. We demonstrated that CKIP-1 could suppress BMP signaling within osteoblasts by promoting the ubiquitination of the Smad1/5 to inhibit bone formation during aging. Furthermore, we showed that therapeutic silencing CKIP-1 within osteoblast could significantly enhance BMP signaling, promote bone formation and increase bone mass in aging rodents, indicating its translational potential as a novel bone anabolic strategy for reversing established osteoporosis during aging.
3. We developed CH6 aptamer–functionalized lipid nanoparticles (LNPs) encapsulating osteogenic CKIP-1 (*Plekho1*) siRNA (CH6-LNPs-siRNA). The osteoblast-specific aptamer CH6 was selected by cell-SELEX. When rendered with nuclease-resistance and conjugated to LNPs encapsulating osteogenic *Plekho1* siRNA, CH6 achieved osteoblast-specific delivery of siRNAs and facilitated bone formation in osteopenic and healthy rodents. CH6 aptamer-functionalized LNPs update targeted delivery systems from a tissue level to a cellular level, which facilitates the clinical translation of an RNAi-based bone anabolic strategy.

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

In this project, we revealed that CKIP-1 could be potential drug target for bone anabolic strategy. In future studies, it is of great significance to screen small molecules targeting CKIP-1 for inhibition to promote bone formation.

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)

The only FDA-approved anabolic agent for stimulating bone formation is parathyroid hormone. However, the dominant bone resorption after 2-year-treatment is a great concern. Thus, it is highly desirable to understand molecular mechanism regulating osteoblastic bone formation and further develop anabolic agents. Casein kinase-2 interacting protein-1 (CKIP-1) abundantly expressed in skeletal system. C-terminal domain of CKIP-1 can interact with linker between WW domains of Smurf1 and enhance the ligase activity of Smurf1. It is a newly discovered negative regulator of bone formation without activating bone resorption. Further, we found that N-terminal PH domain of CKIP-1 was also required for regulating Smurf1 and CKIP-1 could bind to ubiquitin through the PH domain, indicating that both of N-terminal PH domain and C-terminal LZ domain of CKIP-1 may be potential target for bone anabolic strategy. By genetic approach, we showed that CKIP-1 could suppress BMP signaling within osteoblasts by promoting the ubiquitination of the Smad1/5 to inhibit bone formation during aging. Moreover, we developed CH6 aptamer–functionalized lipid nanoparticles (LNPs) encapsulating osteogenic CKIP-1 (*Plekho1*) siRNA (CH6-LNPs-siRNA). CH6 achieved osteoblast-specific delivery of siRNAs and facilitated bone formation in osteopenic and healthy rodents. CH6 aptamer-functionalized LNPs facilitates the clinical translation of an RNAi-based bone anabolic strategy.

<u>Part C: Research Output</u>

8. Peer-reviewed journal publication(s) arising <u>directly</u> 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)*						

2015				**Liang C**, **Guo B**, Wu H, Shao N, Li D, **Liu J**, Dang L, Wang C, Li H, Li S, Lau WK, Cao Y, Yang Z, Lu C, He X, Au DW, Pan X, Zhang BT, Lu C, Zhang H, Yue K, Qian A, Shang P, Xu J, Xiao L, Bian Z, Tan W, Liang Z, **He F**, Zhang L*, Lu A*, **Zhang G***.	Aptamer-functionalized lipid nanoparticles targeting osteoblasts as a novel RNA interference-based bone anabolic strategy. ***Nat Med.*** 2015 Mar;21(3):288-94. doi: 10.1038/nm.3791. Epub 2015 Feb 9.	2016	Yes	Yes	No
	2016			**Liu J**, **Liang C**, **Guo B**, Wu X, Li D, Zhang Z, Zheng K, Dang L, He X, Lu C, Peng S, Pan X, Zhang B*, Lu A*, **Zhang G***	Increased PLEKHO1 within osteoblasts suppresses Smad-dependent BMP signaling to inhibit bone formation during aging. *Aging Cell*.	2016	Yes	Yes	No

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)*
09/2014/Houston, Texas, USA	Aptamer-Functionalized Lipid Nanoparticles (LNPs) Targeting Osteoblasts as a Novel RNAi-Based Bone Anabolic Strategy	ASBMR 2014	2016	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
Liu Jin	Ph.D.	Sep 2016	Aug 2016/Nov 2016

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

NA