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RESEARCH PROGRESS SUMMARY:**COLORECTAL CANCER****A. Molecular Pathogenesis**

1. Single-cell whole-exome sequencing has been carried out to catalogue somatic mutations in 63 cancer cells isolated from a colon cancer specimen. The mutation spectrum was heterogeneous at the single-cell level. The accumulation of mutations was closely related to tumorigenesis based on population genetic analysis. Among them, we identified a high-frequency mutated gene at the single-cell level, which showed low prevalence in an additional cohort study of colon cancer. Functional characterisation of the mutant gene revealed its potential oncogenic effect in colon cancer.
2. Whole-exome sequencing of 10 colon cancer patients identified a novel recurrent mutation. This mutation was detected in 23 out of 118 patients (20.18%) in the validation cohort. Ectopic expression of this mutant in colon cancer cells increased cell proliferation and colony-forming ability, caused accumulation of cells in S-phase, and enhanced *in vivo* tumorigenicity. The mutant protein also exhibited altered subcellular localisation.

3. A bactericidal factor known as cathelicidin secreted by macrophages, polymorphonuclear leukocytes, and colonocytes was reported to contribute to colon cancer suppression by activating a novel pathway of apoptosis mediated by Apoptosis-Inducing Factor (AIF) and Endonuclease G (EndoG) in colon cancer cells.
4. Hydrogen sulphide, a gaseous bacterial metabolite that reaches high levels in the large intestine, was reported to lower proliferation and induces protective autophagy in colon epithelial cells via activation of adenosine monophosphate-activated protein kinase signalling.
5. We identified that miR-7, a microRNA downregulated in colorectal cancer, suppresses colon tumorigenesis by targeting the oncogenic Yin Yang 1 (YY1).

B. Biomarkers and Cancer Screening

1. Our study identified miR-92a and miR-21 in stool samples as potential screening biomarkers for colorectal cancer and polyps. Patients with colorectal cancer had a significantly higher stool miR-21 level and miR-92a level compared with normal controls. Stool miR-92a, but not miR-21, was significantly higher in patients with polyps than in controls. At a cut-off value of 435 copies/ng of stool RNA, miR-92a had a sensitivity of 71.6% and 56.1% for colorectal cancer and polyp, respectively, and a specificity of 73.3%.

2. Multivariate analysis revealed that patients with YY1 protein high expression had a significant decrease in overall survival.
3. Cap-assisted colonoscopy demonstrated marginal benefit over standard colonoscopy for polyp detection and shortened the caecal intubation time.
4. Siblings of patients with colorectal cancer were found to have a higher prevalence of advanced neoplasms (defined as colorectal cancers or adenomas of at least 10 mm in diameter, high-grade dysplasia, with villous or tubulovillous characteristics) than siblings of healthy individuals. Screening is indicated in this high-risk population.

C. Molecular Therapeutics

1. A fragment of the tumour suppressing peptide LL-37 induces caspase-independent apoptosis and autophagic cell death through the common p53-Bcl-2/Bax cascade in colon cancer cells. Our study also uncovered previously unknown reciprocal regulation between these two cell death pathways.
2. Colitis is associated with increased risk for colorectal cancer. Cathelicidin, a pleiotropic peptide, was found to alleviate experimental colitis in mice. The altered tissue levels of pro-inflammatory cytokines, myeloperoxidase activity, apoptosis, and mucus secretion in mice with colitis can be reversed by intrarectal administration of cathelicidin or cathelicidin-encoding plasmid.



