CRF 8G (Revised Sep 15)

RGC Reference HKBU5/CRF/10G
please insert ref. above

The Research Grants Council of Hong Kong
Collaborative Research Fund Group Research Projects
Completion Report

(*for completed projects only*)

Part A: The Project and Investigator(s)

1. Project Title

Mass spectrometry-based metabolomics for the characterization of cellular metabolic pathways associated with the development of hepatocellular carcinoma

2. Investigator(s) and Academic Department/Units Involved *(please highlight approved changes in the composition of the project team and quote the date when RGC granted approval of such changes)*

Research Team	Name/Post	Unit/Department/Institution	Average number of hours per week spent on this project in the current reporting period
Project Coordinator	Prof. Zongwei CAI, Chair Professor	Department of Chemistry, HKBU	
Co-Principal investigator(s)	Prof. Xinyuan GUAN, Professor and Director	Laboratory of Cancer Genetics, Department of Clinical Oncology, HKU	
	Prof. Nathalie WONG	Department of Anatomical and Cellular Pathology, CUHK	
	Dr. Philippe Schmitt-Kopplin	Department of BioGeoChemistry and Analytics, Helmholtz Zentrum München, German Research Center for Environmental Health, Germany	
	Prof. Xiao SUN	School of Biological Science & Medical Engineering, Southeast University, China	
Collaborators/ Others			

3. Project Duration

	Original	Revised	Date of RGC Approval *(must be quoted*)
Project Start Date	April 1, 2011	June 1, 2011	February 17, 2012
Project Completion Date	March 31, 2014	November 30, 2014	October 22, 2013
Duration (*in month*)	36 months	42 months	
Deadline for Submission of Completion Report	December 31, 2014	November 30, 2015	

Part B: The Final Report

5. Project Objectives

5.1 Objectives as per original application

1. To develop mass spectrometry-based methodologies for investigating cellular metabolic profiling by using three cell strains, namely human liver normal cell line LO2, HCC cell line QGY-7703 and metastatic HCC cell line H2M.
2. To conduct metabolomics study by using the cell models and to identify differentiating metabolites in non-targeted approach by using high resolution MS and MS/MS. NMR analysis may be performed for conclusive structure elucidation when sample condition allows. Targeted analysis through absolute quantification will be applied to confirm the proposed metabolic pathways.
3. To characterize metabolic profiling in cells introduced with eIF-5A2 versus vector controls for understanding the phenotypes of eIF-5A2 function in cancer pathogenesis and visualized by the sophisticated mathematical tools. Co-immunoprecipitation will be carried out in the cancer cell lines introduced with eIF-5A2 or PDSS2 for the correlation study.
4. To simulate the metabolic networks after absolute quantification of the metabolites by bioinformatic tools.
5. To investigate protein-metabolite interactions and enzyme mutants or novel enzymatic activities through the analyses using mass spectrometry as well as X-ray and NMR.
6. To unlock the metabolic programming associated with the pluripotency of stem cells and to better understand the HCC progression from a novel vista by adopting liver cancer stem cells.

5.2 Revised objectives

1. To develop mass spectrometry-based methodologies for investigating cellular metabolic profiling by using three cell strains, namely human liver normal cell line LO2, HCC cell line QGY-7703 and metastatic HCC cell line H2M.
2. To conduct metabolomics study by using the cell models and to identify differentiating metabolites in non-targeted approach by using high resolution MS and MS/MS. NMR analysis may be performed for conclusive structure elucidation when sample condition allows. Targeted analysis through absolute quantification will be applied to confirm the proposed metabolic pathways.
3. To characterize metabolic profiling in cells introduced with eIF-5A2 versus vector controls for understanding the phenotypes of eIF-5A2 function in cancer pathogenesis and visualized by the sophisticated mathematical tools. Co-immunoprecipitation will be carried out in the cancer cell lines introduced with eIF-5A2 or PDSS2 for the correlation study.
4. To simulate the metabolic networks after absolute quantification of the metabolites by bioinformatic tools.
5. To investigate enzyme mutants or novel enzymatic activities through the analyses using mass spectrometry as well as X-ray and NMR.
6. To unlock the metabolic programming associated with the pluripotency of stem cells and to better understand the HCC progression from a novel vista by adopting liver cancer stem cells.

Date of approval from the RGC: February 17, 2012

Reasons for the change: Due to the reduced budget, we revised one objective (#5) from the original objectives and would not carry out the protein-metabolite interactions in this project.

2.

3.

