SCHOOL OF BIOMEDICAL SCIENCES

Postgraduate Research Day

2022

Lo Kwee-Seong Integrated Biomedical Sciences Building,
Area 39, The Chinese University of Hong Kong

10th to 11th November 2022

SBS Postgraduate Research Day 2022

Faculty of Medicine

The Chinese University of Hong Kong

Welcome Message from the Director of School of Biomedical Sciences

I am most delighted to welcome you all to the *SBS Postgraduate Research Day 2022*. This annual student-organized event provides an opportunity for the postgraduate students to share their research achievements and expose themselves to new ideas from their peers and other researchers.

This year, we have received 73 posters covering research topics of three Thematic Research Programs and it would be displayed on the first day of event. All postgraduate students, except first-year students at our School are encouraged to give a 5-minute poster presentation on the first day of event. 12 presenters will be nominated by judges to compete for the best presentation prize by giving an oral presentation on the second day of event.

To facilitate our students to achieve research and academic excellence, the School has provided state-of-the-art research facilities as well as professors who are dedicated in training future research scientists. We also strive to provide a holistic education to our students by including classes such as bioethics and scientific writing. I certainly believe that this annual Postgraduate Research Day offers the best opportunity to sharpen their other attributes such as leadership, communication, organization and social skills. And I am sure you will be impressed by the achievement of our students.

With this two-day event, no matter whether you are a first-year student or a final year student busying with your thesis work, I do hope you can gain from the Postgraduate Research Day either as a presenter or a participant. Your active participation through your insightful comments or suggestions will further strengthen the collaborative and sharing spirit among School members.

Organizing such an event is not an easy task. So, I would like to take this opportunity to thank the Organizing Committee and all individuals who are involved in various aspect of organizing this event. Also, I would like to extend my heartfelt gratitude to Prof. Woody Chan, Prof. Zhao Hui and the Graduate Education Office for their continued support to our various postgraduate education missions.

On behalf of the School of Biomedical Sciences, I wish you all a very successful *Postgraduate Research Day 2022*.

Andrew M. Chan, PhD
Professor and Director
School of Biomedical Sciences

Members of the Organizing Committee

President: Ms. He Borong

External Vice Presidents: Mr. LIT Kwok Keung

Internal Vice Presidents: Ms. Gao Ming

Secretary: Mr. Zhang Ruoyu

Publicity Secretary: Ms. Zhu Yao

Treasurer: Mr. Brian Anugerah Urip

Member: Mr. Peter Choi

Advisers:

Prof. CHAN Wood Yee Woody

Ms. OU Fanghong

Special Acknowledgements:

Ms. CHAN Mei Tak Mandy

Ms. LAU Liu Yin Carmen

Ms. NG Sui Ching Nicole

Ms. KWAN Mei Nui Becky

Ms. CHING Tak Man Janis

Ms. LAI Chi Yung Lili

Mr. CHUNG Yiu Wa

Acknowledgements

The organizing committee would like to thank the following professors for serving as adjudicators of Poster and/or Oral presentation:

Prof. BLOCKI Anna Maria	Prof. KER Dai Fei Elmer
Prof. CHAN Hon Fai Vivas	Prof. KO Wing Hung
Prof. CHAN Leung Franky	Prof. KWAN Yiu Wa
Prof. CHAN Man Lok Andrew	Prof. LIN Ge
Prof. CHAN Wood Yee Woody	Prof. POON Ngar Yun Ellen
Prof. CHEN Yang Chao	Prof. SHUM Sau Wun Alisa
Prof. CHENG Sze Lok Alfred	Prof. SO Kam Hei Karl
Prof. CHEUNG Chi Kwan Vincent	Prof. TIAN Xiao Yu
Prof. CHEUNG Hoi Hung Albert	Prof. TONG Man Carol
Prof. FOK Kin Lam Ellis	Prof. WANG Dan Michelle
Prof. GU Shen Linda	Prof. WANG Wu Ming
Prof. HUI Xiao Yan Hannah	Prof. XIA Yin
Prof. IP Pak Kan Jacque	Prof. YAO Xiao Qiang
Prof. JIANG Yangzi	Prof. ZHAO Hui

