Project number: T12-704/16-R

RESEARCH GRANTS COUNCIL
THEME-BASED RESEARCH SCHEME (TRS)

Completion Report on Funded Project

Project start date: *January 1, 2017*
Project completion date: *December 31, 2021*

1. Project Title

Understanding Cancer Stemness in Liver Cancer - From Regulation to Translational Applications

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)	Ng, Irene OL/Chair Professor; Director; Honorary Consultant (1)	Pathology, HKU; State Key Laboratory of Liver Research (HKU) (2); Queen Mary Hospital	12
Co-Principal Investigator(s)	Guan, Xin-Yuan/Chair Professor (3)	Clinical Oncology, HKU	6
	Poon, Randy YC/ Chair Professor (4)	Division of Life Science, HKUST	5
	Sham, Pak-Chung/ Chair Professor	Psychiatry, HKU (5)	4
	Tsui, Stephen KW/ Professor & Division Head	School of Biomedical Sciences, CUHK	4

	Lee, Terence KW/ Associate Professor [6]	Applied Biology & Chemical Technology, PolyU	12
	Wong, Carmen CL/ Assistant Professor	Pathology, HKU	12
Co-Investigator(s)	Chen, George G/ Professor	Surgery, CUHK	1
	Ching, Yick-Pang/ Associate Professor	School of Biomedical Science, HKU	4
	Jin, Dong-Yan/ Professor	School of Biomedical Science, HKU	4
	Ma, Stephanie/Associate Professor [7]	School of Biomedical Science, HKU	4
	Mak, Tak-Wah/HKU Distinguished Visiting Professor	University of Toronto and HKU	1
	Wong, Chun-Ming/ Associate Professor [8]	Pathology, HKU	8
	Wu, Angela R/Assistant Professor	Division of Life Science, HKUST	1
	Zhang, Michael Q/ Distinguished Chair; HKU Visiting Research Professor	Systems Biology Science, U of Texas, Dallas; Tsinghua U, Beijing; HKU	1
	Cheung, Tan-To/Clinical Associate Professor	Surgery, QMH	0.5
	Lo, Irene LO/Associate Consultant	Surgery, Queen Elizabeth Hospital	0.5
	Poon, Ronnie TP/ Honorary Professor	Surgery, HKU & QMH	0.5
	Tang, Chung-Ngai/ Chief of Service	Surgery, Pamela Youde Eastern Hospital	0.5
	Yau, Thomas CC/ Honorary Clinical Associate Professor [9]	Medicine, HKU & QMH	1
Collaborators	Chew, Valerie/Senior Research Scientist	Singhealth Translational Immunology and Inflammation Centre, Singapore	N.A.
	Wang, Hong-Yang/ Professor & Director	National Center for Liver Cancer, China	N.A.
	Wang, Xin-Wei/ Head	Liver Carcinogenesis Section, Lab. of Human Carcinogenesis, NCI, USA	N.A.

Notes:

1. Prof Irene OL Ng's headship of Department of Pathology of HKU and Chief of Service of Pathology of QMH expired by December 31, 2019.
2. State Key Laboratory for Liver Research was renamed as State Key Laboratory of Liver Research (HKU) in September 2018.
3. Prof Xin-Yuan Guan was promoted from Professor to Chair Professor in May 2018.
4. Prof Randy YC Poon was promoted from Professor to Chair Professor in 2019.
5. Directorship of Center for Genomic Sciences expired upon revamping of the Center in end of 2019. Prof Pak-Chung Sham's post in Department of Psychiatry is used instead.
6. Dr Terence KW Lee was promoted from Assistant Professor to Associate Professor in July 2019 and was further promoted to Professor in 2022.
7. Dr Stephanie Ma was promoted from Assistant Professor to Associate Professor in December 2018.
8. Dr Chun-Ming Wong was promoted from Assistant Professor to Associate Professor in August 2018.
9. Dr Thomas Yau has changed the employment mode to Honorary Clinical Associate Professor since 2022.

