Project number: T12-710/16-R

RESEARCH GRANTS COUNCIL
THEME-BASED RESEARCH SCHEME (TRS)

Completion Report on Funded Project

Project start date: *November 1, 2016*
Project completion date: *October 31, 2022*

1. Project Title: Gastric Cancer Genomics and Beyond – Moving from Patient Samples to 3D Organoid Cultures for Integrative Genomics Analysis, Drug Sensitivity Assays, Cell Biological Studies and Animal Models

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 current reporting period
Project Coordinator (PC)	Leung Suet Yi /Chair Professor	Pathology/ HKU	12
Co-Principal Investigator(s)	Zhang Jiangwen /Associate Professor	Biological Sciences/ HKU	6
	Shi Jue /Associate Professor	Physics/ HKBU	6
	Fan Xiaodan /Associate Professor	Statistics/ CUHK	6
	Law Simon /Chair Professor	Surgery/ HKU	6
	Ma Stephanie /Assistant Professor	Biomedical Sciences/ HKU	6
	Clevers Hans /Professor	Molecular Genetics/ Hubrecht	N/A ^

		Institute	
	Mak Tak Wah/Professor	Campbell Family Institute for Breast Cancer Research / University of Toronto	N/A ^
	Zhu Jun /Professor	Genetics and Genomic Sciences/ Mount Sinai	N/A ^
Co-Investigator(s)	Kwong Dora /Professor	Clinical Oncology/ HKU	2
	Chan Danny /Professor	Biomedical Sciences/HKU	2
	Ching Y.P. /Associate Professor	Biomedical Sciences/HKU	2
	Wong Alan S.L. /Assistant Professor	Biomedical Sciences/HKU	2
Collaborators	Stratton M.R./Director	Sanger Institute	N.A.
	Leung S.K./Consultant	Surgery/TMH	N.A.
	Tsang S.H. /Associate Consultant	Surgery/UCH	N.A.
	Ho S.L. /Associate Consultant	Pathology/QMH	N.A.
	McDermott Ultan /Group leader	Sanger Institute	N.A.

^ These overseas Co-PIs are all well-established and run large research labs that are involved in developing methods for and performing research along similar lines as this research project, albeit on other cancer types. They shared their reagents and methods for the development of our project, including providing training for our PhD students or Postdocs. Thus, it was not possible to assess their direct work hours, as some of it was provided by their senior research team members.

\# *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. Deep mechanistic insight into the complex alternative pathways of gastric carcinogenesis through functional genomics study of combination driver alterations in normal organoid culture by creating stepwise alterations through genome editing	100%	Completed successfully
2. Building of a biobank of gastric cancer organoids carrying these	100%	Completed successfully

Objectives*	Percentage achieved	Remarks**
complex genomic alterations for drug sensitivity testing; and validating drug sensitivity of genomic perturbations that are direct drug targets for translational application of genome-guided drug prescription		
3. Identify vulnerability, signalling pathway de-regulation, cancer stem cell phenotype, drug resistant mechanisms consequent to different combination driver alterations and combinatorial genetic screening for therapeutic development using the CombiGEM-CRISPR technology	100%	Completed successfully
4. In depth study of RHO signalling pathway deregulation in diffuse type GC, including the development of novel animal models	100%	Completed successfully
5. Development of novel methods for integrative genomic analysis to identify new cancer driver pathways and predict new drug targets.	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.))

Our project has achieved numerous significant research accomplishments that have contributed greatly to the field of cancer research. Among these achievements are the creation of both gastric and colon cancer organoid biobanks, along with a pre-neoplastic intestinal metaplasia biobank, which provide valuable resources for studying the biology of these cancers. Through drug screening conducted on the biobank, we discovered novel drug sensitivities in distinct molecular subtypes, demonstrating a considerable potential for translation into clinical applications. We have also discovered a molecular mechanism involving RHO signalling that enables gastric cancer cells to evade cell-cell and cell-matrix dependence, as well as a novel mechanism of chemoresistance in gastric cancer. Furthermore, our team has identified a mutational signature associated with ganciclovir plus mycophenolate mofetil treatment and cancer development across various solid organs. Our project also features the creation of an improved CombiGEM-CRISPR platform (v2.0) for conducting functional genomic screens, which aids in the discovery of synergistic drug combinations. In addition, we have established a state-of-the-art 3D single-cell imaging analysis pipeline for quantifying cell-cell interaction dynamics within the tumour microenvironment. Lastly, we have developed innovative bioinformatic methods for integrative genomic analysis, allowing us to identify new cancer driver pathways and predict potential drug targets.

1. Generation of a gastric cancer organoid biobank for drug sensitivity testing (Yan et al., Cell Stem Cell. 2018 Dec 6;23(6):882-897)
One of the most significant accomplishments of this project was the creation of a biobank of gastric cancer (GC) organoids that carry complex genomic alterations for drug sensitivity testing. We published this important achievement in Cell Stem Cell in 2018 entitled, "A comprehensive human gastric cancer organoid biobank captures tumor subtype heterogeneity and enables therapeutic screening". With an impressive impact factor (IF) of 23.9, Cell Stem Cell is a leading journal in the field. Our paper, which has garnered 455 citations, is recognized as a highly-cited work by the ISI Web of Science, further highlighting its significance and influence. We also received the University of Hong Kong Li Ka Shing Faculty of Medicine Outstanding Research Output Prize in 2019 for this publication. Subsequently, Cell Stem Cell invited PC SY Leung to write a spotlight article highlighting groundbreaking publications in the field of organoid research, entitled "Breakthrough Moments: Organoid Models of Cancer", as well as a full review on organoids entitled, "Organoid cultures for cancer modelling". Furthermore, as a result of this paper, PC SY Leung was invited to speak at 14 different conferences/symposiums (Appendix I). Thus, this publication has brought significant global recognition to our research team.

In this paper, we described the generation of a large biobank consisting of 63 gastric organoids, 46 from cancer tissue and 17 from normal tissue, from 34 patients. They span all the common molecular repertoire of gastric cancer (EBV, MSI, diffuse, mixed, and intestinal subtypes) and cover rare driver mutations, such as RHOA mutants and ARHGAP fusions. We performed in-depth characterisation of these organoids through genomic and transcriptomic analysis, comparing the organoids with their corresponding patient tumour tissue, and found that they recapitulate the features of the original tumours, including key driver mutations, copy number aberrations, and gene expression patterns, even after long-term culture.

Moreover, the organoids also preserved the morphology and heterogeneity of the original tumours, displaying glandular, solid, or discohesive growth patterns and varying degrees of intratumoral subclones. We also used the organoids to study tumour evolution and progression, by comparing organoids derived from different tumour regions, dysplasia, or metastasis, and tracing their clonal history and divergence. Lastly, we used the organoids to perform large-scale drug screening and identified sensitivity to some drugs that are FDA approved or in clinical trials, such as Napabucasin and VE-822 (an ATR inhibitor). Hence, this paper provides a comprehensive dataset of exome and transcriptome sequencing of GC organoids and corresponding tumour tissues, as well as drug screening results, which have been made publicly available for further analysis and research by the scientific community.

Since the publication of our Cell Stem Cell paper in 2018, we have continued to expand the biobank and perform more complex multi-omics profiling and analysis. The current number of organoids in our biobank now stands at >90 from 58 patients, including organoids derived from different regions within the same patient, such as lymph nodes, ovarian metastasis, and umbilical cord metastasis. Furthermore, we have continued to enhance the dataset beyond the genomic and transcriptomic profiling that was shown in our paper. Methylation, proteomic and phosphoproteomic profiling, have been performed on the organoids (Figure 1). As a result, this organoid biobank represents the broadest and most comprehensively analysed biobank for GC so far, making it a truly valuable reference resource for the general research community.