Rundown of Postgraduate Research Day 2022
School of Biomedical Sciences (SBS)

November 10th (Thursday – Day 1)		
9:00	**Registration (Research Common, G/F, SBS, Area 39)**	
09:15 – 10:00	**Opening Ceremony (Room G02, SBS)**	
09:15 – 09:20	Introduction (special thanks to VIPs and Guests)	
09:20 – 09:35	Opening remarks by Professor CHAN Wai Yee, Pro-Vice-Chancellor	
09:35 – 09:50	Welcome Speech by Professor CHAN Man Lok, School Director	
09:50 – 10:00	Group photos	
10:00 – 11:10	**Posters CBET 1-10(Venue #1)**	**Posters DRB 1-10 (Venue #2)**
	Break (20 minutes)	
11:30 – 12:40	**Posters CBET 11-21 (Venue #1)**	**Posters DRB 11-20 (Venue #2)**
	Lunch Box	
14:00 – 15:10	**Posters NVMB 1-9 (Venue #1)**	**Posters DRB 21-30 (Venue #2)**
	Break (20 minutes)	
15:30 – 16:40	**Posters NVMB 10-17 (Venue #1)**	**Posters DRB 31-35 (Venue #2)**
November 11th (Friday – Day 2)		
09:00 – 9:10	**Brief introduction: rules of oral presentation (Room G02)**	
09:10 – 10:40	**Oral Presentation No. 1-6**	
	Break (10 minutes)	
10:50 – 12:35	**Oral Presentation No. 7-13**	
	Break (10 minutes)	
12:45 – 12:55	**Prize Presentation Ceremony and group photos (Room G02)**	
	Lunch Box	