 # 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.

3. Project Objectives

Summary of objectives addressed/achieved:

Objectives*	Percentage achieved	Remarks**
1. Understanding liver CSC molecular mechanisms by delineating individual and common consensus pathways of liver CSC subpopulations using next generation sequencing	100%	Completed successfully.
2. Lineage hierarchy inference for single cells isolated from HCC patients' tumors	100%	Completed successfully.
3. To delineate the interaction between tumor microenvironment and liver CSCs and cancer stemness	100%	Completed successfully.

* *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

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.))

Here, because of our fruitful work, we are listing the team's best research accomplishments **in groups**. They were all published in high-impact journals.

1. We established the first single-cell RNA-sequencing analysis on an HCC patient cohort in HK, as is a result of availability of HCC tissues, cutting-edge technical platforms, and data interpretation. We identified novel immune checkpoint axis TIGIT and Nectin family (TIGIT-Nectin1 and TIGIT/Nectin2) and the mechanistic rationale of combined treatment with immune checkpoint inhibitor anti-PD1 and anti-TIGIT and the resistance mechanisms. The two projects were published:

- Ho DW,…Lo IL, Cheung TT, Sham PC, Cheung TT, Wong CC, Ng IO. **Nat Commun** 2021.
- Chiu D…., Ng IO, Yau TC, Wong CM, Wong CC. **Gastroenterology** 2020.

2. Key signaling and metabolic pathways. We identified key therapeutic targets and biomarkers which lead to enhanced cancer stemness. They are novel gene targets to guide precision medicine for liver cancer. These included identification of TSC1/2 mutations, which can be used to stratify HCC patients for treatment with Rapamycin; EphB2 to which kinase inhibitor has been generated and its efficacy is being tested in vitro. In addition, we found that liver cancer cells rely on thioredoxin to maintain REDOX balance; Thioredoxin reductase inhibitor, auranofin, could be repurposed for liver cancer treatment. The Journal publications are:

- Ho DW, Chan LK,…Poon RT, Cheung TT, Tang CN, Lo IL,… Wong CM, Ng IO. **Gut** 2017). (TSC1/2)
- Leung HW, …Ng IO, Ma S, Lee TK. **Cancer Res** 2021. (EphB2)
- Lee D,… Ng IO, Wong CM, Wong CC et al. **Hepatology** 2019. (Thioredoxin)

3. Enriched the understanding of the tumor microenvironment of liver cancer.

HCC grows large and fast and is often hypoxic; hence hypoxia is a major tumor environmental factor contributing to liver cancer stemness. Our team members have comprehensively reviewed on how the tumor microenvironment regulates cancer stemness in HCC. In addition, we have demonstrated that hypoxia induces the hypoxia inducible factor HIF-1 which in turn induces ENTPD2/CD39L1 and promotes myeloid-derived suppressor cells accumulation in HCC to result in immunosuppression and enhance cancer stemness in HCC. Paracrine supply of FSTL1 from activated fibroblasts promotes a metastatic and stemness environment in HCC. Furthermore, we have shown that histone chaperone FACT complex is upregulated in HCC hypoxia and oxidative stress response. FACT is a histone chaperone complex mediating nucleosome disassembly and reassembly during transcription. Targeting FACT complex by its small molecular inhibitor Curaxin effectively suppressed HCC growth and sensitizes HCC towards Sorafenib treatment.