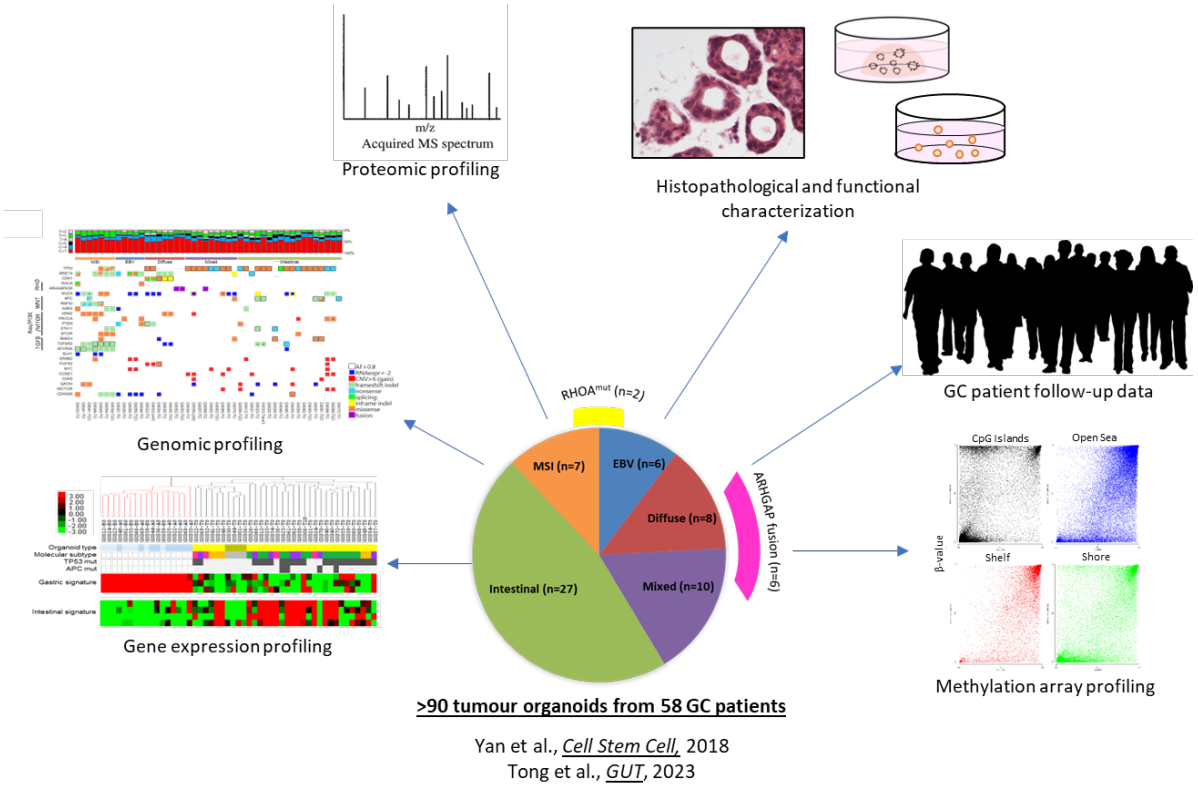

Figure 1. Establishment and characterization of a gastric cancer organoid biobank. Summary of the gastric cancer organoids established from 58 patients for the biobank to date, their range of diverse molecular subtypes and the types of data that have been collected and analyzed.

3. Discovery of a mechanism for GC evasion of cell-cell and cell-matrix dependence that is partly linked to RHO signalling (Tong et al., Gut. 2023 Feb;72(2):242-255)

Using our GC organoid biobank, we performed deep mechanistic and functional studies to understand the pathways of GC carcinogenesis. Specifically, we found that most GC organoids were able to survive without cell-matrix adhesion (CMi) and some were also able to survive without cell-cell adhesion (CCi) or both (CCi/CMi). We published our findings in Gut in 2023 and our paper is entitled, "Escape from cell-cell and cell-matrix adhesion dependence underscores disease progression in gastric cancer organoid models". Gut has a high impact factor of 24.5 and is a leading journal in the field of gastroenterology, with a broad international readership. PC SY Leung and co-corresponding author Dr H Yan were both invited to speak about this paper at international conferences. Therefore, this work has

clearly brough us additional international recognition.

A defining characteristic of cancer is the loss of cell adhesion, enabling cancer cells to detach from their original location and migrate to new sites. In this study, we assessed GC organoids using ROCK inhibitor withdrawal, spheroid formation, and double challenge assays to investigate their cell-matrix (CMi) and cell-cell anchorage (CCi) independent growth capabilities (Figure 4). We then conducted a comprehensive analysis to pinpoint key factors associated with anchorage dependence by integrating our findings with patient clinicopathological parameters and gene expression data. Our results showed that almost all GC organoids were CMi, but only a subset were CCi or CCi/CMi. Moreover, the CCi/CMi phenotype was associated with an infiltrative tumour edge, advancing tumour stage and metastasis, as well as a transcriptomic signature associated with shorter overall and disease-free survival in multiple public datasets of GC patients. Tumour organoids isolated from the deeper submucosa of early-stage GCs were also less dependent on cell-cell adhesion compared to paired tumour organoids established from the superficial mucosa, demonstrating that different parts of the tumour from the same patient can behave differently. Additionally, we observed that CCi/CMi and CCi phenotypes were enriched in diffuse-type GC organoids, especially in those with oncogenic driver perturbation of RHO signalling via *RHOA* mutation or *ARHGAP* fusions. Inducible knockout of *ARHGAP* fusions in CCi/CMi tumour organoids led to resensitisation to CC/CM dissociation-induced apoptosis, upregulation of focal adhesion and tight junction genes, and partial reversion to a more normal cystic phenotype. To test driver gene function and drug response, we developed an animal model involving orthotopic injection of human GC organoids to the mouse stomach, thereby generating xenografts. Utilizing this animal model, we were able to demonstrate that knockdown of *ARHGAP* fusion resulted in the suppression of xenograft formation. Lastly, CRISPR-engineered normal organoids with *RHOA* or *CDH1* mutations became CMi or CCi, respectively. Thus, our findings not only reveal the heterogeneity and dynamics of cell adhesion properties in GC organoids, but also highlight the potential therapeutic implications of targeting specific RHO signalling pathways in the context of diffuse-type GC with distinct oncogenic drivers, ultimately paving the way for more personalized and effective treatment strategies.