6. Research Outcome

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

For this project supported by CRF, we already published a total 15 papers in ***Nature Medicine***, ***Gastroenterology***, ***Hepatology*** and ***Analyst***, etc. Three more manuscripts are under review and other 3 manuscripts are under preparation. These papers cover mass spectrometric analysis and cancer biology research. In the project, we established a metabolomics platform and elucidated the metabolic reprogramming and related molecular mechanisms in the development of hepatocellular carcinoma (HCC). Firstly, we developed metabolomics platform based on mass spectrometry (manuscript submitted to ***Talanta***). The intermediate metabolites in central carbon metabolism and amino

acids can be separated and quantitatively analyzed by using ion-pair reagents in liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis.

Secondly, we found that oncogene eukaryotic translation initiation factor 5A-2 (EIF-5A2) regulates lipogenesis by consuming acetate. Acetate provides a new carbon source for lipid synthesis which was found to be regulated by EIF-5A2 through knockdown of EIF-5A2 in HCC cell lines or enforced expression in normal liver cell line LO2 (manuscript under preparation). It should be noted that EIF-5A2 overexpression under hypoxia contributes to metastasis and angiogenesis of esophageal squamous cell carcinoma (***Gastroenterology*** 2014).

Thirdly, we developed a mass spectrometric method for quantification of both reduced and oxidative forms of CoQ10 in cells and animal tissues (***Analyst*** 2014). This method was applied in elucidation of metabolic mechanisms in HCC regulated by tumor suppressor gene prenyl (decaprenyl) diphosphate synthase, subunit 2 (PDSS2). PDSS2 reprograms mitochondrial metabolism in HCC cell lines and functions anti-tumor activity (manuscript under preparation).

Fourthly, this project also supports our studies in cancer stem cells. We highlighted hexosamine biosynthetic pathway (HBP) in CD133-positive subpopulation compared to CD133-negative cells. Next, we investigated regulatory role of HBP in on hepatic cancer stem cell marker CD133 under low glucose conditions (Manuscript under review). Moreover, the project also supported related studies in metabolomics (***Current Alzheimer Research*** 2015; ***Analytical Chimica Acta*** 2015; ***Trends in Analytical Chemistry*** 2015; ***Analytical Methods*** 2015); as well as liver cancer researches, such as RNA editing (Nature Medicine 2013) and other oncogenes in HCC development (***Hepatology*** 2012; ***Gastroenterology*** 2013; ***Gastroenterology*** 2014).

The CRF project also fostered young scientists including three PhD students and two research assistant professors.

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

The established method and our findings in metabolic reprogramming in HCC are still premature, although more than 15 papers/manuscripts are published and some are prepared. In the future research, we will further develop metabolic flux analysis in cells and animal models, particularly establish the mathematical models. The underlying mechanisms in tumor metabolism should be investigated *in vivo* for the proposed course of action, highlighting tissue-specific metabolic modes.

6.3 Research collaboration achieved *(please give details on the achievement and its relevant impact)*

We achieved the research collaboration successfully between PC and Co-PIs. The PC (Prof. Zongwei Cai) led the metabolomics team and frequently met and discussed with the Co-PIs on the scientific questions raised from this project.

The Co-PI (Prof. Xin-yuan Guan) led a team to carry out the molecular mechanisms in HCC and collaborated with the PC Prof. Cai to uncover the underlying metabolic mechanisms. The Co-PI (Prof. Nathalie Wong) joined the collaborative research in HCC. The Co-PI (Prof. Philippe Schmitt-Kopplin) provided Fourier transform ion cyclotron resonance mass spectrometry and ultrahigh performance liquid chromatography quadrupole time-of-flight mass spectrometry for the cellular metabolomics. The Co-PI (Prof. Xiao Sun) provided suggestion in statistical analysis.

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)

It is well-known that HCC is the deadly disease. For example, there were 1790 new cases of liver cancer in 2012, with 1364 cases of males and 426 cases of females. This CRF project conducted the metabolomics development and application in HCC research. Metabolomics is a powerful tool in systems biology for a broader investigation of cellular metabolism. Metabolic alterations in cancer cells have been gained attention due to the potential therapeutic targets. By combining metabolomics with molecular biology, metabolic mechanisms in HCC have been investigated. Lipogenesis, dysregulated mitochondrial metabolism and hexosamine biosynthetic pathway have been highlighted as the therapeutic targeted in HCC.

