Project number: T12-403/11

RESEARCH GRANTS COUNCIL
THEME-BASED RESEARCH SCHEME (TRS)

Completion Report on Funded Project

Project start date: *1-Dec-2011*
Project completion date: *30-Nov-2016*

1. Project Title:

The Liver Cancer Genome Project: translating genetic discoveries to clinical benefits

2. Names and Academic Affiliations of Project Team Members#

Project team member	Name / Post	Unit / Department / Institution	Average number of hours per week spent on this project in the whole project period
Project Coordinator (PC)	Nathalie WONG Professor	Dept of Anatomical & Cellular Pathology, The Chinese University of Hong Kong	30h
Co-Principal Investigator(s)	Jun YU Professor	Dept of Medicine and Therapeutics, The Chinese University of Hong Kong	10h
	Xin Yuan GUAN Professor	Dept of Clinical Oncology, The University of Hong Kong	10h
	Ting Fung CHAN Associate Prof.	School of Life Sciences, The Chinese University of Hong Kong	8h#
	Ka Fai TO Professor	Dept of Anatomical & Cellular Pathology, The Chinese University of Hong Kong	8h
	Paul B. LAI Professor	Dept of Surgery, The Chinese University of Hong Kong	4h
	Henry L. CHAN Professor	Dept of Medicine and Therapeutics, The Chinese University of Hong Kong	4h

	King L. CHOW Professor	School of Science, The Hong Kong University of Science and Technology	2h
	Tony S. MOK Professor	Dept of Clinical Oncology, The Chinese University of Hong Kong	2h
	Ying Rui LI Professor	Beijing Genomics Institute at Shenzhen, Shenzhen	1h
	Wilfred S. NG Associate Prof.	Dept of Computer Science & Engineering, The Hong Kong University of Science and Technology	1h
Co-Investigator(s)	Stephen CHAN Associate Prof.	Dept of Clinical Oncology, The Chinese University of Hong Kong	6h
	Alfred CHENG Associate Prof.	School of Biomedical Sciences, The Chinese University of Hong Kong	6h
	Vincent WONG Professor	Dept of Medicine and Therapeutics, The Chinese University of Hong Kong	4h
	Kwok Wai LO Professor	Dept of Anatomical & Cellular Pathology, The Chinese University of Hong Kong	4h
	Kwan MAN Professor	Dept of Surgery, The University of Hong Kong	2h
Collaborators	Chung Mau LO Professor	Dept of Surgery, The University of Hong Kong	N.A.
	Yun Fei YUAN Professor	Dept of Hepatobiliary Oncology, Sun Yat-Sen Cancer Center, Guangzhou	N.A.

 # Please highlight the approved changes in the project team composition and quote the date when the RGC granted approval of such changes. For changes in the project team composition, please submit a separate request, together with the justification and the curriculum vitae of the new member(s), to the RGC three months prior to the intended effective date of the change.

Prof. Ting Fung Chan, bioinformatician from School of Life Sciences CUHK, was approved as Co-PI to this project following the first On-Site Visit by the RGC Expert Panel. The application recommending Prof. Chan as team member was made in the first Review Report and approval granted on 7/Jan/2014.

3. Project Objectives

Summary of objectives addressed/achieved:

Objectives*	Percentage achieved	Remarks**
1. To elucidate the genetic basis of viral hepatitis B (HBV)-induced and non-alcoholic steatohepatitis (NASH)-induced liver carcinogenesis	100%	nil
2. To delineate specific molecular events leading to cell invasion and metastasis of hepatocellular carcinoma (HCC)	100%	nil
3. To examine defined genomic alterations for biological basis that promote pathologic pathways during HCC development and progression	100%	nil
4. To evaluate identified genetic markers for clinical usefulness including early disease detection, patient prognosis and as druggable targets	100%	nil

* *Please highlight the approved changes in objectives and quote the date when the RGC granted approval of such changes.*

****** *Please provide reasons for significantly slower rate of progress than originally planned.*

6. Research Highlights and Outputs

(Maximum 20 A4 pages for sections 6 to 9, excluding any appendices and attachments)

6.1 What are the most exciting research accomplishments of the project?

(Please list five or more of the team's best research accomplishments, such as journal and conference papers, software codes, research infrastructure, etc. For each item, please clearly justify how it has achieved international excellence (e.g. best paper award, invited presentation, citations, product licensed to industry, etc.)

The project team has undertaken intense research activity during the course of this TRS project. Together, we have achieved project goals and made key accomplishments from the next-generation sequencing (NGS) analysis of HCC for its genetic blueprint. In this Report, we highlight few exciting endeavours:

Functional Significance of Somatic Abnormalities

We have functionally characterised a number of NGS-derived somatic variants as reflected by the number of publications shown in Section 6.4a. In particular, from the analysis of HCC and adjacent non-tumoral liver pairs, co-PI **Guan XY** discovered the novel presence of frequent RNA editing on the AZIN1 gene, where the overediting contributes to HCC initiation and progression (***Nature Medicine 2013***). The RNA editing machinery is known to be dysregulated in some cancers, but only recently have the mechanistic implications of aberrant editing events in cancer begun to be dissected. The work by Guan's group showed that A→I (G) RNA editing occurs at residue 367 (Ser→Gly) of AZIN1 and that this recoding edited event is predicted to affect protein conformation, leading to changes in subcellular localization and function. While genome studies have provided new insights into the genomic changes that occur in HCC, much less is known, however, with respect to RNA alterations in this cancer. Hence, the findings of Guan XY and co-workers are especially of impact and significance to the field as they unveil, for the first time, a substantial role for RNA editing in HCC and a previously undescribed target AZIN1. Their impressive study was published in Nature Medicine and received commentary on this exciting discovery. This paper was also presented by Guan XY as invited speaker at the National Cancer Centre of China Annual Conference. Beijing in March 2013.

Our work drew attention from scientific community and received highlights & commentaries:

- Cancer Discovery Research Watch 2013: Recoding RNA editing of AZIN1 is oncogenic in HCC
- Nature Medicine 2013: RNA editing enters the limelight in cancer.

A New Layer of Oncogenesis in HBV Insertional Mutagenesis

Chronic infection with chronic hepatitis B (HBV) leads to liver inflammation and cirrhosis, and is associated with significant increased risk of HCC. Despite a direct oncogenic effect from HBV integration has long been postulated, the biologic consequences of HBV integration events remain elusive. The work led by **Wong N** and members **Chan TF, To KF, Chan H and Lai P** illustrated that the mutagenic effect of an HBV integrant can predispose to the risk of HCC development (***Cancer Cell 2014***). Through transcriptome sequencing of HBV-positive HCC cell lines, we showed transcription of viral-human fusions from the site of genome integrations. We showed HBx-LINE1 promotes matrix invasion of HCC cancer cells and tumor growth, and augment of Wnt/β-catenin signaling, which is a major pathway deregulated in HBV-associated HCC. In particular, we found the tumor-promoting properties of HBx-LINE1 intriguingly functions as a hybrid non-coding RNA. Our finding provides pathogenic insights into a hybrid RNA in HCC development and highlights a new layer of molecular complexity in viral oncogenesis. In addition, in the field of RNA biology, our work adds important new information on lncRNA production, and provides novel link between a hybrid lncRNA and the pathological outcome of cancer development.