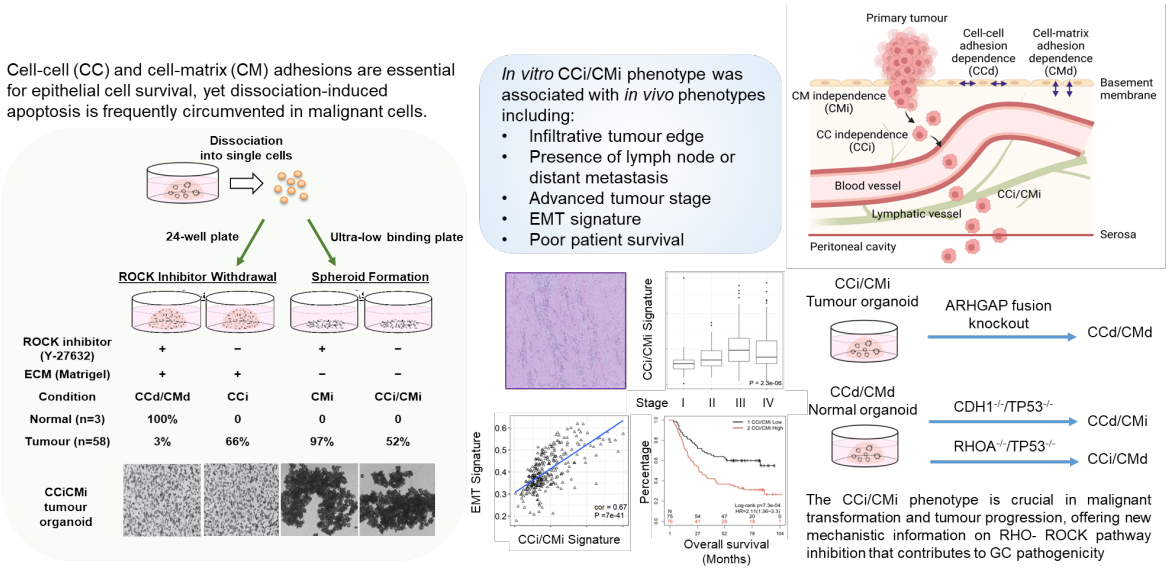

Figure 4. Summary of our mechanistic study on GC organoids. GC organoids were subjected to ROCK inhibitor withdrawal, spheroid formation and double challenge assays to examine their cell-matrix and cell-cell anchorage independent growth capacities. Cell-cell and cell-matrix adhesion independence was correlated with an infiltrative tumour edge, advanced tumour stage, poor patient survival, diffuse gastric cancer subtype, and mutations or fusions in genes involved in the RHO-ROCK signalling pathway, such as CDH1, RHOA and ARHGAP1. ARHGAP fusions, especially CLDN18-ARHGAP26, were responsible for conferring both cell-cell and cell-matrix adhesion independence in gastric cancer organoids by inhibiting RHO-ROCK-mediated apoptosis.

4. Discovery of a mechanism that drives drug resistance and self-renewal in GC (Wong et al., Nat Commun. 2023 May 19;14(1):2861)

Chemotherapy with 5-fluorouracil and cisplatin (5FU + CDDP) is often used to treat GC, but many patients, especially those with intestinal subtype GC, develop resistance to this treatment and relapse. Recently, we discovered a new mechanism that explains how GC cells become resistant to 5FU + CDDP and suggest new therapeutic targets for overcoming it. Our findings were published in 2023 in Nature Communications and is entitled, "ADAR1-mediated RNA editing of SCD1 drives drug resistance and self-renewal in gastric cancer". Nature Communications is considered an impactful journal due to its association with a prestigious publisher, interdisciplinary scope, rigorous peer review, open access model, high impact factor (16.6), and global reach.

In this paper, we established 5FU+CDDP resistant intestinal subtype GC patient-derived organoids by challenging the organoids with increasingly elevated doses of 5FU+CDDP until they stopped proliferating but remained alive. We found that resistant organoids had higher levels of an enzyme called ADAR1, which can modify RNA molecules by changing adenosine (A) to inosine (I). This process, called A-to-I RNA editing, can alter the function of genes and proteins involved in various cellular processes. Whole-exome sequencing and RNA sequencing revealed that ADAR1 edited the 3'UTR of SCD1 to create a binding site for KHDRBS1 (Figure 5). SCD1 is an enzyme that regulates lipid metabolism, which is essential for energy production and cell survival. In our model, SCD1 facilitated lipid droplet formation to alleviate chemotherapy-induced endoplasmic reticulum (ER) stress and enhanced self-renewal through increasing β-catenin stability. Remarkably, pharmacological inhibition of SCD1 reversed the chemoresistance and reduced self-renewal in the GC organoids. Furthermore, analysis of clinical samples from GC patients who received 5FU+CDDP combination chemotherapy showed that high levels of ADAR1 and SCD1 proteins, or high ADAR1 mRNA/SCD1 editing signature scores, predicted worse survival outcomes. Overall, our study provides insights into the molecular mechanisms of RNA editing in GC chemoresistance and self-renewal, and unveiled a novel actionable target to circumvent 5FU+CDDP chemoresistance.

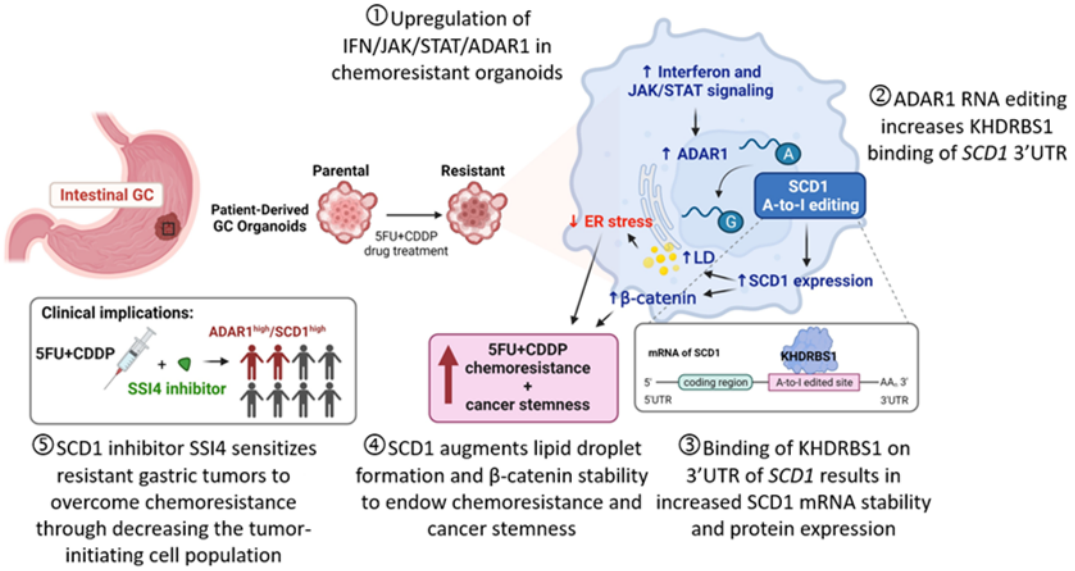

Figure 5. ADAR1-mediated RNA editing of SCD1 drives drug resistance and self-renewal in GC. Chemoresistant tumours harbour activated interferon, JAK/STAT and ADAR1 expression. ADAR1 confers chemoresistance and cancer stemness through RNA editing of SCD1, resulting in its enhanced protein expression. SCD1 facilitates lipid droplet formation to alleviate chemotherapy-induced ER stress and enhances self-renewal through increasing β-catenin expression. Pharmacological inhibition of SCD1 editing/ADAR1 mRNA signature score predicts a worse prognosis. Findings of this study identifies an actionable target to circumvent chemoresistance, inhibiting gastric cancer growth at its roots.

5. Generation of an organoid biobank enriched with early onset colorectal cancer (Yan et al., Gut. 2020 Dec;69(12):2165-2179)

The tools that we developed to study gastric cancer in this project have led to research opportunities for other cancer types. The organoid culture technology developed and experience acquired as part of this project were also utilized for establishing and performing comprehensive molecular characterisation of a colon organoid biobank. Our colon biobank contains 52 organoids, including 18 normal organoids, 2 pre-neoplastic organoids, 29 tumour organoids and 3 metastatic lymph node organoids, from 20 patients (Figure 6). This achievement was published in Gut in 2020 and is entitled, "Organoid cultures of early-onset colorectal cancers reveal distinct and rare genetic profiles". Gut has an impact factor of 24.5 and our paper received the University of Hong Kong Li Ka Shing Faculty of Medicine Faculty Outstanding Research Output Award in 2021. As a result of this paper, PC SY Leung was invited to write a spotlight article in Cancer Cell (impact factor: 50.3) highlighting research on cancer-promoting mutations in intestinal stem cells, entitled "Oncogenic mutations drive intestinal cancer initiation through paracrine remodeling". Additionally, PC SY Leung and her RAP Dr Helen Yan were each invited to speak at an international symposium about our colon biobank paper.