Part C: Research Output

8. Peer-reviewed journal publication(s) arising directly from this research project

(Please attach a copy of the 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) *(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 RGC *(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)						
2012				Ming Liu, Leilei Chen, Tim Hon Man Chan, Jian Wang, Yan	Serum and Glucocorticoid Kinase 3 at 8q13.1 Promotes	2012	Yes	Yes	Yes

				Li,1 Yan Li, Ting-Ting Zeng, Yun-Fei Yuan, Xin-Yuan Guan*****	Cell Proliferation and Survival in Hepatocellular Carcinoma ***Hepatology*** 2012;55:1754-1765.				
2013				Yan Li, Leilei Chen, Tim Hon Man Chan, Ming Liu, Kar-Lok Kong, Ji-Liang Qiu, Yan Li, Yun-Fei Yuan*****, Xin-Yuan Guan*****	SPOCK1 Is Regulated by CHD1L and Blocks Apoptosis and Promotes HCC Cell Invasiveness and Metastasis in Mice ***Gastroentero logy*** 2013;144:179–191.	2012	Yes	Yes	yes
2013				Leilei Chen, Yan Li, Chi Ho Lin, Tim Hon Man Chan, Raymond Kwok Kei Chow, Yangyang Song, Ming Liu, Yun-Fei Yuan, Li Fu, Kar Lok Kong, Lihua Qi, Yan Li, Na Zhang, Amy Hin Yan Tong, Dora Lai-Wan Kwong, Kwan Man, Chung Mau Lo, Si Lok, Daniel G Tenen*****, Xin-Yuan Guan*****	Recoding RNA editing of AZIN1 predisposes to hepatocellular carcinoma ***Nature Medicine*** 2013;19(2):209-216		Yes	Yes	Yes
2014				Ming Liu, Yan Li, Leilei Chen, Tim Hon Man Chan, Yangyang Song, Li Fu, Ting-Ting Zeng, Yong-Dong Dai, Ying-Hui Zhu, Yan Li, Juan Chen,	Allele-Specific Imbalance of Oxidative Stress-Induced Growth Inhibitor 1 Associates With Progression of Hepatocellular Carcinoma ***Gastroentero***		Yes	Yes	Yes

				Yun-Fei Yuan, Xin-Yuan Guan*****	***logy*** 2014;146:1084–1096.				
2014				Yan Li*****, Li Fu, Jian-Biao Li, Yanru Qin, Ting-ting Zeng, Jie Zhou, Zhao-Lei Zeng, Jinna Chen, Ting-Ting Cao, Xiaojiao Ban, Chaonan Qian, Zongwei Cai, Dan Xie, Peng Huang, Xin-Yuan Guan*****	Increased Expression of EIF5A2, Via Hypoxia or Gene Amplification, Contributes to Metastasis and Angiogenesis of Esophageal Squamous Cell Carcinoma ***Gastroenterology*** 2014;146:1701–1713.		Yes	Yes	Yes
2014				Keng Po Lai, Jiawei Chen, Mian He, Arthur K.K. Ching, Coleen Lau, Paul B.S. Lai, Ka-Fai To, Nathalie Wong*****	Overexpression of ZFX confers self-renewal and chemoresistance properties in hepatocellular carcinoma ***International Journal of Cancer*** 2014;135(8): 1790-1799.		Yes	Yes	Yes
2014				Ming Liu, Lingxi Jiang, Xin-Yuan Guan*****	The genetic and epigenetic alterations in human hepatocellular carcinoma: a recent update ***Protein & Cell*** 2014;5(9):673-691		Yes	Yes	Yes
2014				Xian Wang, Shuhai Lin, Zongwei Cai*****	Mass spectrometry-based metabolomics and their developments in China ***Scientia Sinica***		Yes	Yes	Yes

					2014;44(5):724-731.				
2014				Zhi Tang, Shangfu Li, Xinyuan Guan, Philippe Schmitt-Kopplin, Shuhai Lin*****, Zongwei Cai*****	Rapid assessment of the coenzyme Q_{10} redox state using ultrahigh performance liquid chromatography tandem mass spectrometry ***Analyst*** 2014;139(21):5600-5604.		Yes	Yes	Yes
2015				Shuxia Jiang, Yongle Li, Shuhai Lin, Hongbo Yang, Xin-yuan Guan, Haiyun Zhou*****, Tiangang Luan, Zongwei Cai*****	Mass spectrometry-based lipidomics analysis using methyl tert-butyl ether extraction in human hepatocellular carcinoma tissues ***Analytical Methods*** 2015;7,8466-8471.		Yes	Yes	Yes
2015				Shangfu Li, Yibao Jin, Zhi Tang, Shuhai Lin, Hongxia Liu, Yuyang Jiang, Zongwei Cai*****	A novel method of liquid chromatography–tandem mass spectrometry combined with chemical derivatization for the determination of ribonucleosides in urine ***Analytica Chimica Acta*** 2015;864:30–38.		Yes	Yes	Yes