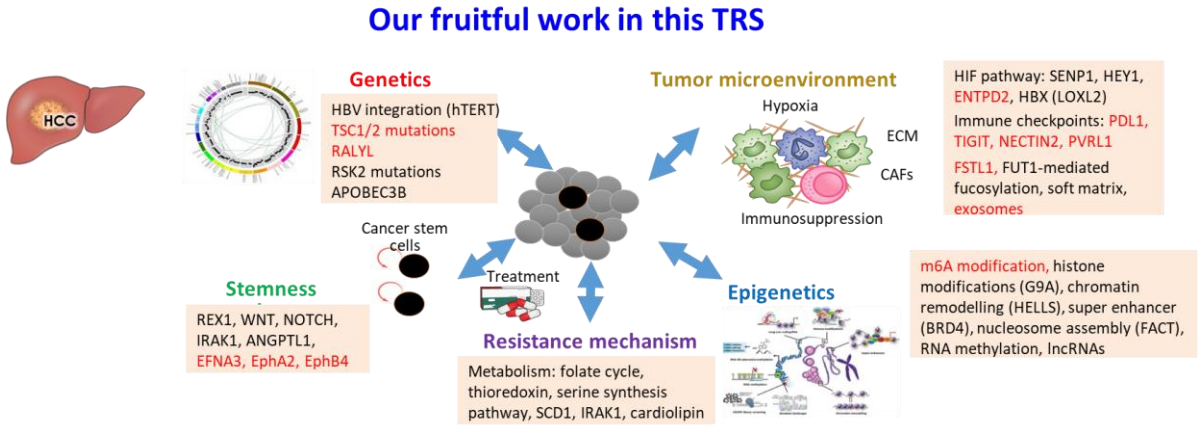
Journal papers:

- Lee TK, Guan XY, Ma S. **Nat Rev Gastroenterol Hepatol** 2022 (tumor microenvironment regulates cancer stemness)
- Chiu DK,…Wong CM, Ng IO, Wong CC. **Nat Commun** 2017. (ENTPD2/CD39L1).
- Loh JJ, … Lee TK, Ma S. **Cancer Res** 2021. (FSTL1))
- Shen J, Ng IO, Wong CC, Wong CM. **Gut** 2020. (FACT) **(Faculty Outstanding Research Output award 2020 and Li Ka Shing Prize Best thesis award of HKU, 2022)**

4. We have also uncovered the extracellular vesicles (EV) as promising therapeutic targets. Polymeric immunoglobulin receptor (pIgR) is identified in HCC exosomes and EV-pIgR promotes cancer stemness, tumor growth and metastasis. Of note, anti-pIgR antibody inhibits HCC tumor growth. Paper: Tey SK,…Ng IOL, Ma S, Lee TK ….Yam JW. **J Hepatol** 2022.

5. Epigenetic regulation contributing to liver cancer stemness
   - Histone methyltransferase G9a promotes liver cancer development by epigenetic silencing of tumor suppressor gene RARRES3 (Wei L et al., *J Hepatol* 2017: 67: 758)
   - m6A methyltransferase METTL3 promotes HCC growth and metastasis. The study demonstrated the new mechanism of epigenetic gene silencing through m6A hypermethylation. Paper: Chen M,…Ho DW, Wong CC, Ng IO, Wong CM. **Hepatology** 2018) **Web of Science Highly cited paper; Citation: > 650.**

6. Published 98 referred research papers, including in Molecular Cancer, Journal of Hepatology, Gut, Gastroenterology, Molecular Cell, Hepatology, and Nature Communications. The following figure gives an overview of the joint publications supported by this TRS. A detailed list of the joint publications with TRS support is provided in **6.4**.



Joint publications supported by TRS

Genetics:
Gut 2017; 66:1496
J Hepatol 2021; 74:360
Hepatology 2022: 76:48
Hepatology 2021; 73:23
Cancer Lett 2020; 492:147
CMGH 2022; 14:513
CMGH 2022; 13:1611
Mol Carcinog 2019; 58:643
Biocell 2021; 45:1495
Hepatoma Res 2021; 7:62

Epigenetics:
Nat Rev Gastroenterol Hepatol 2018; 15:137
Mol Cancer 2020; 19:44
Gut 2020; 69:329
J Hepatol 2017; 67:758
Hepatology 2019; 69:2502
Hepatology 2019; 69:2013
Hepatology 2018; 67:2254
Cell Death Differ 2021; 28:1955
Cell Death Differ 2019; 26:2268
Theranostics 2019; 9:796
Cell Rep 2022; 38:110304
CMGH 2022; 14:1053
Hepatobiliary Surg Nutr 2022; 11:126
Commun Biol 2021; 4:888
Int J Mol Sci 2021; 22:3137