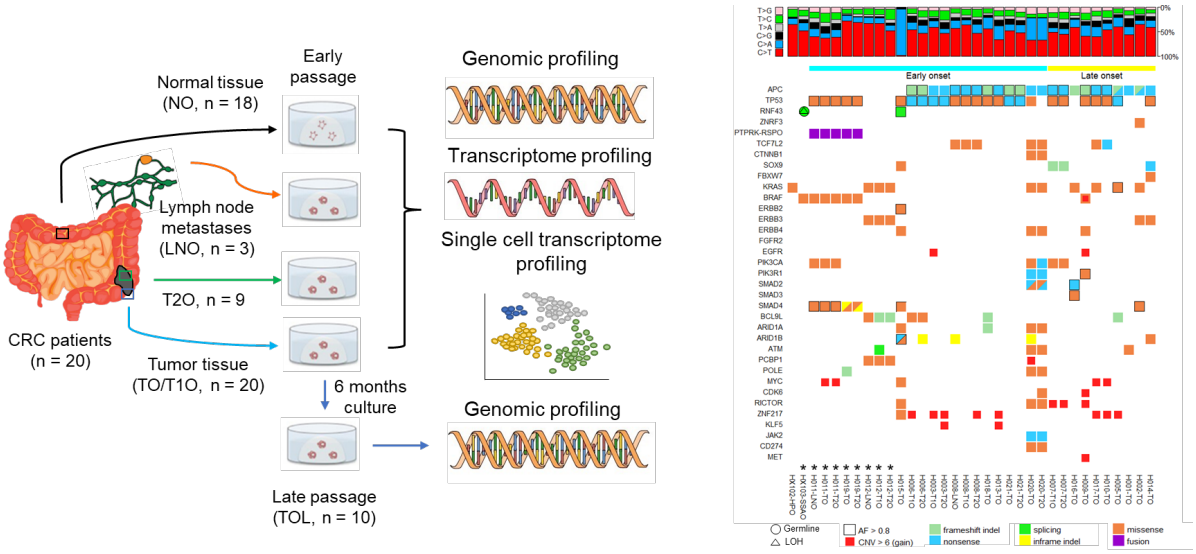

Figure 6. Establishment and characterization of a colorectal cancer (CRC) organoid biobank. Summary of the colon organoids established from 20 patients for the biobank to date, the types of data that have been collected and analyzed, as well as an oncoplot showing the somatic cancer driver alterations in the organoids.

This biobank is unique, as it is enriched in microsatellite-stable early-onset colorectal cancers (EOCRCs), which are cancers that occur before the age of 50 and are poorly represented by existing cell models. We performed whole-exome and whole-genome sequencing on the organoids and compared them with their original tumours and normal tissues. We found that EOCRCs had diverse molecular phenotypes, including PTPRK-RSPO3 fusions, RNF43 splicing mutations, a novel mutational signature in a leukaemia survivor, and a POLE proofreading mutation that displayed an ultramutation phenotype. We also confirmed a reduced incidence of truncating APC mutations and an increased incidence of other Wnt pathway alterations in EOCRCs compared with late-onset CRCs. Transcriptome profiling of the EOCRCs under different growth conditions revealed distinct gene expression patterns. For example, RSPO fusion organoids had a transcriptome that resembled normal organoids and were highly dependent on Wnt-3A, but not R-spondin1. They also retained their differentiation potential upon Wnt withdrawal, unlike APC mutant organoids that were locked in progenitor stages. Moreover, PTK7, a transmembrane protein and a potential therapeutic target, was upregulated in APC mutant organoids, but not in RSPO fusion or RNF43 mutant

organoids. These results suggest that different Wnt signalling-targeted therapies may have differential applications in RSPO fusion versus APC mutant CRCs. Thus, our EOCRC enriched biobank with linked genomic data fills a gap in existing CRC models and revealed distinct genetic profiles and novel pathway cooperativity with potential therapeutic implications.

7. Identification of a ganciclovir-linked mutational signature and cancer development (Fang et al., Genome Med. 2022 Oct 31;14(1):124)

Since organoids can more closely mimic the *in vivo* cellular environment of human organs and tissues compared to traditional 2D cell culture systems, they have enormous potential for application in clinical practice. Our recent study, entitled "Ganciclovir-induced mutations are present in a diverse spectrum of post-transplant malignancies," highlights the value of organoids by using them to investigate the mutagenic effects of ganciclovir on human cells and its connection to post-transplant malignancies. Our study was published in Genome Medicine, which is a reputable and well-regarded journal in the field of genomics and its application to human health. This study was an exciting accomplishment because it clearly demonstrates the potential of organoids for drug toxicity studies, which can significantly advance drug development and personalized medicine, ultimately enhancing patient care and safety.

In this study, we used organoids from our CRC biobank to understand the role of ganciclovir-induced mutations in contributing to the development of cancer in transplant patients. Previously, we observed a novel mutational signature characterized by CA>AA substitutions in both the normal and CRC organoids of a patient. This patient had a history of acute myeloid leukaemia and received a haematopoietic stem cell transplant, followed by ganciclovir (GCV) and mycophenolate mofetil (MMF) as part of her treatment. GCV is an antiviral drug used to treat patients who have received solid organ or haematopoietic stem cell

transplants to prevent or treat cytomegalovirus (CMV) infection, a common complication after transplantation, while MMF is an immunosuppressant that is often co-prescribed with GCV. However, GCV is also a known mutagen and has been shown to incorporate into genomic DNA, causing errors during DNA replication. Since the signature was present in both normal and tumour organoids, we hypothesized that it was a GCV induced signature (GCVsig). We used the GCVsig derived from this patient organoid to analyse publicly available genomic data from 121,771 cancer patients who had undergone targeted sequencing of their tumours, and identified 22 patients with strong evidence of GCVsig. These 22 patients had all previously undergone solid organ transplantation and received GCV+MMF treatment, and showed a diverse range of malignancies. We then used another normal organoid from our biobank as an exposure model to test the effects of GCV and other drugs. We observed that after 4-6 weeks, organoids cultured with GCV showed increased DNA damage and CA>AA mutations, but the addition of MMF resulted in enhanced incorporation of GCV into genomic DNA and an even higher proportion of CA>AA mutations. Interestingly, organoids cultured with acyclovir (ACV), an antiviral that is structurally similar to GCV, did not show mutagenicity. Therefore, prescription of ACV in combination with MMF may represent an alternative treatment option with potential widespread implications for numerous patients requiring bone marrow or solid organ transplantation. Thus, our study successfully showed the clinical utility of organoids for studying the genotoxicity of GCV in transplant patients, demonstrating that MMF can potentiate GCV mutagenicity and calling for a more thorough investigation of the optimal use of these drugs.