Venue #1: Room G01, LKSIBSB **Venue #2: G02A, LKSIBSB**

Map of the Meeting Venue

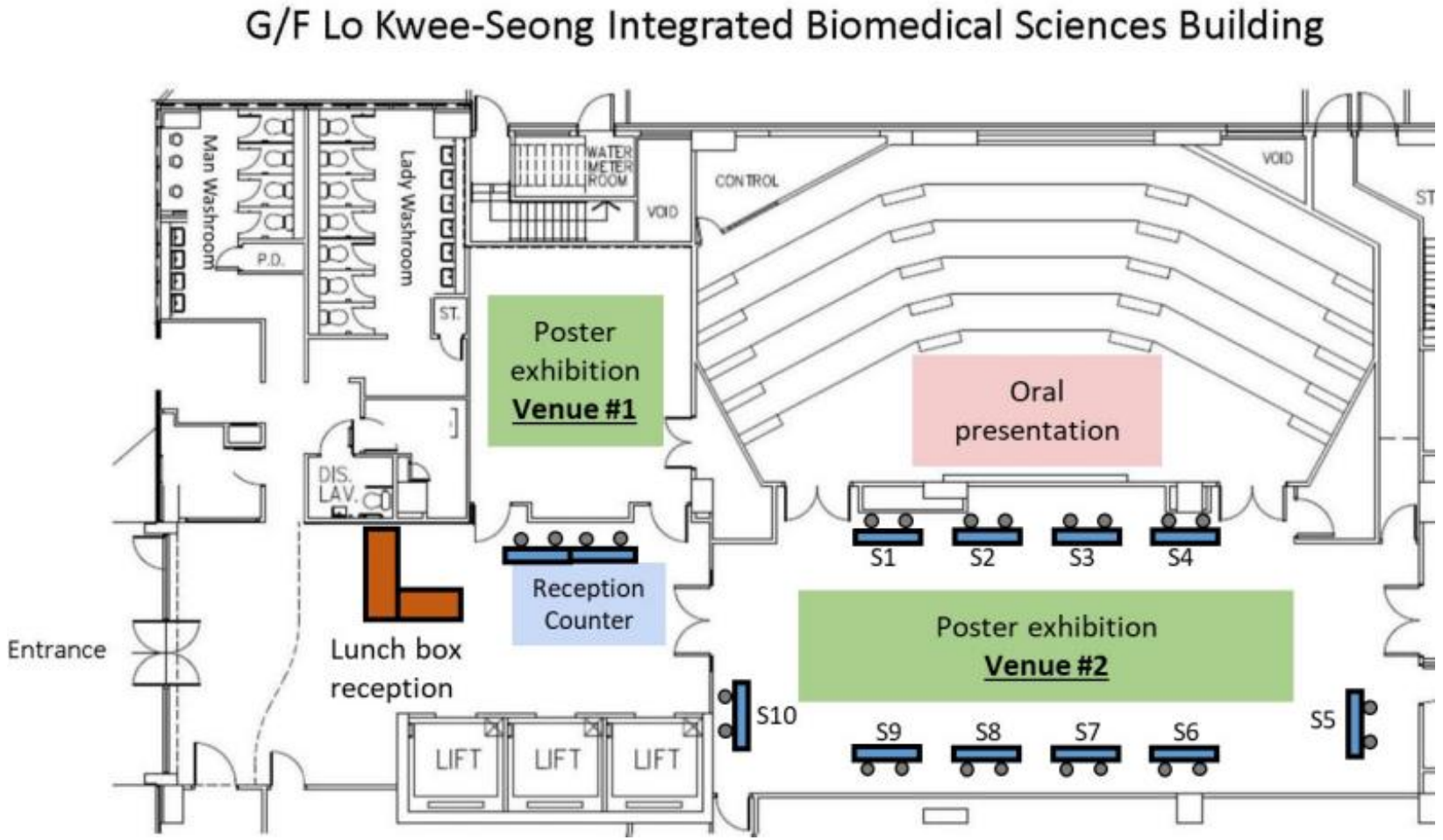
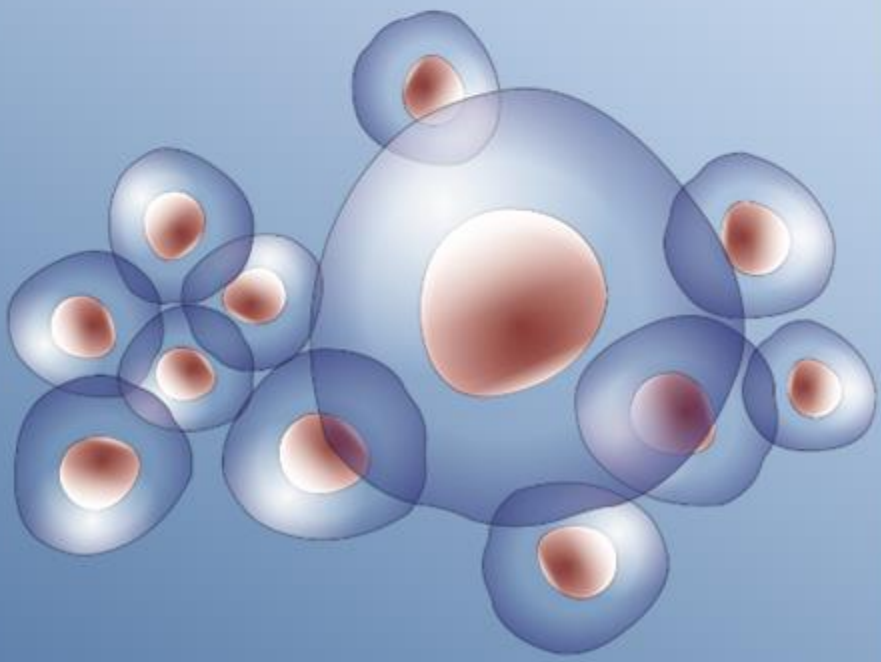


Table of Contents

SCHOOL OF BIOMEDICAL SCIENCES

Postgraduate Research Day 2022

Cancer Biology and Experimental Therapeutics (CBET)