Our work drew attention from scientific community and received a no. of commentaries:

- Cancer Cell Previews 2014: RNA Identity Crisis: Hepatitis B Walks the LINE.
- Cell Host & Microbe Previews 2014: LINE(1)s of Evidence in HBV-Driven Liver Cancer.
- Nature Genetics Research Highlights 2014: Oncogenic chimeric transcript
- Cancer Discovery Research Watch 2014: Hepatitis B viral insertion generates an oncogenic long noncoding RNA.
- Faculty of 1000 Editorial 2014: F1000Prime under Gastroenterology & Hepatology

Development of Bioinformatics Tools and Infra-structure

In the attempt of addressing some of the most basic questions one can expect from a cancer genome project, the CUHK team has been developing newer and better computational tools to not only ourselves but also the research community. The first fruit of our labour is ViralFusionSeq (VFS) for the discovery of viral integration sites within the host genome. As HBV insertional mutagenesis is an important pathogenic risk for the development of HCC, VFS is especially useful in studying the viral integration at the genome and/or transcriptome levels. A paper on VFS was published (***Bioinformatics 2013***) and we have used VFS to study a set of HBV-associated HCC samples, and successfully identified some highly abundant viral-human chimeras involved in heightened risk of HCC development (***Cancer Cell 2014***).

In addition to developing new algorithms and pipelines, project team realised the importance to build a bioinformatics infrastructure unit that would aid our NGS analysis. From a CUHK seed fund, an **Integrated Bioinformatics Laboratory (IBL)** for cancer biology and metabolic diseases was setup. The co-ordinator of this unit is **Wong N**, with members including **Chan TF**, **Lo KW**, **Yu J**, **To KF** and Ma R. This IBL unit aims to develop a bioinformatics core that supports research activities entailed within three Theme-based Research Scheme projects (namely 'The Liver Cancer Genome', 'Systematic Development of Molecular Targets for Nasopharyngeal Carcinoma' and 'An integrated Trans-omics Approach to Diabetic Cardio-renal Complications') and two State Key Laboratories (namely 'State Key Laboratory of Digestive Disease' and 'State Key Laboratory in Oncology in South China'). The laboratory equipped with hardware and personnel dedicate its efforts to integrating bioinformatics interpretations with cancer biology and metabolic diseases. In addition, clinical information will also be incorporated for identifying targets of translational potentials. The expanded computation power, personnel support and integrative efforts have given great impetus to our research activities.

NAFLD/NASH Biology

Non-alcoholic fatty liver disease (NAFLD) affects nearly a quarter of the general population. Perpetuate liver inflammation is crucial in the pathogenesis of NASH from NAFLD, and the subsequent development of HCC. During this Project period, **Yu J** and co-workers investigated cytokine/chemokines signalling pathways for their role in liver inflammation. For instance, using both dietary and genetic mouse models of NAFLD, and cultured steatotic hepatocytes, they showed CXCR3 plays a pivotal role in NASH development by inducing production of cytokines, macrophage infiltration, fatty acid synthesis and causing autophagy deficiency. In another study, they showed OGT, a glycosyltransferase, plays an oncogenic role in NAFLD-HCC through regulating palmitic acid and inducing ER stress, consequently activating oncogenic JNK/c-jun/AP-1 and NF-κB cascades.

This work received a commentary in the same issue as the publication:

- Journal of Hepatology 2017: O-GlcNAcylation: Undesired tripmate but an opportunity for treatment in NAFLD-HCC

In a project led by **Yu J** together with team members **Wong N and Lai P**, the sequelae epoxidase (SQLE) a rate limiting enzyme in cholesterol biosynthesis was identified as a crucial component in the initiation of NAFLD and eventually promotes HCC development. Hepatocyte-specific SQLE transgenic expression in mice led to an accelerated development of high-fat, high-cholesterol diet-induced HCC. In addition to activating endoplasmic reticulum stress and NF-kB signaling pathways, SQLE also drives oxidative stress-induced, DNMT3A-dependent promoter methylation and transcriptional silence of PTEN, leading to the activation of AKT-mTOR and histone H3S10 phosphorylation. Terbinafine, an inhibitor of SQLE, markedly inhibits SQLE-induced signal transduction and oncogenesis in NAFLD-HCC cell lines and animal models. Together, our exciting finding on SQLE led to establish its role as a driver oncogene in NAFLD-HCC that can be targeted to suppress tumor development. This work is currently under review at Science Translational Medicine (*minor revision*)

Clinical Translation of Diagnostic Mutational Profiling using Custom NGS Panel

With the advances made in cancer genomic studies, it becomes evident that driver gene mutations may differ between individuals even within the same cancer type. In addition, identification of these driver mutations or genetic targets has led to the rapid development and success of personalized molecular targeting therapy. In this respect, genetic screening to identify druggable targets has become part of a standard treatment for some cancer types, such as lung non-small cell carcinoma. However, much work still needs to be done for HCC. Globally, Phase 1 clinical trials using molecular pre-screening approach is being conducted in several international major centres. In Asia, the POLARIS program in Singapore and EPOC program in Japan are also running clinical trial to stratify patient treatment based on genomic results. Thus, a genetic screening service coupled with Phase 1/2 targeted therapy clinical trial for HCC could help develop a suitable therapy for patients.

During this Project period, **To KF** and co-workers have launched an **Actionable Cancer Panel for Molecular Diagnostics** that has been put into patient service through the CUHK pathology department. The target enrichment sequencing of a panel of druggable genes integrates with bioinformatics workflow derived from this Project has been optimized for next generation sequencing of fresh frozen and formalin fixed tumor tissue specimens. Currently, the panel consists of ~250 targeted genes, which have been selected on the basis of their known impact as actionable targets of existing and emerging anti-cancer therapies, and their mutation recurrence frequency across known cancer types. These genomic features have been interrogated to achieve a minimum analytic detection-limit of at least 5%. It is anticipated that based on the molecular screening results, patient will receive treatment in our Phase 1/2 clinical trial center. Thereof, patients will be followed up to monitor disease status and treatment response.

In addition to the above research highlights, there are also a number of studies arising from this TRS project that are either finalising for submission or currently under review at journals.

6.2 What was the added value of the TRS funding, rather than standard project grant funding?
(For example, could this work have been achieved with other funding scheme, such as the General Research Fund or Collaborative Research Fund? If not, why?)

This TRS funding has given project team invaluable opportunities to pursue more in-depth investigations and strengthen our links with colleagues at local universities. In addition, through current research many team members have initiated new national and international collaborations. New avenues generated from results of this Project has also led to further funding opportunities and success in obtaining a number of General Research Funds and grants from different schemes. We have also jointly obtained a Collaborative Research Fund. Our new collaborative links are listed in Section 7.1 and new funding attained shown in Section 9.3

6.3 If the project has not met its original objectives, why?

Project team has accomplished and met original research objectives

6.4 (a) Peer-reviewed journal publication(s) arising <u>directly</u> from this 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. Please mark the symbol "#" next to the publications involving inter-institutional collaborations)

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 the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged 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)*						

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 the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged 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)*						
		2017		Liu D, Wong CC, Fu L, Chen H, Zhao L, Li C, Zhou Y, Zhang Y, Xu W, Cheng G, **Lai PB, Wong N**, Sung JY, ***Yu J**	Squalene mono-oxygenase overexpression drives hepatocarcinogenesis and is a therapeutic target in nonalcoholic fatty liver disease-associated hepatocellular carcinoma	2017	No	Yes	No
#2017				Jiang L, Yan Q, Fang S, Liu M, Li Y, **Yuan YF**, Li Y, Zhu Y, Qi J, Yang X, Kwong DLW, ***Guan XY**	Calcium-binding Protein 39 promotes hepatocellular carcinoma growth and metastasis by activating extracellular signal-regulated kinase signaling pathway Hepatology. 2017;66:1529-1545	2017	Yes	Yes	No
#2017				Zhang X, Wu WKK, Xu W, **Man K**, Wang X, Han J, Leung WY, Wu R, Liu K, ***Yu J**	C-X-C motif chemokine 10 impairs autophagy and autolysosome formation in non-alcoholic steatohepatitis Theranostics. 2017;7(11):2822-2836	2017	Yes	Yes	No