GASTRIC CANCER

A. Molecular Pathogenesis

1. We discovered that major aetiological microorganisms of gastric cancer namely *Helicobacter pylori* (Hp) and Epstein-Barr virus (EBV) cause epigenetic dysregulation to promote carcinogenesis. In particular, forkhead box D3 (FOXD3)-mediated transcriptional control of tumour suppressors is deregulated by Hp infection-induced hypermethylation, which in turn perturbs the balance between cell death and survival. Zinc finger E-box binding factor 1 (ZEB1) was reported as a key mediator of the latent-lytic switch of EBV-associated gastric cancer. FOXD3 and ZEB1 may be potential targets for infection-induced gastric cancer therapy.
2. Using genome-wide methylation screening, we identified potential tumour-suppressor genes that play important roles in gastric carcinogenesis. Silencing of these novel tumour-suppressor genes, previously known as hypothetical proteins such as ring finger protein 180 (*RNF180*), Zinc-finger protein 331 (*ZNF331*) and *ZNF545* was reported to activate growth and anti-apoptotic pathways in gastric cancer. Besides, we demonstrated that genes critical for normal cell differentiation and development e.g. *B cell CLL/lymphoma 6 member B (BCL6B)*, *HOXD10*, *paired box gene 5 (PAX5)*, were involved in gastric cancer progression when aberrantly methylated.
3. Novel epigenetic aberrations were found to occur early in gastric carcinogenesis. For example, promoter methylation of *HOXD10*, *RNF180* and *ZNF545* was detected in 30-60% of intestinal metaplasia, a precancerous lesion of gastric cancer. These findings provide promising new strategies for cancer diagnosis and prevention.

B. Biomarkers and Cancer Screening

1. We demonstrated that hypermethylated tumour-suppressor genes as independent biomarkers for prognosis of gastric cancer patients. For example, methylation of *BCL6B*, *PAX5* and *ZNF545* were associated with shortened survival in different stages of gastric cancer patients.
2. We explored the potential clinical utility of *RNF180* methylation for screening gastric cancer patients. Methylated *RNF180* DNA was detected in the plasma of ~60% of gastric cancer patients, but not in healthy controls.

C. Molecular Therapeutics

In addition to tumour-suppressor gene silencing, we found that up-regulation of novel oncogenes activates key signalling pathways in gastric cancer. Stathmin1 (*STMN1*) is a candidate oncoprotein and prognosis marker in several kinds of cancers. We confirmed that *STMN1* is a putative downstream target of miR-223 in gastric cancer. Our findings revealed that *STMN1* is a potential gastric oncogene and might serve as a therapeutic target for gastric cancer.

LIVER CANCER

A. Molecular Pathogenesis

1. We have previously demonstrated that peroxisome proliferator-activated receptor (*PPAR γ*) activation inhibits hepatocarcinogenesis. We further demonstrated that *PPAR γ* exerts an inhibitory effect on the invasive and metastatic potential of hepatocellular carcinoma (HCC) *in vitro* and *in vivo*, thus representing a target for the prevention and treatment of HCC metastasis.
2. We delineated that miR-139, down-regulated in metastatic HCC cells, suppresses HCC migration by targeting the oncogenic c-fos.

B. Biomarkers and Cancer Screening

The diagnosis of non-alcoholic steatohepatitis (NASH), an emerging risk factor for HCC, is limited by the need for liver biopsy. We tested the accuracy of combined serum biomarkers for the non-invasive diagnosis of NASH. A two-step approach using cytokeratin-18 fragment and fibroblast growth factor 21 was reported to accurately diagnose NASH.

C. Molecular Therapeutics

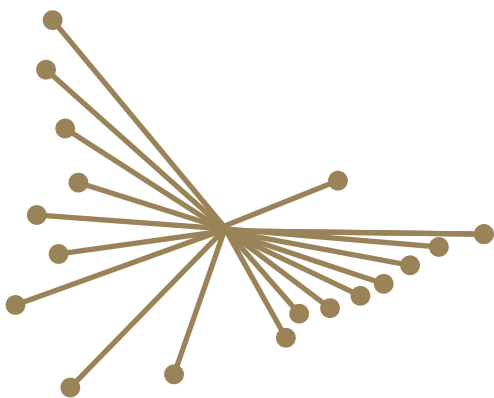
1. For the control of HCC which is more prevalent in obese patients, we are actively engaging in research of NASH therapy. Fuzheng Huayu recipe (FZHY), a compound of Chinese herbal medicine, was reported to improve liver function and fibrosis in patients with hepatitis B virus infection. We demonstrated the protective role of FZHY in ameliorating nutritional fibrosing NASH *in vivo* through regulating key genes related to oxidative stress, inflammation and fibrogenesis.
2. We confirmed the crucial effects of *PPAR γ* agonist in preventing HCC development and spread using pre-clinical models, thus pointing *PPAR γ* as a valid therapeutic target against HCC.