8. Creation of CombiGEM-CRISPR 2.0 and Opti-SpCas9 for functional genomic screens (Zhou et al., STAR Protoc. 2021 Jan 9;2(1):100255, Zhou et al., Cell Rep. 2020 Aug 11;32(6):108020 and Choi et al., Nat Methods. 2019 Aug;16(8):722-730)(2 patents filed)

One of the main goals of this project is to discover new therapeutic targets for GC using CombiGEM-CRISPR screening technology. However, efficient and precise gene targeting is crucial for minimizing the identification of false positive hits from genetic screens. Unfortunately, the original CombiGEM-CRISPR design has many repeated DNA sequences in the CRISPR constructs, potentially causing increased intramolecular recombination frequency during lentiviral transduction. This might result in DNA barcodes separating from their related library elements in combined genetic screens, reducing the screening quality. To improve the screening quality, we have made significant advancements in developing an enhanced platform, CombiGEM-CRISPR 2.0 (Figure 7), which uses different promoter sequences (i.e., human H1, human U6, and mouse U6 promoters) to drive the multiplexed expression of

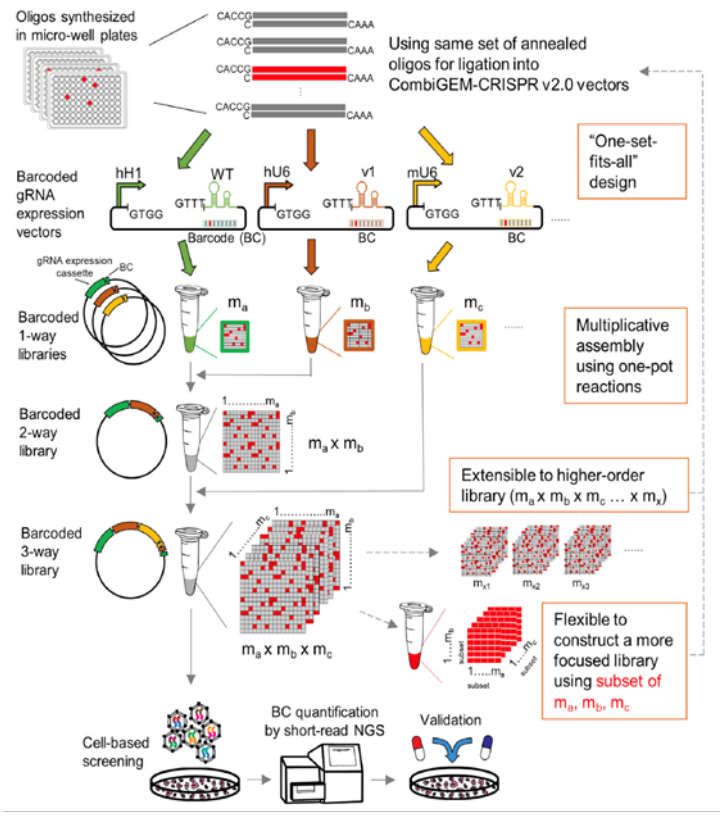

Figure 7. Development of CombiGEM-CRISPR v2.0.

guide RNAs while maintaining knockout efficiency, thereby minimizing recombination events. We also created engineered guide RNA scaffold sequences compatible with the combinatorial assembly strategy of CombiGEM-CRISPR, improving user-friendliness and cost- effectiveness for large-scale screens. These sequences maintain comparable efficiency to wildtype scaffold sequences in knocking out target genes. Our work, entitled "A three-way combinatorial CRISPR screen for analyzing interactions among druggable targets" was published in Cell Reports (IF 8.8), along with a detailed experimental protocol and a complementary computational analysis pipeline (i.e., CombiPIPE) (Figure 8), entitled "Extensible combinatorial CRISPR screening in mammalian cells" in STAR Protocols (IF 1.48). We have filed a patent application based on our new design: "Wong, S.L.A., Zhou, P., Ho, S.Y. A system for combinatorial CRISPR screens for pairwise and three-way therapeutic combinations and methods thereof. WO2021208971 (2021)".

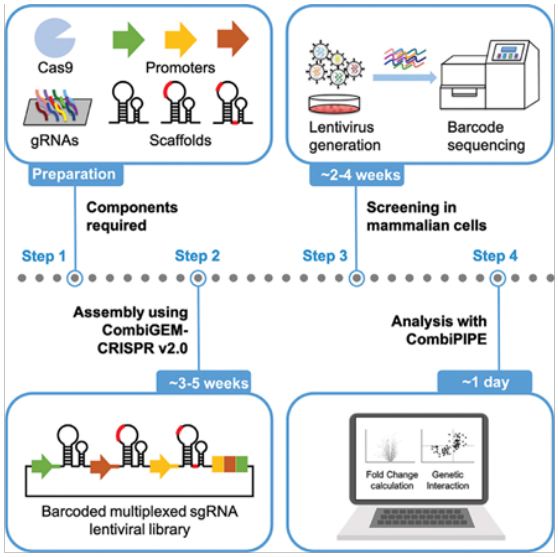

Figure 8. Detailed experimental steps, technical considerations, and analytical pipeline for combinatorial CRISPR screen.

We have also successfully engineered a new high-fidelity version of SpCas9, named Opti-SpCas9. Although SpCas9 is the most commonly used Cas9 protein in CRISPR-Cas9 genome editing, the wildtype SpCas9 can sometimes cause off-target effects. Our Opti-SpCas9 variant maintains on-target activity comparable to wild-type, while exhibiting reduced off-target activity in human cells. We also demonstrated similar on-target activities between Opti-SpCas9 and wildtype SpCas9 in a human GC organoid. Our work, entitled "Combinatorial mutagenesis en masse optimizes SpCas9's genome-editing activities", was published in Nature Methods, which has a high impact factor of 48. We have filed a patent application based on our engineered Cas9 variants "Wong, S.L.A., Choi, G.C.G. Improved high-throughput combinatorial genetic modification system and optimized cas9 enzyme variants. WO2020057481A1, EP3853363A1, CN112955549A, KR20210060541A (2019)". By employing Opti-SpCas9 in conjunction with CombiGEM- CRISPR v2.0, we can achieve greater efficiency and accuracy in gene targeting for our genetic screens in GC organoids.

Utilizing CombiGEM-CRISPR v2.0 and Opti-SpCas9, we conducted a combinatorial CRISPR screen targeting a list of druggable genes in a gastric cancer organoid line (GX058). We chose gRNAs with high on-target scores based on Azimuth 2.0 predictions and included control gRNAs from the GeCKOv2 library without on-target loci in the human genome as references. The pairwise gRNA library pool, consisting of 32 x 32 gRNAs (1,024 total combinations), was delivered into GX058 cells using lentiviruses. High-throughput barcode sequencing confirmed high coverage (>95%) of the pairwise library and strong correlation of barcode representation between the plasmid and infected organoid pools. We compared barcode abundances between day 13 and day 20 groups to obtain log2 values as a measure of cell growth. Our bioinformatics analysis of the CRISPR screen data using MAGecK and GEMINI allowed us to evaluate and define the synergy of the identified combinations. ERBB2+TYMS and mTOR-TYMS emerged as the combinations with the greatest depletion log2 fold change (LFC). While a decrease in observed LFC compared to expected LFC indicated synergy, GEMINI's strong lethality score revealed that this synergy was mild. Our validation experiments confirmed that ERBB2 and mTOR contributed only minimally to the

additive effect on TYMS. In conclusion, our study demonstrates the robustness of our CRISPR screening platform in gastric cancer organoids. Prior to this project, the technical feasibility of such a CRISPR dropout screen in organoids had not been previously reported. Thus, our work establishes a crucial foundation for scaling up screening in organoids to identify effective targets for inhibiting their growth.

10. Uncovering genomic stability and cancer trajectories in Lynch Syndrome (Lee et al., Nat Commun. 2022 May 17;13(1):2710)

Lynch Syndrome (LS) is an autosomal dominant disease conferring a high risk of colorectal cancer due to germline heterozygous mutations in a DNA mismatch repair (MMR) gene. Although cancers in LS patients show elevated somatic mutation burdens, information on mutation rates in normal tissues and understanding of the trajectory from normal to cancer cell is limited. To approach this, we performed whole genome sequencing on 152 crypts from normal and neoplastic epithelial tissues from 10 LS patients covering the stomach and colon. Interestingly, the repertoire of mutational processes and mutation rates in normal tissues of LS patients were found to be similar to those observed in wild type individuals. To verify this finding, we employed an organoid model, creating clonal organoid cultures from normal gastrointestinal adult stem cells of two LS patients and subjecting them to whole genome sequencing. The mutation rates and mutational signatures throughout these patients' lifetimes

were comparable to those of organoids from a wild-type individual. Furthermore, extended culture of these LS organoids for >4 months did not reveal an increased in vitro mutation rate compared to organoids from the wild-type individual, confirming the stability of the genome in normal cells from LS patients. Overall, this study demonstrates the genomic stability of epithelial cells carrying heterozygous germline MMR gene mutations and highlights crucial differences in LS pathogenesis compared to other colorectal cancer predisposition syndromes. The findings also provide guidance for chemoprevention strategies focused on preventing the second hit inactivation of the MMR gene as a cancer initiation event. This work, entitled "Mutational landscape of normal epithelial cells in Lynch Syndrome patients" was published in Nature Communications (IF 16.6).