Stemness pathway:
J Hepatol 2022; 77:880
J Hepatol 2022; 77:383
J Hepatol 2022; 76:883
J Clin Invest 2021; 131:143377
Nat Commun 2021; 12:1518
Adv Sci (Weinh) 2021; 8:2002483
Hepatology 2020; 72:183
Semin Cancer Biol 2022; 82:134
Cancer Res 2021; 81:5692
Cancer Res 2021; 81:3229
Cancer Res 2018; 78:2332
Cancer Res 2017; 77:5831
J Exp Clin Cancer Res 2022; 41:211
J Exp Clin Cancer Res 2022; 41:182
Cell Death Differ 2018; 25:1426
Front Med (Lausanne) 2022; 9:860395
Cancer Lett 2022; 524:70
Cancer Lett 2019; 459:176
Cell Death Dis 2021; 12:148
Cell Death Dis 2021; 12:125
Cell Death Dis 2020; 11:510
Cell Biosci 2021; 11:217
Br J Cancer 2020; 122:1428
Oncogene 2022; 41:732
Oncogene 2021; 40:1147
Oncogene 2019; 38:4061
Essays Biochem 2022; 66:423
Cancers (Basel) 2020; 12:1410
Oncotarget 2021; 12:1727
Oncotarget 2017; 8:39430
Histol Histopathol 2019; 34:1

Resistance Mechanism:
J Hepatol 2017; 67:979
J Clin Invest 2017; 127:1856
Nat Commun 2019; 10:4681
Hepatology 2021; 74:776
Hepatology 2019; 69:1768
Cancer Res 2022; in revision
Cell Rep 2021; 34:108676
Cancers (Basel) 2021; 13:2002
Cancer Gene Ther 2022; Jun
Hepatol Commun 2022; Jul

Microenvironment:
Gastroenterology 2020; 159:609
Gut 2017; 66:2149
J Clin Invest 2021; 131:138699
J Clin Invest 2020; 130:5052
Nat Commun 2021; 12:3684
Nat Commun 2017; 8:517
Hepatology 2021; 73:1631
Sci Adv 2021; 7:8346
J Immunother Cancer 2021; 9:1945
Cell Death Dis 2019; 10:934
Oncogenesis 2018; 7:44
Front Cell Dev Biol 2021; 9:727640
Hepatol Commun 2022; 6:178

Others:
Mol Cell 2020; 80:1123
Small 2020; 16:1905055
Science Advances 2021; under review
Brief Bioinform 2022; 14:167
Cell Rep 2021; 37:109808
Cell Rep 2018 Feb 6; 22:1439
Cell Death Dis 2019; 10:314
Histopathology 2022; 80:720
Cell Cycle 2019; 18:238
BMC Genomics 2021; 22:420
Biomicrofluidics 2020; 14:2
Transl Gastroenterol Hepatol 2019; 4:47

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?)*

TRS funding provides resource and opportunities to explore novel concepts and challenging projects in a multidisciplinary group effort to the extent that are unlikely funded by GRF or CRF. The TRS has provided us with a cutting-edge research platform for sharing of resources and reagents, such as single cell analysis, genome-wide CRISPR library screening platforms, in-depth investigation of genes and pathways, etc, which greatly facilitate the liver cancer research. This project requires conglomeration of efforts and cooperation of multiple parties and research teams. It relies on huge investment of resources, manpower and specialty knowledge that could only be achieved in the TRS. For example, this project requires the collection of resected HCC samples by surgeons of multiple hospitals in HK. The logistics were carefully planned to streamline the processing of the samples for tumor dissociation, FAC-sorting, biobank storage and sample annotation by the experience personnel. Single-cell

RNA-sequencing and valuable bioinformatic analyses were provided by the bioinformatic specialists of two participating universities. Some further in vitro and in vivo experimental work was also carried out by the various laboratories and parties under the support of this TRS. It provides an opportunity for a number of research seminars and facilitates the exchange of research ideas from colleagues and from overseas experts. Considering such large scale of this study, it can hardly be achieved by single GRF or CRF.

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

N/A

6.4 (a) Please provide details of each peer-reviewed journal publication arising directly from this project by completing the table at *Annex I (one for each publication)*.