Cancer Biology and Experimental Therapeutics

Title	Author	Number
Genome Assembly and Annotation of Flathead Grey Mullet (Mugil cephalus) for studying fish allergy and evolution	AO Fu Kiu	CBET1
RAC1 aberrations in head and neck squamous cell carcinoma (HNSCC) affect tumor immune microenvironments	Chan Hoi Yin	CBET2
The role of Th2 in immune surveillance of NAFLD-associated liver metastasis	CHAN Ting Hei Thomas	CBET3
Epigenetic Remodeling of Endothelial Cells underlying Immune Checkpoint Resistance in Hepatocellular Carcinoma	CHEN Shu Fen	CBET4
A study of circadian clock-associated transcription factors and nuclear receptors in prostate cancer	CHOPRA Ria	CBET5
Characterisation of gastrointestinal (GI) motility in zebrafish larvae: establishment of a drug analytical tool to monitor diabetes drug-induced side effects on GI.	HUI Chung Man Jessica	CBET6
Contribution of disrupted gastrointestinal myoelectrical activity to mechanisms of cisplatin-induced acute and delayed nausea and emesis	KHALID Aleena	CBET7
Comparative Mitochondrial Genome and Phylogenetic Analysis of Brain-eating Amoeba: Balamuthia mandrillaris	LAW Cherie Tsz Yiu	CBET8
Targeting BRAF mutations in head and neck cancer	LAW Chun Ho	CBET9
Tumor cell-derived lipids modulate macrophage activity to regulate immunotherapy resistance of hepatocellular carcinoma	LIANG Zhixian	CBET10
Toll-like receptor 4 is involved in morphine-induced nausea and emesis in Suncus murinus (House Musk Shrew)	LIU Luping	CBET11
Molecular mechanism underlying KRAS regulation on STK31 expression in Pancreatic ductal adenocarcinoma	Liu Yuting	CBET12
Modulation of autophagy affects pyrrolizidine alkaloid-induced liver injury	PAN Yueyang	CBET13
The role of circular RNAs on pancreatic cancer cells ferroptosis	PENA Jessica Jazmin	CBET14
The whole genome sequencing and hybrid assembly of black carp (M.piceus) and bighead carp (H.nobilis) and phylogenomic analysis of Cyprinid fishes	Shi Ling	CBET15
Decoding cancer associated fibroblasts in immunotherapy outcomes of hepatocellular carcinoma patients by single-cell RNA sequencing	WONG Patrick Pak Chun	CBET16
Decoding cancer immunotherapy resistance by single-cell multi-omics analysis	WU Haoran	CBET17
Effect of Centrally Administered Ghrelin on Cisplatin-Induced Emesis in Suncus murinus	YANG Lingqing	CBET18

Deciphering the cellular and molecular determinants of immunotherapy resistance in NASH-associated hepatocellular carcinoma by single-cell analysis	ZHANG Lingyun	CBET19
Beta 2 adrenergic receptor (β2-AR): a potential target to inhibit liver metastasis by disrupting the immune suppressive microenvironment	ZHANG Yi Xuan	CBET20
Deletion of 11q curbs ETS1 expression to inhibit cell growth through destabilizing MYCN in neuroblastoma	ZHOU Liangji	CBET21

CBET1

Genome assembly and annotation of Flathead Grey Mullet (Mugil cephalus) for studying fish allergy and evolution

AO Fu Kiu

Supervisor: TSUI Kwok Wing Stephen

Mugil (M.) cephalus, commonly referred to as flathead grey mullet, is one of the major sources of fish in the daily consumption of humans worldwide, including fish meat and delicacies of mullet roe. They primarily occur in brackish water sites, such as river deltas, where freshwater and seawater meet. Their survival in such a varying level of salinity demonstrated their ability in adaptation. On the other hand, despite the ongoing research on fish allergy with the protein parvalbumin, fish allergies resulting from M. cephalus are rarely studied, which may underpin the shortcomings of the current fish allergy diagnosis using skin prick tests.

A mix of next-generation sequencing and the third generation Nanopore sequencing was adopted to sequence the genome of M. cephalus, with an estimated genome coverage of 147.5x and 48.0x respectively. The assembled M. cephalus genome has a size of 658,518,483 bp with 2,093 scaffolds (N50 = 9,224,614 bp) and 2,129 contigs (N50 = 6,885,576). The high-quality genome for M. cephalus also had a high BUSCO completeness (98.1%) from the Actinopterygii dataset (odb10) searching for 3,640 BUSCO groups. The study was followed by annotations of various genome components such as genes and repeat contents.