RECOGNITIONS:

AWARDS AND FELLOWSHIPS

Member's Name	Details
Jun Yu, Joseph Sung, Henry Chan, Vincent Wong, et. al.	<ul style="list-style-type: none"> National Science & Technology Progress Award 2012
Jun Yu, Vincent Wong, Henry Chan, Joseph Sung, et. al	<ul style="list-style-type: none"> Scientific and Technological Progress Award, Class 1, of the Higher Education Outstanding Scientific Research Output Awards 2012, The Ministry of Education, China
Jun Yu	<ul style="list-style-type: none"> Visiting Professor, Chinese Academy of Sciences, Guangzhou Institute of Advanced Technology (December 2012 - November 2014)
Francis Chan, Joseph Sung, Philip Chiu	<ul style="list-style-type: none"> Technological Advancement Award, Class 2, of the Higher Education Outstanding Scientific Research Output Awards 2011, The Ministry of Education, China
Francis Chan	<ul style="list-style-type: none"> Professor Richard Yu Medal, Advances in Medicine 2012
Alfred Cheng	<ul style="list-style-type: none"> Research Excellence Award 2011 - 12, The Chinese University of Hong Kong (May 2012) Travel Grant Award for Basic Scientists, United European Gastroenterology Week (October 2012) Oral Free Paper Prize, United European Gastroenterology Week (October 2012)
Vincent Wong	<ul style="list-style-type: none"> Inaugural Frontline Gastroenterology Abstract Prize, Digestive Disease Foundation, United Kingdom (June 2012) Distinguished Research Paper Award for Young Investigators, Hong Kong College of Physicians (October 2012) Emerging Leader Lectureship, Asian Pacific Digestive Week (December 2012)
Simon Ng	<ul style="list-style-type: none"> European Society of Coloproctology (ESCP) Traveling Fellowship to the American Society of Colon and Rectal Surgeons (ASCRS) Annual Scientific Meeting (June 2012) Best Scientific Paper Award of the Conjoint Scientific Congress of The Royal College of Surgeons of Edinburgh and The College of Surgeons of Hong Kong (September 2012)
Philip Chiu	<ul style="list-style-type: none"> First Prize of the World up of Endoscopy, American Society of Gastrointestinal Endoscopy, United States of America Founding Fellow of Wu Yee Sun College, The Chinese University of Hong Kong
Siew Ng	<ul style="list-style-type: none"> Visiting Professor, Shanghai Medical College, Fudan University (July 2012 - July 2014)