Around 100 peer-reviewed journal publications were published in high impact journals, including in Molecular Cancer, Journal of Hepatology, Gut, Gastroenterology, Molecular Cell, Hepatology, and Nature Communications. For summary of each publication please refer to **Annex I**; for the full article please refer to **Appendix I**.

(b) Please provide details of recognised international conference(s) in which paper(s) related to this project was/were delivered by completing the following table:
(Please attach a copy of each conference abstract)

A total of 36 conference papers were published in international conferences. Full list please refer to **Annex II**. For conference abstract please refer to **Appendix II**.

(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.

Journal paper no. 12, 14, 80, 82, 97: A few publications in the above lists did not acknowledge the TRS project as the research work was not financially supported by RGC but the they reflected the joint expertise in collaboration of the researches related to the TRS project.

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

The TRS required collaboration among the different parties. These include the collection of resected HCC samples by surgeons of the collaborating tertiary hospitals in HK, the logistics for the processing of the samples for tumor dissociation, biobank storage and sample annotation by the experience personnel of the laboratories.
Collaboration among the institutions, on such as single-cell RNA-sequencing, transcriptome sequencing and valuable bioinformatic analyses, was crucial.
The novel findings were shared among the PC, co-PIs and co-Is and led to discovery of important targets in a number of directions and pathways. Such efforts have also led to fruitful multi-disciplinary study including basic-scientists, bioinformaticians and clinical scientists in this TRS project.

6.6 Research students trained (registration/awards):
Over 70 Postgraduate research students were trained during the project period. The TRS project provided a platform for student training in cancer researches and the training and research output were recognised by various internal and external awards that the students

achieved. Full list please refer to **Annex III**.

6.7 Specific products (e.g. software or netware, instruments or equipment developed):
Ng, IO and Ho, DW - scAnalyzer (a comprehensive software package with graphical user interface for single-cell RNA sequencing analysis).
Ng, IO and Ho, DW - Virus-Clip (a fast and memory-efficient viral integration site detection tool at single-base resolution with annotation capability)
Wu, AR - An optimized protocol for liver sample preparation for single-cell RNA-seq.
Wu, AR - FIRM: Flexible Integration of single-cell RNA-sequencing data for large-scale Multi-tissue cell atlas datasets (https://github.com/mingjingsi/FIRM)

6.8 Other education activities and/or training programmes developed:
Various education activities include symposia, seminars, lectures, internal research meetings were developed to encourage knowledge exchange and collaboration. This TRS project also serves a platform to train undergraduate students in their research projects. Full list please refer to **Annex VI**.

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.

N.A.

Project Management

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

The PC oversaw the whole program and chaired the MB meetings, chaired the organizing committee for the TRS symposia, organized TRS seminars, and wrote up the reports after gathering the relevant information from the program investigators.
The PC also organized visits by SAB members to comment on this group's direction and progresses and provide expert advice.

7. Awards and Recognition

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

Around 50 research grants mainly from RGC General Research Fund, RGC Collaborative Research Fund, Health and Medical Research Fund, and National Natural Science Foundation of China were awarded between 2017 - 2022. Full list please refer to **Annex V**.

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

The PC of the project contribute to international conferences including Keystone Symposia, APPLE Meeting as a role of Organizer. Our project team members were also invited to over 80 international conferences 2017 – 2021. Full list please refer to **Annex VI**.

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

The PC and project team member are editors/editorial board members of many renowned medical journals, such as *Journal of Hepatology*, *Nature Reviews Gastroenterology & Hepatology*, *Hepatology*; and were the fellows/members of international associations, such as *APPLE Association*, *International Liver Transplant Society*, *Department of Health.* Full list please refer to **Annex VII**.