The results have provided essential data for studying study the allergen profile of the M. cephalus by mapping to the established food allergen groups in Animalia Chordata from the WHO/IUIS allergen nomenclature database, which will facilitate the ongoing research on the diagnosis and treatment of fish allergy. Comparative analysis between the Mugilidae family is also possible for studying the evolution of M. cephalus and elucidating its allergenicity. The study can also act as a starting point to investigate the adaptation of brackish water species and their genome characteristics for surviving in this habitat.

CBET2

RAC1 aberrations in head and neck squamous cell carcinoma (HNSCC) affect tumor immune microenvironments

CHAN Hoi Yin Helen, LIU Yuchen, LAM Ngan Hoi, LUI Wai Yan Vivian

Supervisor: TSUI Kwok Wing Stephen

RAC1 is a Rho GTPase well-identified as an oncogene overexpressed in various cancer types. RAC1 amplification or gain account for ~40% of head and neck squamous cell carcinoma (HNSCC) while mutation of RAC1 occurs at ~3% according to TCGA-HNSCC cohort. HNSCC patients with RAC1 aberrations, including RAC1 mutations and amplification or gain, are associated with poor overall and disease-free survival in HNSCC. Significantly higher tumor mutational burden is observed in RAC1-mutated group ($p=0.0336$) or in RAC1-amplified/gain group ($p=0.0109$) vs. wildtype patients in TCGA-HNSCC cohort. As increases in TMB are often associated with immune cell infiltration in cancers, we hypothesized that RAC1 aberrations impact the tumor immune microenvironment (TIME) of HNSCC, potentially contributing to disease progression.

PD-L1 (CD274) is an important immunosuppressive immune checkpoint molecule expressed in tumor cells. Here, we first reported that ectopic overexpression of RAC1 p.P29S and p.A159V mutations in a human HNSCC cell line (PECAPJ41 clone D2, ATCC, USA) could cause upregulation of PD-L1 at both mRNA (by RNA-seq) and protein levels (by Western blotting), first demonstrating a direct effect of RAC1 mutations on potential HNSCC TIME modulation via PD-L1. Furthermore, by tumor immune estimation resources (TIMER) analysis, we found that RAC1-mutated tumors have significantly higher level of neutrophils immune infiltration of 38.63% ($p=0.027$) compared to the wildtype tumors. From the CIBERSORTx analysis, M2 Macrophages was significantly increased by 40.42% in RAC1-mutated tumors vs wildtype in the TCGA-HNSCC cohort ($p=0.031$). We also validated this finding by immunofluorescent staining of PD-L1 and M2 Macrophages in immunocompetent mouse HNSCC xenografts models expressing RAC1 p.P29S and p.A159V mutations as compared to controls.

Our in silico, in vitro and in vivo findings first uncover an important role of RAC1 aberrations in HNSCC TIME immunosuppression by regulating PD-L1 expression, neutrophil and M2 macrophages infiltrations. Clinical activities of PD-L1 inhibitors in RAC1-mutated HNSCC patients worth future investigations.

This research is funded by the General Research Fund, Research Grant Council, Hong Kong (#14168517).

CBET3

The role of Th2 in immune surveillance of NAFLD-associated liver metastasis

CHAN Ting Hei Thomas[1]

Supervisor: ZHOU Jingying[1]

[1]School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, China

Non-alcoholic fatty liver disease (NAFLD) is an increasing global epidemic associated with chronic inflammation, metabolic disorders, immunosuppression and importantly, increased incidence of primary and metastatic liver cancers. We have recently demonstrated that NALFD-associated fibrosis and increased cholesterol could promote the expansion and immunosuppressive function of myeloid derived suppressor cell and dysregulate anti-tumor natural killer T cells, resulting in aggressive tumorigenesis and immunotherapy resistance of primary liver cancer. Targeting the liver immunosuppressive microenvironment could significantly restore immune surveillance and potentiate tumor eradication by immune-checkpoint blockade therapy. Given the importance of metabolic and immune imbalance in the formation of metastatic-prone liver microenvironment, we hypothesise that the immunosuppressive nature of NAFLD may also contribute to the liver tropism of cancer metastasis.