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Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
2017				Cao TT, Lin SH, Fu L, Tang Z, Che CM, Zhang LY, Ming XY, Liu TF, Tang XM, Tan BB, Xiang D, Li F, Chan OY, Xie D, Cai Z, ***Guan XY**	Eukaryotic translation initiation factor 5A2 promotes metabolic reprogramming in hepatocellular carcinoma cells Carcinogenesis. 2017;38:94-104	2017	Yes	Yes	No
2017				Xu W, Zhang X, Wu JL, Fu L, Liu K, Liu D, Chen GG, **Lai PBS, Wong N, *Yu J**	O-GlcNAc transferase promotes fatty liver-associated liver cancer through inducing palmitic acid and activating endoplasmic reticulum stress Journal of Hepatology. 2017;67:310-320	2017	Yes	Yes	No
2017				Leung AKY, Jin N, *Yip KY, ***Chan TF**	OMTools: a software package for visualizing and processing optical mapping data Bioinformatics. 2017;33:2933-2935	2017	Yes	Yes	No

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 the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged 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)*						
2016				XU Z, Zhang X, Lau J, ***Yu J**	C-X-C motif chemokine 10 in non-alcoholic steatohepatitis: role as a pro-inflammatory factor and clinical implication Expert Reviews in Molecular Medicine. 2016;18:1-11	2017	Yes	Yes	No
#2016				Zhang X, Han J, **Man K**, Li X, Du J, Chu ESH, Go MYY, Sung JJY, ***Yu J**	CXC chemokine receptor 3 promotes steatohepatitis in mice through mediating inflammatory cytokines, macrophages and autophagy Journal of Hepatology. 2016;64:160-170	2017	Yes	Yes	No
2016				Shen J, Tsoi H, Liang Q, Chu ESH, Liu D, Yu ACS, **Chan TF,** Li X, Sung JJY, ***Wong VWS, *Yu J**	Oncogenic mutations and dysregulated pathways in obesity-associated hepatocellular carcinoma Oncogene. 2016;35:6271-6280	2017	Yes	Yes	No

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Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
2016				Leung AKY, Kwok TP, Wan R, Xiao M, Kwok PY, *Yip KY, ****Chan TF**	OMBlast: Alignment tool for optical mapping using a seed-and-extend approach Bioinformatics.2016;33:311-319	2017	Yes	Yes	No
2016				***Chan SL**, Wong AM, Lee K, **Wong N**, Chan AKC	Personalized therapy for hepatocellular carcinoma: Where are we now? Cancer Treatment Reviews. 2016;45:77-86	2017	Yes	Yes	No
#2016				Liu M, Chen L, Ma NF, Chow RK, Li Y, Song Y, Chan TH, Fang S, Yang X, Xi S, Jiang L, Li Y, Zeng TT, Li Y, **Yuan YF**, ***Guan XY**	CHD1L promotes lineage reversion of hepatocellular carcinoma through opening chromatin for key developmental transcription factors Hepatology. 2016;63:1544-59	2017	Yes	Yes	No

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 the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged 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)*						
2016				Tang S, *Wu WKK, Li X, Wong SH, **Wong N**, Chan MTV, Sung JJY, ***Yu J**	Stratification of digestive cancers with different pathological features and survival outcomes by MicroRNA expression Scientific Reports. 2016; 6:24466	2017	Yes	Yes	No
#2015				Jiang L, Kwong DLW, Li Y, Liu M, **Yuan YF**, Li Y, Fu L, ***Guan XY**	HBP21, a chaperone of heat shock protein 70, functions as a tumor suppressor in hepatocellular carcinoma Carcinogenesis. 2015;36:1111-1120	2017	Yes	Yes	No
2015				Tian Y, Wong VWS, Wong GLH, Yang W, Sun H, Shen J, Tong JHM, Go MYY, Cheung YS, **Lai PBS**, Zhou M, Xu G, Huang THM, **Yu J, To KF**, ***Cheng ASL, *Chan HLY**	Histone deacetylase HDAC8 promotes insulin resistance and β-catenin activation in NAFLD-associated hepatocellular carcinoma Cancer Research. 2015;75:4803-16	2017	Yes	Yes	No

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 the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged 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)*						
#2015				Song Y, Pan G, Chen L, Ma S, Zeng T, Chan TH, Li L, Lian Q, Chow R, Cai X, Li Y, Li Y, Liu M, Li Y, Zhu Y, **Wong N, Yuan YF,** Pei D, ***Guan XY**	Loss of ATOH8 increases stem cell features of hepatocellular carcinoma cells Gastroenterology. 2015;149(4): 1068-1081	2015	No	Yes	No
2015				Sun T, ***Wong N**	Transforming growth factor-β-induced long noncoding RNA promotes liver cancer metastasis via RNA-RNA crosstalk Hepatology. 2015;61:722-4	2015	No	Yes	No
#2015				Wang J, Chu ES, Chen HY, **Man K**, Go HY, Huang XR, Lan HY, Sung JJ, ***Yu J**	MicroRNA-29b prevents liver fibrosis by attenuating hepatic stellate cell activation and inducing apoptosis through targeting PI3K/AKT pathway Oncotarget. 2015;6:7325-38	2015	No	Yes	No

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 the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged 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)*						
2015				***Chan SL**, Chan AW, Yeo W	Novel therapeutic targets and predictive markers for hepatocellular carcinoma Expert Opinion on Therapeutic Targets. 2015;19:973-83	2015	No	Yes	No
#2015				Wang J, Liu M, Chen L, Chan TH, Jiang L, **Yuan YF**, ***Guan XY**	Overexpression of N-terminal kinase like gene promotes tumorigenicity of hepatocellular carcinoma by regulating cell cycle progression and cell motility Oncotarget. 2015;6:1618-30	2015	No	Yes	No
#2014				Lau CC, Sun T, Ching AK, He M, Li JW, Wong AM, Co NN, Chan AW, Li PS, Lung RW, Tong JH, **Lai PB, Chan HL, To KF, *Chan TF, *Wong N**	Viral-human chimeric transcript predisposes risk to liver cancer development and progression Cancer Cell. 2014;25:335-49	2014	No	Yes	No

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Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
#2014				Jiang L, Yang YD, Fu L, Xu W, Liu D, Liang Q, Zhang X, Xu L, **Guan XY**, Wu B, Sung JJ, ***Yu J**	CLDN3 inhibits cancer aggressiveness via Wnt-EMT signalling and is a potential prognostic biomarker for hepatocellular carcinoma Oncotarget. 2014;5:7663-76	2014	No	Yes	No
#2014				Liu M, Li Y, Chen L, Chan TH, Song Y, Fu L, Zeng TT, Dai YD, Zhu YH, Li Y, Chen J, **Yuan YF**, ***Guan XY**	Allele-specific imbalance of oxidative stress-induced growth inhibitor 1 associates with progression of HCC Gastroenterology. 2014;146:1084-96	2014	No	Yes	No
#2014				Liu L, Dai Y, Chen J, Zeng T, Li Y, Chen L, Zhu Y, Li J, Li Y, Xie D, **Yuan YF**, ***Guan XY**	Maelstrom promotes hepatocellular carcinoma metastasis by inducing epithelial to mesenchymal transition via Akt/GSK-3β/Snail signalling Hepatology. 2014;59:531-43	2014	No	Yes	No