12. Development of innovative bioinformatic methods for integrative genomic analysis

Throughout this project, we have developed multiple products for analysing extensive multi-omics datasets, such as our various organoid datasets, as well as publicly available data. These products have been published in high-impact journals and are freely accessible to the scientific community, enabling fellow researchers to gain deeper insights into complex biological systems. Publication of these products is briefly summarized below:

- DMRMark **(Shen et al., Bioinformatics. 2017 Dec 1;33(23):3701-3708. IF 5.8)**: a novel Bayesian framework for detecting differentially methylated regions (DMRs) in DNA methylation array data. It improves upon existing methods by rigorously modelling the correlation of nearby CpG sites, handling both paired and unpaired samples, and detecting DMRs from a single pair of samples.
- CR2Cancer **(Ru et al., Nucleic Acids Res. 2018 Jan 4;46(D1):D918-D924; IF 14.9)**: a comprehensive database annotating chromatin regulators (CRs) in human cancer, which integrates genomic, transcriptomic, proteomic, clinical, and functional data for over 400 CRs across various cancer types. It establishes cancer type-dependent and -independent relationships, such as CR-target and protein-protein interactions.
- MICMIC **(Tong et al., Genome Biol. 2018 Jun 5;19(1):73; IF 12.3)**: a web tool for identifying genome-wide DNA methylation in distal regions that may have causal effects on tumourigenesis. MICMIC complements existing knowledge on promoter methylation, enabling a comprehensive understanding of cancer methylomes.

- miRNACancerMAP **(Tong et al., Bioinformatics. 2018 Sep 15;34(18):3211-3213, IF 5.8)**: a computer server to analyze deregulated microRNA-mRNA-lncRNA gene networks associated with tumorigenesis. Utilizing large-scale data integration, miRNACancerMAP implements various algorithms and pipelines to construct dynamic miRNA-centered networks using rigorous systems biology approaches and advanced visualization tools. The server can visualize miRNA-mRNA interaction networks and perform functional enrichment analysis, providing valuable insights into cancer-related gene interactions.
- MR4Cancer **(Ru et al., Bioinformatics. 2019 Feb 15;35(4):636-642; IF 5.8)**: is a web server for identifying master regulators (MRs) that control cancer initiation and progression. The server ranks MRs based on enrichment testing against predefined regulons. It conducts Gene Ontology and pathway analyses, offers dynamic network visualization for MR-target relations, and enables users to interactively explore the network, providing novel insights into tumorigenesis and therapeutic intervention.
- DM-miRNAs **(Pian et al., Mol Ther Nucleic Acids. 2020 Mar 6:19:1423-1433; IF 8.8)**: a method for detecting cancer-related miRNAs, so called "dark matter miRNAs", that are non-differentially or only weakly differentially expressed. This approach enables the discovery of previously undetectable cancer-related miRNAs, providing valuable insights into potential therapeutic targets.
- miR+Pathway **(Pian et al., Brief Bioinform. 2020 Mar 23;21(2):699-708; IF 9.5)**: a database for visualizing human experimentally validated miRNA-target interactions and KEGG pathways, addressing the previous lack of miRNA information in KEGG pathways. This tool provides a comprehensive understanding of miRNA functional mechanisms and aids in constructing complex miRNA-gene regulatory networks.
- MM-6mAPred **(Pian et al., Bioinformatics. 2020 Jan 15;36(2):388-392; IF 5.8)**: a web server for identifying DNA N6-methyladenine (6mA) sites, which play an important role in epigenetic modification and are closely related to embryonic development, stress response, as well as other important biological functions.
- BaySub **(Fan et al., Brief Bioinform. 2023 Jan 19;24(1):bbac568; IF 9.5)**: a novel Bayesian model for subtyping cancers based on paired methylation array data. The model can capture the methylation changes associated with different cancer subtypes and cluster patients accordingly.

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?)

The TRS funding had several advantages over standard project grant funding. Both the length and the amount of the TRS funding was beneficial to the success of this project. First, with long-term, significant financial support, we were able to undertake more ambitious, complex, and high-impact projects that require a longer timeline to yield results. For example, we not only established one of the largest biobank to date of patient-derived gastric tumour, metaplasia and normal organoids and with the most comprehensive molecular cancer subtypes, including those not previously exist in cancer cell lines, but also rare in other organoid cohorts. We also performed comprehensive multi-omics profiling, functional studies and large-scale drug screening on the organoids. The funding also enabled us to perform high-quality CRISPR screens and other functional studies using organoid culture, which are more close to

patient sample compared with cancer cell lines. The scale and expense of such a project could not have been achieved with GRF or CRF funding alone. Second, TRS funding fostered cross-disciplinary and inter-institutional collaborations. This project brought together gastric cancer researchers, molecular biologists, stem cell biologists, biophysicists, bioinformaticians, bioengineers, pathologists, physicians, surgeons and clinicians across 6 different institutes. Such extensive collaboration would not be possible with standard funding.

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

We have met the original objectives of the project.

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

Please see the Annex

(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)

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)*
Please see 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.

All publications/conference papers listed in (a) and (b) acknowledge RGC funding.

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

This project has significantly strengthened inter-institutional collaborations and other partnerships amongst the various labs of our overall team. Each of the Co-PIs labs contributed their expertise and worked together to achieve the common goal. For example, members of J Shi's lab at BU and S Ma's lab at HKU received organoid culture training from PC SY Leung's lab at HKU. On the computational side, X Fan at CUHK, J Zhang at HKU and PC SY Leung worked together to analyse the gastric cancer organoid multi-omics data. Notably, PC SY Leung's and Co-PI J Zhang jointly sponsored a post-doctoral fellow during this project. Dr Yin Tong was previously a PhD student in Co-PI J Zhang's lab and was a key player in the development of many of the bioinformatic tools developed in his lab. To facilitate the application of more of our bioinformatic tools for analysing our data, Dr Yin Tong split his time between both labs, putting theory into practice by applying the developed tools to actual data in order to generate meaningful results. His presence in both labs also bolstered interaction, discussion and teamwork between the two labs. Furthermore, all three of our overseas Co-PIs, H Clevers, T Mak and J Zhu, from Utrecht University, The University of Toronto and Mount Sinai, respectively, have visited Hong Kong, in addition to frequent communication via Skype and phone conferences (Appendix III). Notably, during year 5 of

this project, Co-PI T Mak relocated to Hong Kong and spent 50% of his time here (Appendix). These collaborations culminated in the publication of a large study in Cell Stem Cell, which detailed the establishment of our gastric organoid biobank with comprehensive multi-omics analysis. This was a large group effort that required frequent contact between senior and junior team members across institutions. Our collaborations are also reflected in other papers that have been published by our team (Annex), the poster presentations that have been presented by the students and postdocs at international conferences (Appendix II), as well as the invited talks given by the PC and Co PIs (Appendix I). The initiation of this project also enabled broader collaboration between overseas Co-PI H Clevers and various local Co-PIs, including S Ma with H Clevers and SY Leung on human primary hepatocellular carcinoma (HCC) organoid establishment, and extending to other research groups in HKU. Specifically, several groups subsequently collaborated with H Clevers on airway organoids, leading to important studies in COVID and influenza virus infection.