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

- Filing of a provisional US patent on a novel LCSC surface marker as a diagnostic and therapeutic target is supported by TTO of HKU and is in progress. (by IOL Ng and YM Tsui)
- USPTO Patent pending: Angela Ruohao Wu, Lei Yu. Simultaneous Amplification of DNA and RNA from Single Cells, filing number 63/093,368, filing date 19/10/2021. (by Angela R Wu)
- Filing of a provisional US patent on "The S100A10 neutralizing antibodies for the treatment of liver cancer" is supported by TTO of HKU and is in progress. (by IOL Ng and X Wang)

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

Ng, Irene OL & Guan, Xin-Yuan	▪ Our State Key Laboratory of Liver Research was awarded "Outstanding" (the highest rank) by the HKRGC/Innovation Technology Commission and Chinese Ministry of Science & Technology in the 2017/18 re-assessment exercise
Ng, Irene OL	▪ Ranked the Best Female Scientist by Research.com (#8 nationally and #517 internationally) ▪ Appointed as Associate Editor of Hepatology (the 2nd top ranking journal in the field of Hepatology) 2018- 2021, and Associate Editor of Cellular and Molecular Gastroenterology & Hepatology 2021-2024 ▪ Faculty Outstanding Research Output Awards, HKU, 2019, 2021, 2022
Wong, Carmen CL	▪ UBC Alumni Builder Award, 2017 ▪ Croucher Innovation Award 2017 ▪ Outstanding Young Researcher Award (OYRA) 2017/18 ▪ Awarded China' s Excellent Young Scientists Fund 2020 ▪ Junior Investigator Award 2019, ILCA ▪ Faculty Outstanding Research Output Award, HKU, 2019, 2021
Cheung, Tan-To	▪ Faculty Outstanding Research Output Award, HKU, 2021
Ma, Stephanie	▪ Croucher Senior Researcher Award 2022 ▪ Rising Star Award - Ton Duc Thang University Scientific Prize ▪ UBC Alumni Builder Award, 2017 ▪ Elected as Founding Member of Young Academy of Sciences of Hong Kong ▪ Appointed as Board Member (2020-2022) of HK Science& Technology Park ▪ Awarded RGC Research Fellow Scheme 2021/22
Ma, Stephanie & Guan, Xin-Yuan	▪ Patent granted for "Use of annexin A3 as a diagnostic and prognostic biomarker and therapeutic target for treating HCC" (Patent no.: US 10000558 B2)
Wong, Chun-Ming	▪ Faculty Outstanding Research Output Award, HKU, 2020, 2021
Wu, Angela R	▪ Outstanding Young Investigator award for invited talk at IEEE EMBS Nano and Microtechnology Conference in USA, Dec 2018
Yau, Thomas CC	▪ Faculty Outstanding Research Output Award, HKU, 2021
Ho, DW (Assistant Professor)	▪ Young investigator award, Oral Poster Session, 9th APPLE Meeting, Korea (2018)
Chan, LK (RAP)	▪ Young investigator award, Oral Poster Session, 9th APPLE Meeting, Korea (2018)
Poon, Randy YC	▪ Croucher Senior Research Fellowships 2021

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.)?

Patents:
Published
- Simultaneous amplification of DNA and RNA from single cells (WO2022084742A1) - by Angela R Wu

In progress (Provisional US patents filed)
- A novel LCSC surface marker as a diagnostic and therapeutic target - by IOL Ng and YM Tsui
- The S100A10 neutralizing antibodies for the treatment of liver cancer - by IOL Ng and X Wang

- Our work has allowed us to understand the biology of liver cancer stemness and cancer stem cells, which are fundamental to the development of novel therapeutic approach targeting this population of liver cancer cells.
- Using cutting-edge technologies, we have identified key therapeutic targets and biomarkers that may guide precision medicine for liver cancer for translational work.
- Also, understanding of the tumor microenvironment in HCC resolves the current challenges in identifying potential responders to therapeutic agents such as molecular targeted drugs and immunotherapy agents.
- For example, the identification of TIGIT/Nectin family in HCC lends a strong support to the use of anti-TIGIT antibody in HCC patients. Anti-TIGIT has very recently been approved by FDA for use in lung cancer patients.
- For example, thus far, there are no inhibitors targeting EPHB2 available in the market. We are synthesizing a novel kinase inhibitor against EPHB2. After confirming in vitro and in cell culture model, we will submit a US patent for new EPHB2 inhibitor.
- Extracellular vesicles (EVs) are non-invasive sources of biomarker for the detection and prognosis of various diseases, as well as being promising therapeutic targets for cancer patients. The information gained in the study will be potentially translated into clinical applications with commercial opportunity. The outcome of the project will have potential to work with external organizations and attract R&D investment for the development of commercial products based on the knowledge gained in the study. We anticipate opportunities to produce diagnostic kits for using EV proteins as biomarkers of HCC. The ultimate goal is to achieve licensing, commercialization and utilization of the newly developed technology or product in clinical settings.
- New initiatives on cancer stem cell immunology that are currently being followed up.