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Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
#2014				Yu C, **Yu J**, Yao X, Wu WK, Lu Y, Tang X, Li X, Bao L, Li X, Hou Y, Wu R, Jian M, Chen R, Zhang F, Xu L, Fan F, He J, Liang Q, Wang H, Hu X, He M, Zhang X, Zheng H, Li Q, Wu H, Chen Y, Yang X, Zhu S, Xu X, Yang H, Wang J, Zhang X, Sung JJ, ***Li YR**, Wang J	Discovery of biclonal origin and a novel oncogene SLC12A5 in colon cancer by single-cell sequencing Cell Research. 2014;24:701-12	2014	No	Yes	No
#2014				Zhang X, Shen J, **Man K**, Chu ES, Yau TO, Sung JC, Go MY, Deng J, Lu L, **Wong VW**, Sung JJ, Farrell G, ***Yu J**	CXCL10 plays a key role as an inflammatory mediator and a non-invasive biomarker of non-alcoholic steatohepatitis Journal of Hepatology. 2014;61:1365-75	2014	No	Yes	No
#2014				Liang Q, Yao X, Tang S, Zhang J, Yau TO, Li X, Tang CM, Kang W, Lung RW, **Li JW, Chan TF**, Xing R, Lu Y, **Lo KW, Wong N, To KF**, Yu C, Chan FK, Sung JJ, ***Yu J**	Integrative identification of Epstein-Barr virus-associated mutations and epigenetic alterations in gastric cancer Gastroenterology. 2014;147:1350-62	2014	No	Yes	No

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2014				Zhang N, Chu E, Zhang J, Li X, Liang Q, Chen J, Chen M, Teoh N, Farrell G, Sung JJ, ***Yu J**	Peroxisome proliferator activated receptor alpha inhibits hepatocarcinogenesis through mediating NF-κB signalling pathway Oncotarget. 2014;5:8330-40	2014	No	Yes	No
2014				Lai KP, Chen J, He M, Ching A, Lau C, **Lai PB, To KF, *Wong N**	Overexpression of ZFX confers self-renewal and chemo-resistance properties in hepatocellular carcinoma Int J Cancer. 2014;135:1790-9	2014	No	Yes	No
2014				Liu M, Jiang L, ***Guan XY**	The genetic and epigenetic alterations in human hepatocellular carcinoma: a recent update Protein Cell. 2014;5:673-91	2014	No	Yes	No

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Year of publication	Year of acceptance *(for paper accepted but not yet published)*	Under review	Under preparation *(optional)*						
2014				Wang J, Dong L, Xu L, Chu ES, Chen Y, Shen J, Li X, Wong CC, Sung JJ, ***Yu J**	B cell CLL/lymphoma 6 member b inhibits hepatocellular carcinoma metastases in vitro and in mice Cancer Lett. 2014;355:192-200	2014	No	Yes	No
2013				***Yu J**, Shen J, Sun TT, Zhang X, ***Wong N**	Obesity, insulin resistance, NASH and hepatocellular carcinoma Seminars in Cancer Biology. 2013;23:483-91	2013	No	Yes	No
2013				Tian Y, **Wong VW, Chan HL, *Cheng AS**	Epigenetic regulation of hepatocellular carcinoma in non-alcoholic fatty liver disease Seminars in Cancer Biology. 2013;23:471-82	2013	No	Yes	No
#2013				Chen L, Li Y, Lin CH, Chan T, Chow R, Song Y, Liu M, **Yuan YF**, Fu L, Kong K, Qi L, Li Y, Zhang N, Tong A, Kwong D, **Man K**, **Lo CM**, Lok S, Tenen DG, ***Guan XY**	Recoding RNA editing of AZIN1 predisposes to hepatocellular carcinoma Nature Medicine. 2013;19:209-16	2013	No	Yes	No

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 the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged 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)*						
#2013				Li Y, Chen L, Chan TH, Liu M, Kong KL, Qiu JL, Li Y, **Yuan YF**, ***Guan XY**	SPOCK1 is regulated by CHD1L and blocks apoptosis and promotes HCC cell invasiveness and metastasis in mice Gastroenterology. 2013;144:179-191	2013	No	Yes	No
2013				Law PT, Qin H, Ching AK, Lai KP, Co NN, He M, Lung RW, Chan AW, ***Chan TF**, ***Wong N**	Deep sequencing of small RNA transcriptome reveals novel non-coding RNAs in hepatocellular carcinoma Journal of Hepatology. 2013;58:1165-73	2013	No	Yes	No
#2013				Wang J, Zhao J, Chu ES, Mok MT, Go MY, **Man K**, Heuchel R, Lan HY, Chang Z, Sung JJ, ***Yu J**	Inhibitory role of Smad7 in hepatocarcinogenesis in mice and in vitro Journal of Pathology. 2013;230:441-52	2013	No	Yes	No

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 the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged 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)*						
2013				Cheung KF, Zhao J, Hao Y, Li X, Lowe AW, **Cheng AS**, Sung JJ, ***Yu J**	CITED2 is a novel direct effector of peroxisome proliferator-activated receptor γ in suppressing hepatocellular carcinoma cell growth Cancer. 2013;119:1217-26	2013	No	Yes	No
2013				Li JW, Wan R, Yu CS, Co NN, **Wong N**, ***Chan TF**	ViralFusionSeq: accurately discover viral integration events and reconstruct fusion transcripts at single-base resolution Bioinformatics. 2013;29:649-51	2013	No	Yes	No
#2013				Li JW, Bolser D, Manske M, Giorgi FM, Vyahhi N, Usadel B, Clavijo BJ, **Chan TF**, **Wong N**, Zerbino D, Schneider MV	The NGS WikiBook: a dynamic collaborative online training effort with long-term sustainability Brief Bioinform. 2013;14:548-55	2013	No	Yes	No

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 the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknow-ledged 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)*						
2013				Tso KK, Yip KY, Mak CA, Chung GT, Lee SD, Cheung ST, **To KF, Lo KW**	Complete genomic sequence of Epstein-Barr virus in nasopharyngeal carcinoma cell line C666-1 Infect Agent Cancer. 2013;8:29	2013	No	Yes	No

(b) Recognised international conferences in which papers related to this project were delivered:

Month/Year/ Place	Title	Conference name	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of the RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
18-22 April 2015, Philadelphia, USA	Histone Deacetylase 8 impairs insulin sensitivity and activates β-catenin signaling in NAFLD-Associated HCC.	AACR Annual Meeting 2015	2015	No	Yes	No
18-22 April 2015, Philadelphia, USA	HCC-derived exosomes promote motility of immortalized hepatocyte through transfer of oncogenic proteins and RNAs.	AACR Annual Meeting 2015	2015	No	Yes	No
3 - 5 Nov 2013, Heidelberg, Germany	Transcriptome sequencing in hepatocellular carcinoma reveals chimera transcription of viral-human sequences at site of HBV integration	EMBL Conference Cancer Genomics. EMBL	2014	No	Yes	No
1-5 Nov 2013, Washington DC, USA	Development of a combined algorithm of Fibroscan and Enhanced Liver Fibrosis (ELF) to detect liver fibrosis in chronic hepatitis B	64th American Association for the Study of Liver Diseases (AASLD).	2014	No	Yes	No
5-9 April 2014, San Diego, USA	ATOH8 depletion can reprogram non cancer stem cells into cancer stem cells	AACR Annual Meeting 2014.	2014	No	Yes	No
5-9 April 2014, San Diego, USA	TTC36, a novel chaperone of heat shock protein 70, functions as a tumor suppressor in hepatocellular carcinoma".	AACR Annual Meeting 2014.	2014	No	Yes	No

Month/Year/ Place	Title	Conference name	Submitted to the RGC *(indicate the year ending of the relevant progress report)*	Attached to this report *(Yes or No)*	Acknowledged the support of the RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
12–15 Mar 2014, Brisbane, Australia	High dietary fat and cholesterol accelerate liver carcinogenesis by inducing DNA damage and promoting hepatocyte proliferation	Asian Pacific Association for the Study of the Liver (APASL),	2014	No	Yes	No
30 Sept – 2 Oct 2013, Toronto, Canada	Whole-genome sequencing identifies recurring CTNNB1 and PNLIP mutations in NASH-related liver cancer".	8th Scientific Workshop International Cancer Genome Consortium (ICGC).	2014	No	Yes	No
24-25 Nov. 2012, Hong Kong.	Biomarker Discovery in Hepatocellular Carcinoma.	The 17th Annual Scientific Symposium of the Hong Kong Cancer Institute: Clinical Application of Biomarker in Cancer Therapy	2013	No	Yes	No
9-13 Nov. 2012, Boston USA	Non-invasive diagnosis of non-alcoholic steatohepatitis by combined serum biomarkers	The Liver Meeting 2012: American Association for the Study of Liver Diseases (AASLD) 63rd Annual Meeting	2013	No	Yes	No
9-13 Nov. 2012, Boston USA	The cirrhosis risk score is not associated with liver fibrosis/cirrhosis and fibrosis progression in Chinese non-alcoholic fatty liver disease patients	The Liver Meeting 2012: American Association for the Study of Liver Diseases (AASLD) 63rd Annual Meeting	2013	No	Yes	No

(c) RGC funding should have been acknowledged in all publication(s)/conference papers listed in (a) and (b) above. If no acknowledgement has been made in any of the publications/ papers, please indicate and provide explanations.