6.6 Research students trained (registration/awards):

Name	Degree registered for	Date of registration	Date of thesis submission/ graduation
Please see Appendix IV			

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

Several products have been developed as a result of this project. Co-PI J Zhang developed 4 user-friendly, interactive web interface tools: 1) CR2Cancer (Nucleic Acids Research, 2018), a comprehensive annotation and visualization database for chromatin regulators (CRs) in human cancer, 2) MICMIC (Genome Biology, 2018), a tool for identifying genome-wide DNA methylation in distal regions that may have causal effects on tumourigenesis, 3) miRNACancerMAP (Bioinformatics, 2018), a computer server for identifying deregulated microRNA- mRNA-lncRNA gene networks associated with tumourigenesis, 4) MR4Cancer (Bioinformatics 2019), a server for identifying master regulators (MRs) that control cancer initiation and progression. Co-PI X Fan has also developed several products, including: 1) DMRMark (Bioinformatics, 2017), a method for detecting differentially methylated regions (DMRs) in DNA methylation array data, 2) DM-miRNAs (Mol Ther Nucleic Acids, 2020), a method for detecting cancer-related miRNAs that are non-differentially or only weakly differentially expressed, 3) miR+Pathway (Brief Bioinform, 2020), a database for visualizing validated miRNA-target interactions and KEGG pathways, 4) MM-6mAPred (Bioinformatics, 2020) a web server for identifying DNA N6-methyladenine (6mA) sites, 5) BaySub (Brief Bioinform, 2023) a novel Bayesian model for subtyping cancers based on paired methylation array data and 6) NetMIM, a network-based integrative Bayesian framework for biomarker selection and disease outcome prediction based on multi-omics data. Lastly, Co-I ASL Wong developed SpCas9 variants, including Opti-SpCas9 and OptiHF-SpCas9, and the vectors for expressing these proteins (Nature Methods, 2019) (deposited on Addgene: https://www.addgene.org/131736/ and https://www.addgene.org/131737/).

6.8 Other education activities and/or training programmes developed:

The Hong Kong Academy of Sciences, the Hong Kong Academy of Gifted Education and the Hong Kong Education Bureau jointly co-organised: Educational Talk Series on Emerging Technologies – Science, Opportunities and Challenges (2019-2020) – “Nature’s Red Pencil: Writing and Re-Writing Genomes”. Co-PI S Ma gave an overview of the talk and acted as a moderator of the event, while Co-I ASL Wong gave a talk on CRISPR editing. In addition,

our various other knowledge exchange interactions (Appendix III) have provided significant educational value for the researchers locally.

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.

Nil

Project Management

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

Even under difficult circumstances, such as local political protests and a global health pandemic, the PC kept the project on track. The PC kept track of all the deadlines and important dates, keeping the management team on target. The PC has organized management team meetings, Zoom meetings and phone conferences. Furthermore, the PC organized 2 SAB visits and 2 RGC site-visits, all of which received very positive reviews and comments. Furthermore, the PC organized several research retreats and symposiums to encourage discussion and inspire new ideas. The PC kept the moral and motivation high among the entire team.

7. Awards and Recognition

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

- Co-PI J Shi received an RFS grant for a project titled: Antitumor Dynamics of Natural Killer Cell and Its Response to Immune Checkpoint Inhibitors in 3D Tumor Organoid Models.
- Co-PI J. Shi received a CRF equipment grant (J. Shi as PC and SY Leung as co-PI) for a new 3D live-cell imaging system that used in this TBRS project to perform long-term high-resolution 3D imaging of gastric organoid drug response dynamics.
- Co-PI S Ma received an RGC Research Impact Fund (RIF) for a project titled: Development and applications of a driver-dependent tumor organoid biobank for translational liver cancer research.
- A postdoctoral fellow in Co-PI S Ma's lab received an HMFR grant, with S Ma as a Co-I and SY Leung and H Clevers as collaborators, for a project titled: Establishment and applications of organoid cultures from primary hepatic carcinomas – a pilot study.
- A RAP, subsequently turned AP, in PC SY Leung's lab received a GRF grant with SY Leung as Co-PI, for a project titled: Unravelling clonal evolution and subclonal heterogeneity of serrated colorectal neoplasia through single-cell RNA sequencing.
- PC SY Leung, together with Co-PI T Mak received funding from the Innovation and Technology Commission to establish the Centre for Oncology and Immunology, Ltd.

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

Please see Appendix I

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

Please see Appendix V

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

In this project, we established a gastric cancer (GC) organoid biobank containing complex genomic alterations for drug sensitivity testing. This achievement was published in the high-impact journal Cell Stem Cell in 2018 and highly cited, thus the experience we shared in the paper has help many other research laboratory or industry to improve the success rate of their biobanking. We also identified new drug sensitivity with patent pending. This project has also led to the development of several innovative genome analytical tools, listed in section 6.7 above, as well as improved SpCas9 variants for performing genetic screens. Lastly, the success of our GC organoid biobank in this project has also enabled us to assist other researchers in developing similar biobanks for various cancer types. By sharing our expertise and methodologies, our team has contributed to the advancement of cancer research in a broader context.

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

- Co-PI S Ma received a Croucher Senior Research Fellowship in 2023
- Co-PI Jue Shi received a Research Fellow Scheme (RFS) grant in 2021
- The University of Hong Kong Li Ka Shing Faculty of Medicine Faculty Outstanding Research Output Award (2021) for our research paper entitled, "Organoid cultures of early-onset colorectal cancers reveal distinct and rare genetic profiles", Gut 2020
- The University of Hong Kong Li Ka Shing Faculty of Medicine Outstanding Research Output Prize (2019) for our research paper entitled, "A comprehensive human gastric cancer organoid biobank captures tumor subtype heterogeneity and enables therapeutic screening", Cell Stem Cell 2018

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.)?

This project has generated several landmark bio-resources that are important for the cancer research community worldwide, including a gastric cancer organoid biobank covering comprehensive molecular subtypes with linked genomic data and paired original tumour (Cell Stem Cell 2018), a colon cancer organoid biobank enriched in early-onset and rare genotypes (Gut 2020) and a gastric intestinal metaplasia biobank. Through drug screening, we identified new drug sensitivity that could be follow up with clinical trial for translational application to benefit gastric cancer patients. Through mechanistic studies we identified new drug resistance mechanisms. This unique resource holds great potential in advancing the practice of precision medicine and improve the treatment outcome of gastric cancer. We have also developed an improved CombiGEM-CRISPR platform (v2.0) (Cell Reports 2020), with accompanying detailed protocol (STAR Protocols, 2021) to share our knowledge with the research community. Moreover, we have generated several bioinformatics tools and software and an a new high-fidelity version of SpCas9, named Opti-SpCas9 (Nature Method, 2019) with wide application. Looking towards the future, these accomplishments, tools and publications reinforce our leading position in gastrointestinal cancer research. Thus, our work will be important for the long-term development of biotechnology in Hong Kong, as we attract more funding and research collaborations from world-renowned scientists and pharmaceutical

companies. Through this grant, we have enhanced our collaboration with various external collaborators, leading to the establishment of the Centre for Oncology and Immunology funded by the ITC, to identify new drug target and develop new drugs to treat hard-to-treat cancer.

8.2 Others (please specify):

Nil

9. Sustainability of the Project

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

- Co-PI J Shi had the idea to use the gastric tumor organoids biobank to investigate interactions between immune cells and tumor organoids in variable 3D tumor microenvironments. She has already established novel co-culture assays and 3D live-cell imaging pipelines to explore the phenotypes and rate-limiting kinetics underlying tumor organoid interactions with different immune cell types, such as natural killer cells (NK cells).
- As a result of this project, Co-PI S Ma had new ideas that extended to the establishment of organoid cultures from human and mouse liver cancers, with acquisition of a RGC Research Impact Fund (RIF) for a project titled: Development and applications of a driver-dependent tumor organoid biobank for translational liver cancer research.
- The lesson we learned from the organoid culture of GI cancer and its pre-cancerous counterparts has induced new ideas to study their cellular dynamics and growth trajectories to understand lineage plasticity in cancer development and drug resistance.