GRANTS AND CONSULTANCY

Grants

Details	Member's Name	Amount (HK\$)
<p>Research Grants Council (RGC) General Research Fund (GRF) (2012 - 2015)</p> <p>Title: A double-blind, randomized, placebo controlled trial of misoprostol for healing of small bowel ulcers in aspirin users with small bowel bleeding.</p>	Francis Chan	1,380,000
<p>Health and Medical Research Fund (HMRF) (2012 - 2014)</p> <p>Title: A novel androgen receptor oncogenic circuitry in hepatitis B virus-associated hepatocarcinogenesis.</p>	Alfred Cheng	685,020
<p>SK Yee Medical Foundation (2012 - 2014)</p> <p>Title: Grant for the use of self-expandable-metallic-stents (SEMS) for the relief of upper gastrointestinal obstruction in patients suffering from advanced malignant upper gastrointestinal diseases.</p>	Philip Chiu	241,500
<p>Research Grants Council (RGC) General Research Fund (GRF) (2010 - 2012)</p> <p>Title: Early selective angiographic embolization to severely bleeding peptic ulcers after their initial endoscopic hemostasis – a randomized controlled trial.</p>	James Lau	589,493
<p>Research Fund for the Control of Infectious Diseases (RFCID) (2012 - 2014)</p> <p>Title: Role of quantiferon in monitoring for tuberculosis during anti-TNF therapy in inflammatory bowel disease.</p>	Siew Ng	447,132
<p>Ferring, Hong Kong (2011 - 2013)</p> <p>Title: Asia-pacific Crohn's and colitis epidemiology study (ACCESS).</p>	Siew Ng	342,000
<p>Pfizer Corporation Hong Kong Limited, Hong Kong (2010 - 2013)</p> <p>Title: A phase III placebo-controlled trial of Cclecoxib in genotype positive subjects with familial adenomatous polyposis.</p>	Simon Ng	247,274
<p>Health and Medical Research Fund (HMRF) (2011 - 2012)</p> <p>Title: The application of transcutaneous electric nerve stimulation on acupoints (Acu-TENS) for pain relief during colonoscopy: a prospective, randomized, placebo-controlled study.</p>	Simon Ng	368,490
<p>Research Fund for the Control of Infectious Diseases (RFCID) (2012 - 2014)</p> <p>Title: Liver fibrosis progression in patients with chronic hepatitis B: a prospective study with paired transient elastography examination.</p>	Vincent Wong	891,776
<p>Research Fund for the Control of Infectious Diseases (RFCID) (2012 - 2014)</p> <p>Title: Elucidating the role of autophagy in hepatitis B virus X protein (HBx)-mediated liver inflammation and carcinogenesis.</p>	William Wu	994,040
<p>Research Fund for the Control of Infectious Diseases (RFCID) (2012 - 2013)</p> <p>Title: Functional significance and clinical application of Epstein-Barr virus-driven novel promoter hypermethylated genes (SSTR1, REC8, IL15RA) in gastric cancer.</p>	Jun Yu	998,984



Innovation and Technology Fund, Hong Kong (2012 - 2013) Title: A novel approach for the non-invasive diagnosis of colorectal cancer in stool.	Jun Yu	851,627
Shenzhen Basic Research Program, China (2012 - 2013) Title: Study on expression, function, and clinical application of DACT1 in gastric cancer.	Jun Yu	RMB 80,000
National 863 Program, China (2011 - 2015) Title: 重大疾病的基因組學技術	Jun Yu (Co-I)	RMB 12,000,000
National 863 Program, China (2012 - 2017) Title: 腸道微生態與感染及代謝的研究	Jun Yu (Co-I)	RMB 5,000,000

Consultancy

Member's Name	Details
Henry Chan	<ul style="list-style-type: none"> Advisory Board Member of Abbott, Bristol Myers Squibb, FuRui, Gilead, Merck, Novartis, Roche, Vertex
Vincent Wong	<ul style="list-style-type: none"> Advisor, Gilead Pharmaceuticals
Vincent Wong	<ul style="list-style-type: none"> Honorary Consultant, Regeneration Society, Hong Kong

