(Revised 07/09)

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(please insert ref. above)

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

Molecular and cellular mechanisms of hypoxia/HIFα pathway in regulating biological behavior of mesenchymal stem cells
(低氧/HIFα 通路调节间充质干细胞生物学行为的细胞与分子机制)

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

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Prof. WAN Chao （万超）	Prof. DENG Lianfu （邓廉夫）
Post	Assistant Professor （助理教授）	Professor, Director （教授，所长）
Unit / Department / Institution	School of Biomedical Sciences/Faculty of Medicine/The Chinese University of Hong Kong （香港中文大学医学院生物医学学院）	Shanghai Institute of Traumatology and Orthopaedics/School of Medicine/Shanghai Jiaotong University （上海交通大学医学院上海市伤骨科研究所）
Co-investigator(s) *(with title)*		

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	01/01/2011		
Project Completion date	31/12/2013		
Duration *(in month)*	36		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. Determine the role of deletion of HIF-1α or accumulation of HIFα in mesenchymal stem cells in regulating cranial bone development and the angiogenic-osteogenic coupling in the developing cranium.

2. Investigate the essential function of Hif-1α in cranial mesenchymal stem cell proliferation and differentiation, and examine how hypoxia and Hif-1α control critical steps of extracellular matrix synthesis through regulation of collagen prolyl-4-hydroxylase.

3. Explore the effects of pharmacologic activation of the HIFα pathway using the bone-seeking complex (SF-DFO) on cranial or tibia large bone defect reconstruction.

5.2 Revised Objectives

Date of approval from the RGC: N/A

Reasons for the change: _____

_____

(Revised 07/09)

1.
2.
3. ....

6. Research Outcome

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

We analyzed the effects of disrupting HIF-1α in the condensing mesenchyme using the Cre/loxp strategy. Our results indicates that HIF-1α serves as a key to couple angiogenesis to osteogenesis during skeletal development and regeneration while angiogenesis during the cranium bone development is not affected due to deletion of HIF-1α in the mesenchyme. We showed that the bones of the mutant mice were smaller and less mineralized than the controls, with disorganized mesenchymal condensation. Deletion of HIF-1α impaired self-renewal and osteoblast lineage differentiation of mesenchymal stem cells indexed by colony forming unit assay and reduced expression of osteogenic marker genes, in accord with our findings *in vivo*. Chromatin immunoprecipitation assays showed direct occupancy of the osterix promoter by HIF-1α. Western blot showed that deletion of HIF-1α affected cyclin D1 levels and its phosphorylation states in MSCs. Further molecular characterization indicates that deletion of HIF-1α is associated with impaired calcium signaling. The results suggest that deletion of HIF-1α impairs MSCs self-renewal and osteoblast differentiation and maturation. HIF-1α is required for normal cranial bone development but functions independent of angiogenesis to control the biological behavior of mesenchymal stem cells. Hypoxia condition facilitates MSCs expansion for MSCs-based therapy for skeletal repair. Activation of HIF-1α using small molecules serves as a novel approach to promote skeletal repair or regeneration. EPO, the downstream target of HIF-1α, functions as an agent to promote skeletal repair through enhanced angiogenesis and cartilaginous callus formation. Hypoxia promotes MSCs expansion for skeletal repair and regeneration. Three abstracts were accepted for presentations in international and national conferences, and two publications under this project were published in Plos One. One manuscript is under preparation. One PhD student was trained under this project with one publication in Plos One as first author. Unpublished data is under preparation for another manuscript for submission (Figures 1-6 enclosed separately).

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

Based on the results obtained both groups have the consensus to further develop the research project, we expect that small molecule HIF-α activator serves as an efficient therapy to promote skeletal regeneration for patients with impaired skeletal repair. We propose to further consolidate the results in large size animal models to test the efficacy and safety of the candidate small molecule, and aim to a potential drug candidate for promoting skeletal repair and regeneration.

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)

In clinic Orthopaedics, impaired bone repair (e.g. large bone defects or fracture non-union) is a difficult skeletal disorder that causes tremendous suffer and cost to the patients and society.

The pathology of the impaired bone healing is characterized by less capacity of bone formation associated with decreased vascular supply and oxygen availability, and lacking of reparative stem cells. This project identify that deletion of HIF-1α in the mesenchyme impairs MSCs self-renewal and osteoblast differentiation and maturation. HIF-1α is required for normal cranial bone development but functions independent of angiogenesis to control the biological behavior of mesenchymal stem cells. Activation of HIF-1α using small molecules serves as a novel approach to promote skeletal repair or regeneration.

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)*
Year of publication	Year of Acceptance *(For paper accepted but not yet published)*	Under Review	Under Preparation *(optional)*					

2013				**Tsang WP, Shu Y,** Kwok PL, **Zhang F,** Lee KKH, Tang MK, Li G, Chan KM, Chan WY, **Wan C*.**	$CD146^{+}$ human umbilical cord perivascular cells maintain stemness under hypoxia and as a cell source for skeletal regeneration. *PLoS One.* 2013, 8(10):e76153.		Yes	Yes
2014				**Wan L, Zhang F,** He Q, Tsang WP, Lu L, Li Q, Liu Z, Qiu G, Zhou G, **Wan C*.**	EPO promotes bone repair through enhanced cartilaginous callus formation and angiogenesis. *PLoS One.* 2014, 9 (7):e102010		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)*

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)*

Dec/2011/ Guangzhou, China	Oxygen sensing in regulation of mesenchymal stem cell self-renewal and tissue regeneration	The 4th Guangzhou International Conference on Stem Cell and Regenerative Medicine	2012	Yes	Yes
April/2012/ Beaver Run, USA	Hypoxia inducible factor-1α regulates mesenchymal stem cell self-renewal and osteoblast differentiation	Keystone Symposium on Regenerative Tissue Engineering and Transplantation	2012	Yes	Yes
Nov/2013/ Guangzhou, China	Erythropoietin promotes cartilaginous callus formation and angiogenesis during bone regeneration	2013 Guangdong-Hong Kong-Macau Science, Education and Culture Collaboration Symposium and Guangdong Postgraduate Research Symposium-Regenerative Medicine Session		Yes	Yes
Nov/2013/ Beijing, China	Erythropoietin Promotes Cartilaginous Callus Formation during Bone Healing	8th International Congress of Chinese Orthopaedic Association		Yes	Yes
Nov/2013/ Hong Kong, China	Hypoxia promotes expansion of mesenchymal stem cells for skeletal tissue regeneration	3rd CUHK International Symposium on Stem Cell Biology and Regenerative Medicine		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
Lin Wan	PhD	01/01/2011	31/12/2013

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

For RPg student training, in MOE Key Laboratory for Regenerative Medicine Postgraduate Research Symposium on Regenerative Medicine 2013, the student Wan Lin obtained the First Prize for oral presentation. During performance of the project national and international collaborations have also been developed. The collaborative institutions include Department of Orthopaedics, Beijing Union Medical Hospital (Drs. GX Qiu, ZH Wu), Department of Orthopaedic Surgery, The Johns Hopkins University (Dr. TL Clemens). These collaborations will further promote the development of future joint projects and research student or research fellow training programs.