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

- Co-PI S Ma is currently using the chemoresistant gastric organoids trained in this study to explore whether and how IFN-1 may drive enrichment and de novo induction of gastric cancer stem cells during chemotherapy-induced immunogenic cell death.
- Co-PI J Shi received an RFS grant for a new project titled: Antitumor Dynamics of Natural Killer Cell and Its Response to Immune Checkpoint Inhibitors in 3D Tumor Organoid Models.
- PC SY Leung and Co-PI Tak Mak started the Centre for Oncology and Immunology, Ltd. to further utilize the organoid models and the functional genomics screen as some of the components in the broad approach of the Centre for new cancer biomarkers and drug development tools.

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

- Co-PIs J Shi from BU and X Fan from CUHK have continued collaborating outside of this project.
- Co-PI S Ma has been collaborating with overseas Co-PI Hans Clevers on projects pertaining to liver cancer organoids.
- Co-PI T Mak of Princess Margaret Cancer Centre, Toronto and HKU, is collaborating with PC SY Leung and Co-I ASL Wong on immune-oncology studies utilising organoid models.
- PC SY Leung is collaborating with Professor Andy Futreal, MD Anderson Cancer Centre,

on single cell analysis and *in vivo* transcriptomics.

- PC SY Leung is collaborating with Professor MR Stratton, Sanger Institute, on single crypt study of gastric tissue.
- PC SY Leung and Co-I ASL Wong is collaborating with Dr Naoto Hirano, Princess Margaret Cancer Centre, Toronto, on cancer organoid immune cell interactions.
- PC SY Leung is collaborating with Dr Tracy McGaha, Princess Margaret Cancer Centre, Toronto, on cancer organoid macrophage interactions.
- PC SY Leung is collaborating with Dr Clive Chung (School of Biomedical Sciences, The University of Hong Kong) on anti-cancer drug development.
- PC SY Leung is collaborating with Dr David Shih (School of Biomedical Sciences, The University of Hong Kong) on cancer genomics.
- PC SY Leung is collaborating with Dr. Yuanhua Huang (School of Biomedical Sciences, The University of Hong Kong) on single cell genomics.

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.

- PC SY Leung and overseas Co-PI T Mak are now co-directors of the Centre for Oncology and Immunology (COI) Ltd., while Co-I ASL Wong and Dr H Yan are principle investigators at COI. This company aims to continue to explore using organoid models and functional genomic screens as part of the technology platforms for cancer biomarkers and drug development. Financing, in the amount of HK$501 million, for establishing the Centre for Oncology and Immunology was funded by the Innovation and Technology Commission of HKSAR, under the InnoHealth initiative.
- Co-PI J Shi has received an RFS grant in 2021, in the amount of HK$5,155,380, to continue her work on exploring the interaction dynamics of natural killer cells and distinctive gastric tumor organoid subtypes in 3D, which was started during this project. The RFS project title is “Antitumor Dynamics of Natural Killer Cell and Its Response to Immune Checkpoint Inhibitors in 3D Tumor Organoid Models”.
- Co-PI S. Ma received an RGC Research Impact Fund (RIF) for a project titled: Development and applications of a driver-dependent tumor organoid biobank for translational liver cancer research (HK$4.1M).

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	28	9	0	3 pending	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
All parts in section 6.1 that are marked “manuscript in preparation” or “patent pending”. These include: 6.1.2, 6.1.6, 6.1.9, 6.1.11, and 1 publication in 6.1.12.	The information within the specified sections should be restricted from public access, as patent applications and several manuscripts are in progress. Access should only be granted upon the completion of these processes.

12. The Layman's Summary

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

Gastric cancer, also known as stomach cancer, poses a significant global health challenge. As the fifth most common cancer worldwide, it accounts for approximately 1 million new diagnoses annually. Furthermore, gastric cancer is the third leading cause of cancer-related deaths, with nearly 769,000 fatalities each year. In Hong Kong, over 1,000 new stomach cancer cases emerge annually, resulting in more than 700 deaths. Moreover, China faces a particularly high prevalence, with its incidence contributing to almost half of the global cases. Factors such as dietary habits, Helicobacter pylori infections, and genetic predispositions influence the disease's incidence. Although diagnostic and therapeutic advancements have been made, the primary treatment remains surgical removal of the cancer, supplemented by chemotherapy. This approach has limited success for late-stage patients, as early-stage stomach cancer often goes unnoticed due to a lack of symptoms. Consequently, over two-thirds of patients discover their condition at advanced stages, where no effective cure exists. The complexity of stomach cancer lies in its numerous subtypes, each with unique characteristics and genetic mutation combinations. A one-size-fits-all treatment approach proves ineffective, highlighting the urgent need for tailored strategies.

Historically, researchers studied stomach cancer using a few cell lines that were grown flat on plastic dishes, but this did not accurately represent the 3D structure of an actual organ. However, with new technology, our team has been able to grow stomach organoids, which are like miniature 3D stomachs grown in the lab. In this project, our team has successfully created one of the largest biobanks of gastric cancer organoids, containing all known sub-types of gastric cancers and their different mutational profiles. We also performed extensive characterisation of them and used them for drug testing. This ground-breaking study was published in a leading scientific journal, Cell Stem Cell, and has led to subsequent discoveries that could have implications for patient treatment.

Specifically, we utilized the organoids to test a wide range of drugs, including those currently in clinical trials or already approved by the FDA. Since the organoids are grown from the patients' cancer tissue and maintains almost the same morphology, mutations and gene expression patterns, the drugs can be tested on the organoids without harm to the patient, while offering a prediction for how the patient might respond to the drug or drug combinations. Therefore, this biobank of stomach cancer organoids is an immense resource for the scientific community for the biological study of stomach cancer. Several drugs exhibited promising responses, but strikingly, a drug currently approved for treating other cancer type has demonstrated effectiveness in treating a subset of gastric cancer patients with a specific mutation. Analysis of the growth conditions of the organoids also revealed a mechanism that allows gastric cancer cells with *RHOA* mutation or *ARHGAP* fusions to detach from their original location and migrate to new sites. Furthermore, we have discovered a new mechanism that explains how gastric cancer cells can become resistant to conventional chemotherapies and cause patients to relapse. These findings suggest that an organoid drug screening platform could guide patient treatment and clinical trials, accelerating anti-cancer drug development.

Our experience establishing the gastric cancer organoid biobank also paved the way for us to develop other biobanks of interest. We established a colorectal cancer organoid biobank enriched in early onset cases, as well as a biobank of pre-cancerous gastric intestinal metaplasia organoids for studying cancer prevention. Along the way, we have also developed a host of advanced algorithms and web servers to analyse the complex genetic data collected from the organoids. We have also made improvements on existing molecular tools used for

genetic screening. Thus, our project has achieved numerous significant research accomplishments that have contributed greatly to the field of cancer research.

In conclusion, the development of our gastric cancer organoid biobank has opened new avenues for understanding the complexities of stomach cancer and its various subtypes. Our team's breakthroughs in drug testing and the discovery of new treatment options have the potential to revolutionize personalized medicine for gastric cancer patients. Ultimately, our various organoid biobanks, linked genomic and molecular studies, drug screening platform, and analytical tools developed have the potential to significantly advance personalized medicine, leading to better patient care, improved treatment outcomes, and enhanced quality of life for those affected by gastric cancer.