2015				Yang Shen, Tohidi Fatemeh, Leihan Tang, Zongwei Cai*****	Quantitative metabolic network profiling of escherichia coli: an overview on analytical methods for measurement of intracellular metabolites ***Trends in Analytical Chemistry*** 2015;doi:10.1016/j.trac.2015.07.006		Yes	Yes	Yes
	2015			Zhi Tang, Liangfeng Liu, Yongle Li, Jiyang Dong, Min Li, Jiandong Huang, Shuhai Lin*****, Zongwei Cai*****	Urinary Metabolomics Reveals Alterations of Aromatic Amino Acid Metabolism of Alzheimer's Disease in the CRND8 Transgenic Mice. ***Current Alzheimer Research.***		Yes	Yes	Yes
2015				Jian Wang, Ming Liu, Leilei Chen, Tim Hon Man Chan, Lingxi Jiang, Yun-Fei Yuan, Xin-Yuan Guan*****	Overexpression of N-terminal kinase like gene promotes tumorigenicity of hepatocellular carcinoma by regulating cell cycle progression and cell motility ***Oncotarget*** 2015;6(3):1618-1630		Yes	Yes	
2015				Lingxi Jiang, Dora Lai-Wan Kwong, Yan Li, Ming Liu, Yun-Fei Yuan, Yan Li, Li Fu, Xin-Yuan Guan*****	HBP21, a chaperone of heat shock protein 70, functions as a tumor suppressor in hepatocellular carcinoma		Yes	Yes	Yes

					Carcinogenesis 2015;36 (10): 1111-1120.				
		√		Iris Ming Jing Xu , Robin Kit Ho Lai , Shu-Hai Lin , Aki Pui Wah Tse , David Kung Chun Chiu , Hui Yu Koh , Cheuk-Ting Law , Chun Ming Wong , Zongwei Cai , Carmen Chak Lui Wong*****, Irene Oi Lin Ng*****	Transketolase counteracts oxidative stress to drive cancer development. ***Proceedings of the National Academy of Sciences of the United States of America***		No	Yes	
		√		Shuhai Lin, Tengfei Liu, Xiaoyan Ming, Zhi Tang, Li Fu, Philippe Schmitt-Koppl in, Basem Kanawati, Stephanie Ma, Xin-Yuan Guan*****, Zongwei Cai*****	Regulatory role of hexosamine biosynthetic pathway on hepatic cancer stem cell marker CD133 under low glucose conditions. ***Scientific Reports.***		No	Yes	
		√		Zhi Tang, Tingting Cao, Shuhai Lin, Li Fu, Shangfu Li, Xin-Yuan Guan, Zongwei Cai*****	Characterization of oncogene-induced metabolic alterations in hepatic cells by using ultrahigh performance liquid chromatography-tandem mass spectrometry ***Talanta***		No	Yes	
			√	Zhi Tang, Tengfei Liu, Tingting Cao, Li Fu, Yongle Li, Jiyang Dong, Xin-Yuan Guan, Shuhai Lin, Zongwei Cai*****	The O-GlcNAc modification directs coordinated switches of glucose metabolism in hepatocellular carcinoma		No	Yes	

					cell line revealed by targeted metabolomics				
			√	Shuhai Lin*, Zhigang Luo, Hongzhi Zhao, Jingjing Liu, Xin-Yuan Guan, Jianlin Wu, Yan Li, Zongwei Cai*	Proteomics and metabolomics reveal the gender disparity in human hepatocellular carcinoma.		No	Yes	

9. Recognized international conference(s) in which paper(s) related to this research project was/were delivered *(Please attach a copy of each conference abstract)*

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 RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
10/2013/ Beijing	Mass spectrometry-based metabolomics and their developments for research in biological sciences	International Beijing Conference and Exhibition on Instrumental Analysis		Yes	Yes	No
05/2014/ Taiwan	Metabolomics for investigation on TCDD toxicity and associated diseases	2014 Workshop on Environmental and Analytical Chemistry		Yes	Yes	No
04/2015/ Shanghai	Determination of the coenzyme Q_{10} redox state using ultrahigh performance liquid chromatography tandem mass spectrometry	The 31st International Symposium on MicroScale Bioseparations		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
TANG Zhi	PhD	16 August 2011	3 December 2015
CAO Tingting	PhD	1 September 2011	31 August 2014
JIANG Lingxi	PhD	1 September 2012	31 August 2015
LI Shangfu	PhD	July 1, 2012	

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

This project has been carried out in an international collaboration with Prof. Philippe Schmitt-Kopplin at Helmholtz Munich Zentrun in Germany as well as Prof. Xiao Sun at Southeast University in mainland China.

Project Coordinator

Signature: _____

Name: _____

Date: _____

Contact Information:

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