8.2 Others (please specify):

N/A

9. Sustainability of the Project

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

- Novel combination of drugs for treatment of HCC.
- Development of gut microbiota-targeted treatment strategy in non-alcoholic fatty liver disease (NAFLD)-associated HCC.
- The role of protein kinases in immune evasion in HCC

- The discovery of immunosuppressive landscape in HCC inspired us to further investigate the utility of immune checkpoint inhibitor and the determinants of their efficacy in HCC treatments.
- All the above will lead to translational studies of HCC to improve treatment in future.

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

- Broad-spectrum kinase profiling identifies CDK6 upregulation as driver for lenvatinib resistance in HCC
- Development of gut microbiota-targeted treatment strategy in non-alcoholic fatty liver disease (NAFLD)-associated HCC.
- Systematic in vivo kinome-wide screening for protein kinases critical for immune evasion in HCC
- The identification of novel functional cell surface LCSC markers and biomarkers, including blood-based and biopsy-based ones, for determinants of immunotherapy efficacy.
- Performance of state-of-the-art experiment on scRNA sequencing to deconvolute the complexity of the tumor microenvironment.

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

New collaborations developed directly from this project:
- Dr. David Baker (School of Biological Sciences, The University of Hong Kong)
- Professor Dan Lindholm (Medicum, Department of Biochemistry and Developmental Biology, Faculty of Medicine, University of Helsinki, Helsinki, Finland)
- Professor Yu Jun (Institute of Digestive Disease, Department of Medicine and Therapeutics, Prince of Wales Hospital, the Chinese University of Hong Kong)
- Professor Yun Jing Ping (Department of Pathology, Sun Yat Sen University Cancer Center Guangzhou, China)
- Professor Ding Jing (Eastern Hepatobiliary Surgery Hospital, The International Cooperation Laboratory on Signal Transduction, Shanghai)
- Professor Zhu Hui Lian (School of Public Health, Sun Yat Sen University, Guangzhou)
- New collaborations at Stanford University/Chan-Zuckerberg Biohub, Peking University, Tsinghua University regarding the new technology, scONE-seq.
- Dr. Alejandro Chavez (Columbia University, USA)
- Dr. Sho Goh (Shenzhen Bay Laboratory, China)
- Prof. Li Xiang (Department of Chemistry, The University of Hong Kong)
- Jing-Ping Yun (Sun Yat-Sen University Cancer Centre)
- Wen Ning (Nankai University)
- Dr. Zhao Qian (Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University)
- Dr. MA Cong (Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University)
- Professor Xu Aimin (Department of Pharmacology and Pharmacy, The University of Hong Kong)
- Dr Clive Chung (School of Biological Sciences, The University of Hong Kong)
- Dr Ken Ma (Department of Pathology, The University of Hong Kong)

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.

To continue the work started under this project, the following funding are obtained:
- Delineating and Translating the Mechanistic Determinants to Improve the Clinical

Management of Liver Cancer (**RGC TRS 2023-2027; T12-716/22-R**; IO Ng as PC) (HKD43.011M)

