(Revised 07/09)

RGC Ref.: N_HKU 754/10 NSFC Ref. :
(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

p38 MAPK in human lung cancers and its potential involvement in treatment resistance to tyrosine kinase inhibitors
p38 MAPK在肺癌中的表达与肺癌耐酪氨酸蛋白激酶抑制剂治疗的机制研究

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

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Dr Maria Pik Wong 王碧醫生	Prof Jiahuai Han 韓家淮教授
Post	Associate Professor 副教授	Professor 教授
Unit / Department / Institution	Pathology/University of Hong Kong 病理系/香港大學	Key Laboratory of Ministry of Education for Cell Biology and Tumor Cell Engineering, School of Life Science, Xiamen University 細胞生物學與腫瘤細胞工程教育部重點實驗室/生命科學學院廈門大學
Co-investigator(s) *(with title)*	Dr Kexia Cai 蔡克瑕博士 Dr Elaine LH Leung 梁麗嫻博士 Pathology/University of Hong Kong 病理系/香港大學	

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	1st Jan 2011		

Project Completion date	31st Dec 2012		
Duration *(in month)*	24 months		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. To study the functional role and effects of p38 modulation, as well as relation with tyrosine kinase inhibitor treatment response with regard to cell survival and epithelial mesenchymal transition in lung cancers using cell-based models.

2. To study the in vivo p38 gene expression/activation status and their correlation with signaling markers and clinicopathological parameters in a panel of clinical lung cancers.

5.2 Revised Objectives

Date of approval from the RGC: Not applicable

Reasons for the change: Not applicable

6. Research Outcome

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

1. Phosphorylated p38 (P-p38) expression was associated with well differentiated compared to poorly-differentiated carcinomas with statistical significance (p=0.019, Chi square test). Also, using Kaplan Meier survival analysis, tumours expressing P-p38 were found to associate with longer disease-free survival (p<0.001) and overall survival (p=0.030) compared to tumours without P-p38 expression. No association was found between nuclear activated p38 expression and tumour type, EGFR status, patients' gender, smoking history or tumour pathological stage. These findings suggested p38 expression had a negative role in lung cancer progression.
2. Pharmacological suppression of p38 in lung cancer cell lines did not lead to increased apoptosis in EGFR-mutant, tyrosine kinase inhibitor (TKI)-sensitive or TKI-resistant lung cancer cell lines, suggesting p38 does not play a tumour-supportive role.
3. In view of the association of p38 with better tumour differentiation, the expression of p38 in lung cancer stem cells or tumour initiating cells (TIC) identified by $CD44^{high}/ALDH^{high}$ markers were analyzed and results showed the non-TIC (differentiated cancer cells) had higher p38 expression, implicating p38 drives lung cancer differentiation. Consistently, p38 suppression alone or in combination with TKI treatment led to increased TIC, supporting a low p38 level mediates TIC maintenance.
4. Overall, the results suggest instead of a tumour-supportive role, p38 plays a tumour-suppressive role in lung cancer through driving cancer cell differentiation

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

The current findings suggest p38 suppresses TIC in lung cancer. Further investigation would be performed by genetic modulation of p38 expression in lung cancer cell lines and observing for effects on TIC properties. In cell lines with high p38 expression, p38 would be knocked-down to test for any increase in TIC proportion. Conversely, in cell lines showing low p38 expression, p38 would be over-expressed followed by detection of TIC reduction. TIC proportions would be analyzed using flow cytometry detection of TIC markers such as CD44, ALDH, CD133 and CD166. TIC properties would be studied using cell sphere assay, serial xenograft transplantability at limiting cell dose, pleuripotency gene expression, cell invasion/migration ability, etc.

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 p38 MAPK pathway regulates body responses to a wide range of stress stimuli in a context-dependent and tissue-specific manner. The isoform p38α (p38) has been found to mediate both

tumour suppressive and tumor-supportive functions in various cancer models. However, its role in lung cancer development is not clear. In view that EGFR is frequently mutated in lung adenocarcinomas and p38 is a downstream target of EGFR signaling, we hypothesize p38 could play a tumour- supportive role in lung cancer by augmenting pro-survival pathways. We analyzed activated p38 expression by immunohistochemistry in a panel of primary lung adenocarcinomas and found its expression was significantly associated with higher tumour differentiation ($p<0.001$) and better patient outcome ($p=0.019$, disease-free survival). *In vitro* p38 suppression in lung cancer cell lines did not lead to enhanced cell death. On the other hand, p38 suppression alone or in combination with EGFR inhibition led to increased $CD44^{high}/ALDH^{high}$ lung tumour initiating cells (TIC); isolated lung TIC also showed higher p38 expression. Together, the data implicate p38 plays a tumour suppressive role in lung cancer by driving tumour cell differentiation. Disrupting pathways that inhibit p38 expression or enhancement of p38 expression might be useful for lung cancer treatment.

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)*					
			Manuscript under preparation	Jing Liu, Lai Han Leung, Zhijie Xiao, Pui-Chi Tin, Fang-lin Zhang, Jia-huai Han and Maria Pik Wong	P38MAPK plays a tumour suppressive role through differentiation of $ALDH^{high}/CD44^{high}$ tumour iniating cells in primary human lung adenocarcinoma	no	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)*

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
WONG Wai Keung	MSc in Biomedical Science, Napier University (Dissertation Project)	Sept 1, 2010	Aug 31, 2011

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