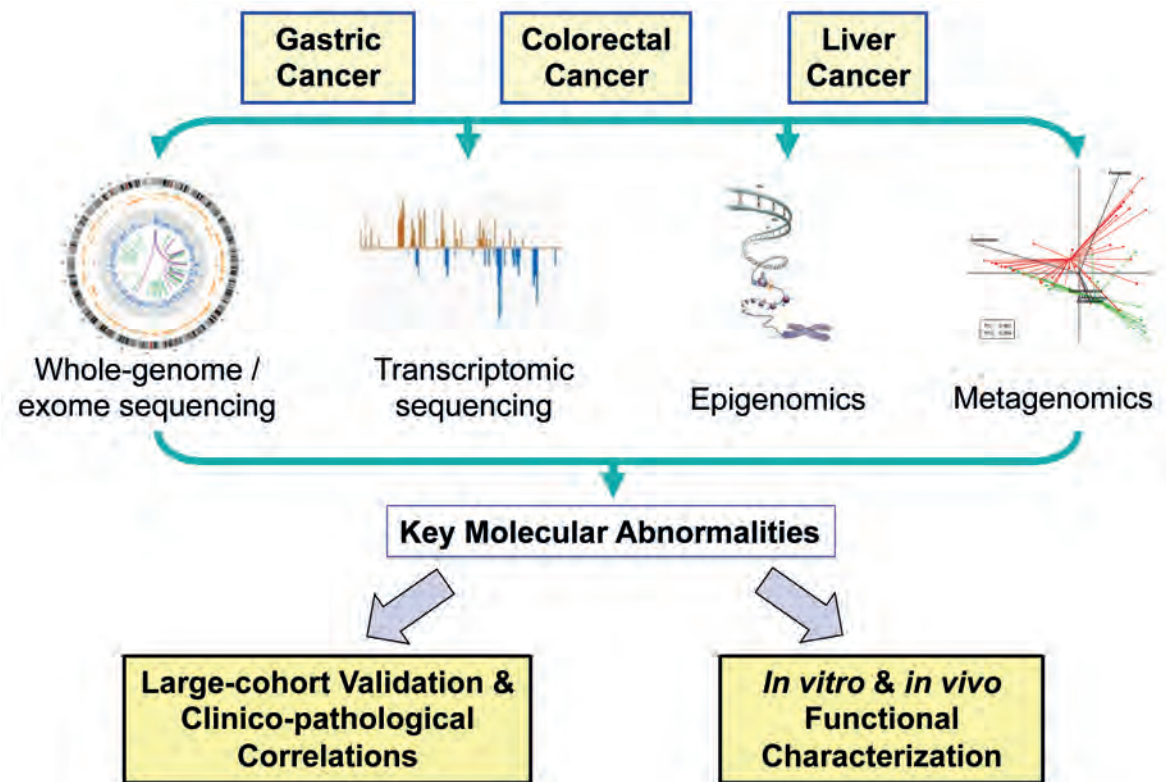


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2. Dong, C. G., Wu, W. K., Feng, S. Y., Wang, X. J., Shao, J. F., & Qiao, J. (2012). Co-inhibition of microRNA-10b and microRNA-21 exerts synergistic inhibition on the proliferation and invasion of human glioma cells. *International Journal of Oncology*, 41(3), 1005-1012.
3. Feng, S. Y., Dong, C. G., Wu, W. K., Wang, X. J., Qiao, J., & Shao, J. F. (2012). Lentiviral expression of anti-microRNAs targeting miR-27a inhibits proliferation and invasiveness of U87 glioma cells. *Molecular Medicine Reports*, 6(2), 275-281.
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6. Klionsky, D. J., Sung, J. J., Wu, W. K., et al. (2012). Guidelines for the use and interpretation of assays for monitoring autophagy. *Autophagy*, 8(4), 445-544.
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11. Lu, D., Wu, Y., Wang, Y., Ren, F., Wang, D., Su, F., Zhang, Y., Yang, X., Jin, G., Hao, X., He, D., Zhai, Y., Irwin, D. M., Hu, J., Sung, J. J., Yu, J., Jia, B., & Chang, Z. (2012). CREPT accelerates tumorigenesis by regulating the transcription of cell-cycle-related genes. *Cancer Cell*, 21(1), 92-104.
12. Lu, J., Yi, L., Zhao, J., Yu, J., Chen, Y., Lin, M. C., Kung, H. F., & He, M. L. (2012). Enterovirus 71 disrupts interferon signaling by reducing the level of interferon receptor 1. *Journal of Virology*, 86(7), 3767-3776.
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Integrative genomic study of major gastrointestinal cancers.

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