As an immunologically complex organ, the liver has a large number of resident and infiltrating immune cells that may be regulated in NAFLD. To dissect the cellular complexity of NAFLD livers, we analysed bulk and single-cell RNA-sequencing (scRNA-seq) datasets from mice and patients with NAFLD. Compared to healthy liver controls, our analysis pinpointed that the CD4+ T helper-2 (Th2) population and its master transcription factor GATA3 were remarkably reduced in NAFLD. Using a high-fed-high-carbohydrate-diet induced NAFLD mouse model, we observed increased hepatic metastasis of colorectal cancer cells via tail vein injection compared to control-diet-fed mice. Of note, hepatic Th2 cells were significantly downregulated and negatively correlated with cholesterol levels. In parallel, consistent reduction of Th2 cell and GATA3 score were also observed in livers with metastatic tumour compared to tumour-free livers from scRNA-seq analysis. Hence, we aim to further delineate the metabolic and molecular mechanisms regulating Th2 cells in NAFLD and NAFLD-associated liver metastasis, which may provide new perspective and therapeutic approaches in targeting the immunometabolic liver microenvironment.

CBET4

Epigenetic remodeling of endothelial cells underlying immune checkpoint resistance in hepatocellular carcinoma

Shufen CHEN[1], Xiaoyu LIU[1], Haoran WU[1], Jianquan CAO[1], Zhewen XIONG[1], Stephen CHAN[2], Alfred Sze-Lok CHENG[1]

Supervisor: CHENG Sze-Lok Alfred

[1]*School of Biomedical Sciences, The Chinese University of Hong Kong,*

[2]*Department of Clinical Oncology, The Chinese University of Hong Kong*

Although immune checkpoint blockade (ICB) has shown promise in cancers, only a modest proportion of hepatocellular carcinoma (HCC) patients showed desirable and durable responses. The recent success of combining ICB and anti-vascular endothelial growth factor (VEGF) as the first-line treatment for advanced-stage HCC highlights the great potential of endothelial cell (EC) normalization in enhancing immunotherapy response. However, around 70% HCC patients still do not benefit from this combinatory regimen. Epigenetic regulation by bromodomain-containing protein 4 (BRD4) plays critical roles in cell differentiation implicated in cancer progression. Therefore, we aim to investigate the epigenetic remodeling of EC underlying immune checkpoint resistance in HCC and explore the therapeutic approach for enhancing HCC immunotherapy. Single-cell RNA sequencing (scRNA-seq) was performed on Phase II clinical trial of pembrolizumab (anti-Programmed cell death 1(PD-1)) in hepatitis B virus (HBV)-related HCC patients (NCT03419481). ICB-resistant HCC mouse model via repeated selection was used to investigate the underlying mechanisms. scRNA-seq analysis and immunofluorescence staining were conducted for distinct EC proportions and BRD4 expression analysis. Immune profiles were determined by high-dimensional flow cytometry. We found that macrovascular-like endothelial cell (MaVEC) highly expressing disorganization signatures was associated with ICB resistance in HCC patients and mouse model. Interestingly, MaVEC-intrinsic BRD4, an epigenetic reader, was related to ICB non-responsiveness in HCC patients. More importantly, a clinically-trialed BRD4 specific inhibitor AZD5153 could suppress HCC development and ameliorate the immunosuppressive microenvironment, which was accompanied by reversal of the normal liver endothelial cell (LEC) to MaVEC transdifferentiation in ICB-resistant HCC mice. Mechanistically, BRD4/activator protein-1 axis in MaVEC may be a potential molecular pathway contributing to HCC ICB resistance. To conclude, our data demonstrated that MaVEC-intrinsic BRD4 may contribute to ICB resistance and epigenetic regulation of LEC-to MaVEC transdifferentiation. Targeting BRD4 may be a readily-translatable combinatory immuno-epigenetic strategy to overcome ICB resistance in HCC. (Funded by GRF (14120621) & AstraZeneca)