All papers listed have acknowledged the Theme-based Research Scheme (TRS). For conference abstracts, the support of TRS fund was acknowledged on the poster or presentation slides.

6.5 To what extent this project has strengthened inter-institutional collaborations and other partnerships?

Members of this TRS have had close communications and collaborations since the commencement of this project. In addition to the quarterly group meetings, team members have also met on their own and on regular basis to update ongoing studies. Some of these activities include our joint efforts in case selection for sequencings and verification work for incidence score **(Lai P, To KF, Yu J, Man K, Wong N**), bioinformatic analyses of NGS data and development of new algorithms (**Chan TF, To KF, Guan XY, Yu J, Wong N**), defining actionable drug targets (**To KF, Chan S, Mok T, Wong N**), development of biomarkers and cancer panel for personalized medicine (**Wong V, Chan H, Chan TF, Wong N**) and functional biology of somatic variants in-vitro and in-vivo (**Guan**

XY, Yu J, Chow K, Cheng A, Wong N). Many of the collaborative efforts have already resulted in joint publications (Section 6.4a) and grant applications (Section 7.1)

6.6 Research students trained (registration/awards):

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
Qian YAN	PhD	1/Sept/2014	31/Aug/2018 (tentative)
Hanyong SUN	PhD	1/Aug/2014	31/July/2018 (tentative)
Xiaodong YANG	PhD	1/Sept/2013	31/Aug/2017
Jingwan ZHANG	PhD	1/Aug/2012	31/July/2016
Yanjiang GUO	PhD	1/Aug/2011	31/July/2016
Mian HE	PhD	1/Aug/2011	31/Aug/2015
Yuan TIAN	PhD	1/Aug/2012	31/July/2015
Xiang ZHANG	PhD	1/Aug/2011	31/July/2015
Xiaojuan WANG	PhD	1/Aug/2012	31/July/2016
Xiangchun LI	PhD	1/Aug/2012	31/July/2016
Lixia XU	PhD	1/Sep/2011	31/Aug/2014
Yang Yang SONG	PhD	1/Sept/2010	31/Aug/2014
Ling Xi JIANG	PhD	1/Sept/2010	31/Aug/2014
Jun HE (BGI)	PhD	1/Aug/2011	31/July/2014
Gary CHEN	PhD	1/Aug/2010	1/Dec/2013
Jiayun SHEN	PhD	1/Aug/2009	31/July/2013
Yeung Ming WAI	MPhil	1/Aug/2014	31/July/2016
Matthew MAN	MPhil	1/Aug/2013	31/Aug/2015
Shaoqing SZE	MPhil	1/Aug/2013	31/Aug/2015
Senwei TANG	MPhil	1/Aug/2013	31/July/2015
Gordon CHAN	MPhil	1/Aug/2013	31/July/2015
Hao Hao, DENG	MPhil	1/Aug/2013	31/July/2015
Coleen LAU	MPhil	1/Aug/2012	1/Dec/2014
Hector WANG	MPhil	1/Feb/2011	31/Aug/2013

Student Awards

1. Jingwan ZHANG (PhD) awarded **Yu To Sang & Yu Shing Keung Memorial Fund Scholarship 2014/2015,** Senate Committee on University Scholarships, Hong Kong, May 2015.
2. Xiaojuan WANG (PhD) awarded **Oral Presentation and Travel Prize** for abstract entitled 'Deficient autophagosomal-lysosomal function induced p53 and hepatic apoptosis in experimental nutritional steatohepatitis', UEG Week Vienna 2014, Austria. October 2014.
3. Lixia XU (PhD) awarded **Global Scholarship Programme for Research Excellence 2013-14**, Global Scholarship Programmes Steering Committee, CUHK, May 2013.
4. Lixia XU (PhD) awarded **Poster Distinction Award** for abstract entitled 'High dietary fat and cholesterol accelerate liver carcinogenesis by inducing DNA damage and promoting hepatocyte proliferation', APASL Brisbane. March 2014.
5. Xiang ZHANG (PhD) awarded **Yu To Sang and Yu Shing Keung Memorial Fund Scholarship 2012-13,** Senate Committee on University Scholarships, Hong Kong, July 2013.
6. Yuan TIAN (PhD) awarded **3rd prize for oral presentation** at CUHK School of Biomedical Sciences Postgraduate Research Day. Her work was also selected for presentation at the Young

Investigator Award session, International Digestive Disease Forum 2014 in Hong Kong.

6.7 Specific products (e.g. software or netware, instruments or equipment developed):

The CUHK-bioinformatics team supported by this TRS project has developed the ViralFusionSeq (VFS) tool for discovery of viral integration sites in host HCC genome. The paper was online in Jan 2013 and the tool itself has been well received. Since its publication, there has been >700 downloads. A majority of downloads from United States along with 13 other geographical regions. VFS is distributed to the scientific community at http://hkbic.cuhk.edu.hk/software/viralfusionseq

Patents (Wong N)

Detection of HBx/8p11 Hybrid Sequence in Human Hepatocellular Carcinoma. United States Patent and Trademark Office, Application No.: 61/530,223

ABRAXIS Methods of Treatment of Hepatocellular Carcinoma. United States Patent and Trademark Office, Application No. 11760333.2-1216

ABRAXIS Methods of Treatment of Hepatocellular Carcinoma. European patent application. Application No.: 14200645.1-1464

6.8 Other education activities and/or training programmes developed:

The broadly based research developments entailed in this TRS project provide ideal training grounds for postgraduate students, post-doctoral fellows and clinical trainees. Team members offer coherent training programs in a number of basic and clinical aspects of HCC. During this Project period, we are collectively supervising 15 postdoctoral fellows in the areas of cancer genomics, functional biology and bioinformatics. Within the same period, training of visiting scholars from China and Japan has also been offered (2 gastroenterologists and 4 postdoctoral fellows).

Nathalie Wong gave a public lecture on 'Cancer Genomic and Personalized Medicine' in Sept-2012 at the Hong Kong Science Museum. The lecture was mainly for the general public and high school students with messages conveyed on the importance of genome information in personalized medicine. Major tools for genome analysis and examples of their application in cancer types were highlighted. At the same time, challenges from both scientific and clinical perspectives in promoting personalized health care were also discussed.

Technological progress in the diagnosis and therapy of gastrointestinal conditions is rapidly changing. To promote clinical expertise specialising in liver diseases, Henry Chan and Vincent Wong have been providing 'Specialist Training Program in Gastroenterology and Hepatology' for clinical fellows. In addition, they also run an MSc course in Gastroenterology for physicians, nurses and other healthcare practitioners to help equip them for the future multidisciplinary practice in digestive diseases. This MSc course is currently the first and only postgraduate programme in Gastroenterology in Asia. Both programs aim to provide a comprehensive and structured higher medical training in gastroenterology, and opportunities for research engagement, for those who have completed general professional training.