- Strategic Importance Project Fund (2020/2021) from Hong Kong Polytechnic University Development of gut microbiota-targeted treatment strategy in non-alcoholic fatty liver disease (NAFLD)-induced HCC. (HKD 1,500,000) (TK Lee, HK PolyU)
- Development and Applications of a Driver-Dependent Tumor Organoid Biobank for Translational Liver Cancer Research (Project number: R7022-20; Funded HK$4.1M) (HK$0.3M transferred to PolyU; 2021.6 – 2024.5) (TK Lee, HK PolyU)
- To continue application of the technology scONE-seq that we developed partially using funds from this project, we have now received further funding from the HKUST Center for Aging Science, the Southern Marine Science and Engineering Guangdong Laboratory (Guangzhou), the Lo Ka Chung Foundation through the Hong Kong Epigenomics Project, and the Chau Hoi Shuen Foundation. We also collaborate in other CRFs led by other PCs (C4001-18G; $6,269,185) to utilize this technology (Angela R Wu, HKUST).
- RGC General Research Fund 2021-22 (Project No. 17100021, Funded $899,732) "Elucidation of the immunosuppressive landscape and subclonal heterogeneity in HCC by single-cell RNA sequencing" (WH Ho, HKU)
- RGC General Research Fund 2021-22 (Project No. 17112021, Funded $1,125,732) "Role of the lipid metabolic regulator DGKH in driving stemness in HCC" (KY Ma, HKU)
- RGC Research Fellow Scheme 2021-22 (Project No. RFS2122-7S05, Funded $5,155,380) "An Immune Perspective on Exploiting Stemness as a Cancer Cell Vulnerability for the Treatment of Liver Cancer" (KY Ma, HKU)
- RGC General Research Fund 2022-23 (Project No. 17119322, Funded $1,179,746) "ADAR1-mediated RNA editing of GLI1 drives hepatocellular carcinogenesis by increasing cancer stemness " (XY Guan, HKU)

10. Statistics on Research Outputs

(Please ensure the statistics in this section are consistent with the information presented in other sections of this report.)

	Peer-reviewed journal publications	Conference papers	Scholarly, books, monographs and chapters	Patents awarded	Other research outputs (please specify)	
					Type	No.
No. of outputs arising directly from this research project	98	36	0	3	N/A	N/A

11. Public Access of Completion Report

(PCs of projects funded in 2010/11 academic year or onwards are required to release the completion reports to the public through the RGC website. Completion reports containing information such as abstracts in non-technical terms, objectives, research output including the list of conference papers / publications / journals and research findings and contact information of PCs should be open to public access.)

Please specify the information, if any, that cannot be provided for public access and give the reasons.

Information that cannot be provided for public access	Reasons
N/A	N/A

12. The Layman's Summary

(describe in layman's language the abstracts and research impact of the project.)

Liver cancer (hepatocellular carcinoma, HCC) is a common malignancy worldwide and very prevalent in Southeast Asia and China including Hong Kong, due to the high prevalence of hepatitis B viral infection. Its incidence is also one of the most rapidly increasing in the west. It is an aggressive cancer with high rate of recurrence even after surgical resection and frequent metastasis. In HCC, cancer stemness endows the cancer cells with the ability of self-renewal, tumor propagation, tumor spread, and resistance to therapies. On the other hand, as cancer cells are maintained in a specialized niche microenvironment, which can interact with and influence the cancer cells. Treatment that can target the pivotal molecular targets in the cancer cells and the tumor microenvironment may lead to cancer cures or control. In this project, we have identified important molecular targets both in the cancer cells and tumor microenvironment that may be potentially targeted by inhibitor drugs or antibodies. We have also identified specific molecular mutations in the cancer cells which create vulnerability that can be targeted therapeutically. Our findings in this TRS have been published in almost 100 research publications, many of them being in high-impact journals. These findings have also lent strong support to our further investigation on this cancer and have generated new ideas and new collaborations with other overseas/local centers. Of note, they have resulted in external grant awards, including a new RGC TRS in 2023 on liver cancer ('Delineating and Translating the Mechanistic Determinants to Improve the Clinical Management of Liver Cancer'). Some of our findings have been translated into patents. During the course of the studies, we have trained numerous young scientists in the research on liver cancer stemness and cancer biology, shared our findings with professional communities, as well as educated the general public. In summary, our TRS project has generated high-quality research findings, which will be potentially translated into new HCC treatments to benefit patients with this deadly cancer.