6.9 Please highlight any deliverables indicated in the project implementation timetable endorsed by the RGC which have not been covered or achieved as per sections 6.1 to 6.8 above, and explain/ elaborate.

Research progresses have adhered to project milestones and timeline during this Project period.

Project Management

6.10 Please elaborate how the PC has played his/her role in coordinating and managing the project.

Since the commencement of this TRS project, PC and the management team has undertaken a governance role in structuring the project and engaging team members in research collaborations. To ensure timely generation of sequencing datasets and pave the downstream research directions,

PC has observed the process of cases selections, logistics in samples transfer and dissemination of analyzed data to group members for different lines of investigations. In terms of resource, the collective decision from PC and the management team had been to be placed major budget allocation during the first 24 months for the NGS work of whole-genome and whole-transcriptome. Following the initial phase, PC has redistributed funds to co-PIs of respective areas of laboratory and clinical studies for downstream investigations, and clinical accruals. PC and the management team has also been responsible to address problems that arose during the research processes and monitor progress of various projects. The overall coordinated activities have been synergistic, collaborative and effective.

7. Awards and Recognition

7.1 Have any research grants been awarded that are directly attributable to the results obtained from this project?

There are a no. of grants awarded to team members during this Project period, below briefly listed:

Collaborative Research Fund

Cheng A (PC), Wong N, To KF (co-PIs). Functional liver cancer epigenomics: exploiting epigenetic vulnerabilities for therapeutics. RGC Collaborative Research Fund, $7,418,375 (2015-2018)

General Research Fund

Cheng A (PI), Wong V (co-I). Dissecting an inflammatory-CCRK circuitry in non-alcoholic fatty liver disease-related hepatocarcinogenesis. RGC General Research Fund, $763,612 (2017-2018)

Paul Lai (PI). Regulation of hepatic cancer stem cells by the zinc finger transcription factor ZBP-89 in hepatocellular carcinoma. RGC General Research Fund, $941,042 (2017-2018)

Wong V (PI). Incidence of non-alcoholic fatty liver disease and advanced fibrosis in patients with type 2 diabetes: A prospective cohort study using paired controlled attenuation parameter and liver stiffness measurements. RGC General Research Fund, $658,050 (2017-2019)

Yu J (PI), Wong N (co-I). Functional characterization of squalene epoxidase in promoting fatty liver disease-associated liver cancer. RGC General Research Fund, $1,141,432 (2016-2018)

Paul Lai (PI). Cellular mechanism for reduced expression of the enzyme cytochrome P450 1A2 (CYP1A2) in hepatocellular carcinoma. RGC General Research Fund, $843,044 (2016-2018)

Chan TF (PI), Wong N (co-I). Integrative analyses of RNA-sequencing, bioinformatics and biological studies to define cancer-associated long intergenic noncoding RNAs in hepatocellular carcinoma. RGC General Research Fund, $749,433 (2015-2017)

Guan XY (PI). Characterization of the regulatory roles of CHD1L in stemness and differentiation in hepatocellular carcinoma. RGC General Research Fund, $1,039,239 (2014-2017)

Cheng A (PI). Mechanistic characterization of liver cancer epigenome mediated by androgen receptor signaling. RGC General Research Fund, $887,850 (2014-2017)

Wong V (PI), Chan H (co-I). Factors associated with incident nonalcoholic fatty liver disease in the general population: A follow-up study with proton-magnetic resonance spectroscopy and transient elastography. RGC General Research Fund, $850,000 (2013-2014)

Chan S (PI). Development of staging system based on circulating inflammatory marker for hepatocellular carcinoma. RGC General Research Fund, $389,715 (2013-2015)

Other Funds:

Wong V (PI). Dietary determinants of endotoxemia and nonalcoholic fatty liver disease – a population study. Health and Medical Research Fund, Hong Kong. $914,146 (2014-2015)

Cheng A (PI). Targeting H3K27 trimethylation epigenome for liver cancer prevention. Health and Medical Research Fund, Hong Kong. $738,206 (2014-2015)

Guan XY (PI). Analysis of the role of ATOH8 in HCC cancer stem cell and somatic cell reprogramming. NSFC/RGC Joint Research Scheme, $1,022,2120 (2013-2016)

Yu J (PI). State Key Laboratory of Cancer Biology (CUHK Joint Research Base in Shenzhen). Shenzhen Virtual University Park Support Scheme, Shenzhen. RMB$1,00,000 (2013-2017)

7.2 Have any project team members participated as invited speakers in or organisers of international conferences as a result of this project?

Team members have been invited as speakers to many international conferences and presented distinguished lectures during this Project period. Below highlights some of these events:

Invited Talks

Wong N. 'Genome Sequencing in Hepatocellular Carcinoma'. 3rd LMU-China Academic Network Scientific Forum, Munich, Germany, Oct 27-29 2017

Wong N. 'Genomic Profiling of HCC using next generation sequencing'. The 76th Annual Meeting of the Japanese Cancer Association, Yokohoma, Japan, 28-30 Sept 2017

Wong N. 'Genome Research and Liver Cancer'. The 5th Cross-strait Digestive Disease Forum, Xiamen, China 18-20 Nov 2016

Wong N. 'Hepatocarcinogenesis: Demystifying molecular pathogenesis. Asian Pacific Association for the Study of the Liver (APASL) Brisbane, 12-15 Mar 2014.

Wong N. 'Next generation sequencing in HCC'. APASL Brisbane, 12-15 Mar 2014.

Wong N. 'Genome sequencing of HCC'. International Conference on Advanced Molecular Technologies. The Hong Kong Polytechnique University, 7-9 March 2014.

Wong N. 'Identification of somatic variants of hepatocellular carcinoma by genome sequencing'. Joint International Symposium on Biomedical Research across the Continents - Insight and Innovation, Tsinghua University, Beijing, China, 10-11 April 2014

Guan XY. 'ATOH8 depletion can reprogram non-cancer stem cells into cancer stem cells'. The 2nd International Proteomics Symposium on Inflammation and Cancer. Guangzhou, China, 10-11 May 2014.

Guan XY. 'The effects of cancer microenvironment on cancer progression'. The 5th Shanghai symposium of breast cancer research. Shanghai, 17 September 2014.

Guan XY. 'Advances of cancer research and their clinical applications'. The 7th Symposium of general surgery across the straits. Dalian, China, 17-19 October 2014.

Guan XY. 'Recent advances in hepatocellular carcinoma study'. Hong Kong – Shenzhen International Cancer Congress. Shenzhen, China, 22 November 2014.

Guan XY. 'Recording RNA editing of AZIN1 predisposes to hepatocellular carcinoma'. The 2013 National Cancer Center of China Annual Conference. Beijing, China, 16 March 2013.

Yu J. 'Gut Microbial Dysbiosis' 3rd LMU-China Academic Network Scientific Forum, Munich, Germany, 27-29 Oct 2017

Yu J. 'Identification of genomic and epigenomic aberrations and signaling pathways in experimental NAFLD-associated HCC". APASL Brisbane, 12-15 Mar 2014.

Yu J. 'Molecular diagnostic and prognostic bio-markers of gastrointestinal cancers'. Shandong Lin Yi Conference of Gastroenterology and Endoscopy Shandong, China, 31 Oct-2 Nov 2014.

Yu J. 'New insight into the molecular pathogenesis of gastrointestinal cancers'. Shanghai Jiaotong University, Shanghai, China, 23-24 Dec 2014.

Chan S. 'When patients fail on molecular targeted therapy: what to do in 2013?'. 3rd APASL Single Topic Conference: HCC in 3D. Cebu, Philippines. 23 Nov 2013.

Chan S. 'Update in the management of inoperable hepatocellular carcinoma'. Hepatobiliary joint meeting in Centro Hospitalar Conde de São Januário, Macau. 21 Mar 2014

Chan S. 'Systemic therapy for HCC: Beyond Sorafenib'. Hepatology Society of the Philippines 2013 HSP Convention, Manila, Philippine. Jan 25, 2013.

Chan S. 'Overview of research for HCC in CUHK'. Hepatobiliary Task Force Meeting 2012 Chicago, USA, June 1, 2012.

Wong V. 'Asian insights into NAFLD in children and adults'. APASL Brisbane, 12-15 Mar 2014.

Wong V. 'NASH in lean patients'. Postgraduate Course of the 50th Annual Meeting of the European Association for the Study of the Liver (EASL). Vienna, Austria. 22 April 2015.

Wong V. 'Transient elastography: From practice to research'. Expert Roundtable Discussion, Korean Association of Clinical Ultrasound. Seoul, Korea. 9 May 2015.

Wong V. Emerging Leader Lecture: 'Non-alcoholic fatty liver disease in Asia: A story of growth'.

Asian Pacific Digestive Week 2012. Bangkok, Thailand. December 8, 2012.

Chan H. 'HCC screening is worthwhile – Yes', The 2nd World Congress in Controversies in Gastroenterology, Xian, China. 12-14 Sept 2014

Chan H. 'Does treatment reduce the HCC risk in Eastern countries?' EASL special conference 'Optimal management of hepatitis B virus infection', Athens, Greece. 26-27 Sept 2014.

Chan H. 'Treatment as a form of liver cancer prevention – clinical efficacy and cost effectiveness of treatment across Asia' World Cancer Congress, Melbourne, Australia. 3-6 Dec 2014

Chan H. 'What is the impact of antiviral drugs on hepatocellular carcinoma', 24th conference of the Asian Pacific Association for the Study of the liver, Istanbul, Turkey. 12-15 March 2015.

Conference Organizations

Team members have jointly organized local scientific meetings and participated in international conference organizations as well. Below some of these events:

- CANCER'17: 'Translating Cancer 'Omics' to Precision Medicine', Hong Kong, 22 June 2017
- CANCER'16: 'Frontiers in Cancer Research', Hong Kong, 1 Nov 2016
- International Digestive Disease Forum, Hong Kong (2013-2016) One of the leading academic meetings on gastroenterology and hepatology in the Asia Pacific region. Clinical managements, basic research and innovative therapeutics discussed.
- Mini-symposium 'Cancer Epigenetics', Hong Kong, 21 July 2016
- Hong Kong-Germany Cancer Genomics and Precision Medicine Summit, Hong Kong, 2 Dec 2015
- Joint Symposium by the State Key Laboratory of Digestive Disease and CANCER'15, Hong Kong, 7 June 2015,
- CANCER'14: 'Advances in EBV and Nasopharyngeal Carcinoma Research', Hong Kong, 31 Oct 2014,
- CANCER'13: 'Genomic Landscape of Cancer', Hong Kong, 19 April 2013.
- Joint scientific meeting 'Genomic approaches to investigate clinical phenotypes'. Hong Kong, 28 April 2014.
- International Symposium on Hepatology, Hong Kong (2013-2015)
- 6th Global Conference of Alliance for Healthy Cities, Hong Kong, 29 Oct-1 Nov 2014
- 2nd International Hepatitis Cure & Eradication Meeting, Vancouver, Canada, 11-12 Nov 2015

7.3 Have any project team members taken leadership positions in editorial boards, scientific and professional organisations?

Nathalie Wong

- Associate Editor, Journal of Pathology
- Panel Member, RGC Research Assessment Exercise 2014 - Health Sciences Panel
- Panel Member, RGC General Research Fund (GRF) and Early Career Scheme (ECS) - Biology and Medicine Panel (2013-present)

Jun Yu

- Editorial Board Member, Gut
- Editorial Board Member, Oncogene
- Editorial Board Member, Journal of Gastroenterology and Hepatology
- Editorial Board Member, Oncogenesis
- Advisory Editorial Board member of Science News and Information

Henry Chan

- Coordinating editor in Hepatology, Journal of Gastroenterology and Hepatology
- Associate editor, Journal of Hepatology
- Associate editor, Hepatology International
- Chairman of Strategic Technical Advisory Committee (STAC), Regional Office for the Western Pacific, World Health Organization, 2015
- Chairman of EASL-ALEH Clinical Practice Guideline committee on non-invasive tests for evaluation of liver disease severity and progression, 2015

Vincent Wong
- Editorial Board Member, Alimentary Pharmacology & Therapeutics
- Editorial Board Member, Journal of Gastroenterology and Hepatology
- Editorial Board Member, Clinical Gastroenterology and Hepatology

King L. Chow
- Editorial Board Member, ISRN Developmental Biology
- Editorial Board Member, Animal Cells and Systems

Ka-Fai To
- Advisory Board, Novartis APECHO 2015
- Advisory Board, MSD Asia Pacific NSCLC & Biomarker
- Advisory Board, MSD Asia-Pacific Oncology Biomarker

7.4 Any documentary proof of the application of technologies arising directly from this project?

The bioinformatics team of this TRS has developed standard pipelines to analyse the genomic and transcriptomic datasets generated from next generation sequencing. They have setup bioinformatic strategies, including utilization of different algorithms, software, scripts, database packages and reference sequences, so to consolidate genomic events and improve somatic variant callings. The same algorithmic approach has also been used to support colleagues working on other cancer types. The technology sharing can be exemplified by publications from team members on colon cancer, gastric cancer and nasopharyngeal carcinoma (papers shown in Section 6.4a)

7.5 Other awards and recognitions as a result of this project (please specify):

Wong N: Outstanding Fellow of the Faculty of Medicine 2012-2017 and 2017-2022, CUHK
Wong N: Scheme for High Impact Publications 2014/15, Faculty of Medicine, CUHK
Wong N: Faculty Annual Report for Outstanding Research 2014
Wong N: Invited commentary by prestigious journal Hepatology
Yu J and co-workers: First-class MOE Higher Education Outstanding Scientific Research Output Award (Natural Science Award) Ministry of Education, China, 2014.
Yu J: Scheme for High Impact Publications 2013/14, Faculty of Medicine, CUHK
Wong V and co-workers: First-Class MOE Higher Education Outstanding Scientific Research Output Award (Scientific and Technological Progress Award), Ministry of Education, China 2013
Wong V: Distinguished Research Paper Award for Young Investigators, Hong Kong College of Physicians, 2013
Guan XY and co-workers: Science and Technology Prize of Higher Education of China (Natural Science, Second-class) Ministry of Education, China, 2014
Cheng A: Most Promising Young Investigator Award, Food and Health Bureau, The Government of Hong Kong SAR, 2014

8. Impacts

8.1 What are the current and expected impacts of the project on the long-term development of Hong Kong (social or economic development, e.g. patent, technology transfer, collaboration with external organisations, etc.)?

HCC is a major health-care issue in Hong Kong. We believe the research into the HCC genome will have major impact in both basic research and clinical services for Hong Kong and in areas where HCC shows a high incidence. The broad research activities entailed in this TRS project cover comprehensive investigations and integration of multiple datasets to achieving depth and resolution in disease pathogenesis. From a clinical perspective, additive genomic events hold the key to targeted therapy and an indispensable element in the development of personalised medicine. Understanding these acquired genomic events is therefore elemental in unveiling the cancer biology and clinical translations, and thus knowledge gained from this research will have both academic and social impact in Hong Kong. We focused our investigative approaches that are of high promises in cancer genomics (including base substitutions, copy number variations, alternate splice variants and

transcription) and their clinical translations. These study initiatives are expected to offer knowledge-based information that can offer patents, technology transfer, and in the long-term increase Hong Kong's competiveness in the field of liver cancer research.

8.2 Others (please specify): *No further, already addressed in various Sections in this report*

9. Sustainability of the Project

9.1 Whether there are new ideas evolved directly from this project?

A number of new ideas have risen from this TRS project. For instance, (1) common genetic mutations of HCC involve components of Wnt/β-catenin, specifically the gain-of-function (GOF) mutations of β-catenin. Based on large-scale bioinformatic analysis of TCGA and in-house data, a strong correlation between an immune 'cold' tumor microenvironment of HCC and GOF β-catenin was found. Functionally, we showed a striking effect for β-catenin activation in the HCC immune evasion by suppressing intra-tumoral T-cell infiltration; (2) New function for GOF p53 mutations in the direct cross-talk with histone methylation and acetylation was illustrated. We showed transcriptional regulation of key histone modifying enzymes by GOF p53 mutants in HCC and further illustrated the effect can also translate to a global aberrant DNA methylation; (3) NAFLD is currently the most common cause of liver disease in many developed countries and it is fast becoming a health issue worldwide. Sequelae epoxidase (SQLE) was found to be a novel gene that links aberrant cholesterol mechanism to the induction of chronic liver inflammation, thereby promoting NAFLD development.

9.2 Whether there are new projects evolved directly from this project?

New projects have evolved during the course of this TRS research, and applications for further funding have been sought. In developing the above-mentioned new ideas, we are currently conducting in-depth studies into the alternate mechanisms of genetic addiction in β-catenin and p53 activated tumors by comprehensively integrating genomic data from ChIP-seq, transcriptome, in-house and public NGS repository datasets, ENCODE information and biological investigations. Specifically, we are exploiting areas of genetic dependencies related to T-cell suppressed microenvironment and epigenetic switch for therapeutic windows, and to explore the combinatory effects of Wnt inhibitors, chromatin drugs and immune checkpoint blockade (such as anti-PD-1 and anti-CTLA-4) for therapy. For SQLE, we have developed a *Sqle* hepatocyte-specific conditional transgenic mouse, of which allows us to conduct in-depth investigations into the mechanism of SQLE activation of aberrant cholesterol metabolism and its associated pro-inflammatory ER stress and NF-kB pathways.

9.3 Whether there are new collaborations developed directly from this project?

A network of collaborative links has also established by team members through this TRS project:

Nathalie Wong has initiated collaboration with Prof. Charles Lee Director of The Jackson Laboratory for Genomic Medicine, Farmington, USA on the study of immune evasion in HCC

Nathalie Wong has initiated collaboration with Prof. Jeong-Sun Seo on genome sequencings. He is CEO of Macrogen Inc., which is one of the largest sequencing Centres in Asia.

Nathalie Wong has initiated collaboration with Prof. Paul Johnston from Dept of Pharmaceutical Sciences, University of Pittsburgh, USA, on small molecule inhibitors screening for HCC.

Nathalie Wong is collaborating with Prof. Xin Wang, a computational biologist from City University Hong Kong, on cancer bioinformatics and integrative analysis of multilevel ~omics.

Nathalie Wong and Ka-Fai To have initiated collaboration with Dr. Timothy Yip from Dept. of Clinical Oncology, Queen Elizabeth Hospital, Hong Kong on the examination of polymorphic detoxification enzymes in NASH-HCC

Nathalie Wong and Jun Yu are collaborating with Prof. Narci Teoh from Department of Gastroenterology & Hepatology, The Canberra Hospital, Australian National University, on

NAFLD/NASH HCC animal studies

Jun Yu initiated projects on 'Liver Fibrosis and HCC Development' with Prof. Matthias Ebert, Department of Medicine II, Heidelberg University, Germany.

Jun Yu collaborates with Prof Youyong Lu from the Oncology Hospital, Beijing University, China, on cancer genome studies.

Xin-Yuan Guan is collaborating with Prof. Daniel G Tenen from the Cancer Science Institute of Singapore, National University of Singapore on cancer biology studies

Vincent Wong and Prof Geoff Farrell (IAB member of this TRS) are working closely on NAFLD in Eastern and Western populations

Henry Chan and Vincent Wong have established the Liver Fibrosis Assessment (LiFA) Study Group to develop non-invasive tests of cirrhosis. This Study Group includes 6 centers from Europe and Asia. They are also collaborating with Prof. Jacob George from The University of Sydney in developing biomarkers of NASH and HCC.

Stephen Chan and Paul Lai are collaborating with Prof. Philip Johnson (IAB member of this TRS) on the assessment score for liver function in patients with HCC.

Stephen Chan has a collaborative study with Novartis on 'Works on TSC2 loss as predictive biomarker for mTOR inhibitor for HCC'

Alfred Cheng is collaborating with Prof. Tim Huang, Chair of Dept. Molecular Medicine at The University of Texas Health Science Center at San Antonio on the topic of MethylCap-seq on human HCC tissues and epigenome analysis

Alfred Cheng, Ka-Fai To and Nathalie Wong are collaborating with Prof Patrick Tan from Duke-NUS Graduate Medical School, Singapore on nano-ChIP seq studies of primary HCC tumours. Prof Tan is also a collaborator of a current CRF project that is jointly obtained by the 3 team members

9.4 Please give details on how much money and from which sources has been obtained/requested for the specific purpose of continuing the work started under this project.

Currently, we have a no. of ongoing GRFs that are supporting the downstream work of this TRS project. Few team members have also jointly put in an RGC Collaborative Research Fund application on the immune evasion of HCC that is modulated by tumor-intrinsic oncogenic pathways. In addition, members of State Key Laboratory of Oncology in South China have put in a renewal application at the Ministry of Science and Technology of China (MOST) and Innovation and Technology Commission (ITC) Hong Kong, which if successful could provide some steady funding for 5 years.

Project Title: The Liver Cancer Genome Project: Translating Genetic Discoveries to Clinical Benefits
Project Coordinator: Prof Nathalie Wong (CUHK)

Summary

Liver Cancer (also commonly known as hepatocellular carcinoma, HCC) is a highly aggressive malignancy and a major health-care issue in China, including Hong Kong. The research activities entailed in this Theme-based Research Scheme project cover broad investigations of multiple datasets to achieve understanding in the genetic characteristics during HCC pathogenesis. Analysis of paired tumor and adjacent nontumoral liver showed major transformation events of *TP53*, *CTNNB1* and *TERT* promoter mutations. These diver mutations represent trunk events in the clonal expansion of tumor progression, and together they constitute >70% of HCC tumors. Next generation sequencing analysis also shed new concepts in the liver carcinogenesis where chromatin remodelling through mutations in the SWI/SNF complex (*ARID1A*, *ARID2* and *SETD2*) and aberrant promoter/enhancer methylations are highlighted. We examined the clonal progression of HCC and realised the degree of genomic concordance between primary and recurrence tumors, and within tumor sectors. Our data suggested ~50% of recurrent tumors are in fact multicentric occurrence with an independent genetic background from the primary HCC. Interestingly, progressive tumors of the same clonal origin that relapsed after surgery showed high dependence on JAK/STAT pathway through common oncogenic mutations. This represents an attractive target for therapeutic intervention. From a clinical perspective, additive genomic events hold the key to targeted therapy and an indispensable element in the development of personalised medicine. Analysis for intratumoral heterogeneity (ITH) revealed high genetic variability in cases with ubiquitous mutations (*ATRX*, *MSH2*) in the DNA mismatch pathway. Non-alcoholic fatty liver disease (NAFLD) affects nearly a quarter of the general population. Perpetuate liver inflammation is crucial in the pathogenesis of NAFLD, and subsequent development of HCC. In examining the transcriptomic background of NAFLD-HCC in human tissues and mouse models, we identified novel genes (*SQLE* and *OGT*) that link aberrant cholesterol mechanism to the induction of chronic liver inflammation, which promote NAFLD development. In sum, our study initiatives provide a new perspective to understanding the mechanisms behind the HCC tumor development and evolution, and defines targets that can be translated to patient benefits.