CBET5

A study of circadian clock-associated transcription factors and nuclear receptors in prostate cancer

CHOPRA Ria

Supervisor: CHAN Leung Franky

Prostate cancer (PCa) is the most common hormone-related male cancer in many developed countries including China and Hong Kong. Genetic drivers involve TMPRSS2-ERG fusion, together with the amplification, mutations and deletion of MYC oncogene, PTEN and TP53 genes and androgen receptor (AR). After prostatectomy and androgen-deprivation therapy (ADT), patients mostly develop resistance to ADT followed by progression to metastatic clinical phenotypes such as castration-resistant prostate cancer (CRPC) and lethal neuroendocrine prostate cancer (NEPC). In emerging reports, disruption of the circadian clock, that functions to sustain the normal rhythmic cycles of expression of core circadian-controlled genes (CCCG) in order to regulate numerous biological and physiological activities, is identified as a risk factor for cancers. Disrupted neuroendocrine-pituitary-gonadal axis contributes to uncontrolled release of androgens and hence promotion of prostate cancer. Our preliminary results identify that a significant number of these CCCG and nuclear receptors (NRs) display differential expression patterns in CRPC and NEPC tumours. Furthermore, we observed differential rhythmic expression patterns in normal mouse prostate tissue and our transgenic mouse adenocarcinoma prostate cancer model (TGMAP) over the 12 hours light/dark cycle. We hypothesize that some of these CCCG and NRs display differential expression patterns in prostate cancer models and play respective roles in the pathogenesis of advanced prostate cancer. The present study aims to determine the significant differential expression patterns of CCCG and NRs in normal prostate and TGMAP models followed by validation in clinically relevant CRPC models.

CBET6

Characterisation of gastrointestinal (GI) motility in zebrafish larvae: establishment of a drug analytical tool to monitor diabetes drug-induced side effects on GI

HUI Chung Man Jessica[1], RUDD John[1], DU Peng[2], LIU Yuen Hang Julia[1]

Supervisor: RUDD Anthony John

[1]*School of Biomedical Science, The Chinese University of Hong Kong,*

[2]*Bioengineering Institute, University of Auckland, New Zealand*

Background: Zebrafish, *Danio rerio,* have been an established animal model in biomedical studies, and share about 70% similarity with the human genome. Larvae are transparent providing opportunities for gastrointestinal (GI) studies, *in vivo*. We previously used a standardised diet to characterize gastric motility in zebrafish larvae at 7 days post fertilization (dpf). In the present studies, we aim to investigate motility differences in normal and diabetic larvae and to establish a drug testing protocol for the detection GI effects. Exendin-4, a selective GLP-1 receptor agonist, and tirzepatide, a dual GLP-1/GIP receptor agonist, are used clinically and have been reported to be associated with reduced gastric emptying. However, the action mechanism is unclear. Hence, in this study will use advanced imaging techniques of the entire GI tract to further characterise the mechanisms involved.

Methodology: Before imaging with light microscopy, larvae were anaesthetised with 100 mg/L Ms-222. Exendin-4 ($1.5x10^5$ – 1.5 pmol) or tirzepatide ($1.5x10^5$ – 1.5 pmol) or vehicle (0.01% DMSO or saline) were microinjected directly into the upper GI tract. Domperidone (0.015 – 1.5 pmol) and atropine (0.15 – 4.5 g per larvae) were used as reference prokinetic and constipating drugs, respectfully. Motility was quantified by pixel differences to track displacement along a one-dimensional centreline of the fore-gut, mid-gut and hind-gut.

Results: Domperidone at high doses (1.5 & 0.15 pmol) increased velocity in all GI regions. Atropine decreased GI contractions and velocity (mainly in the mid- and hindgut). Motility analysis showed differences in peristaltic activity between the diabetic models, where HFD exhibited increased contractions and faster velocity while SDG showed a reduced motility indicating subtypes of GI dysfunction between the diabetic models. Exendin-4 decreased counts and velocity at higher concentrations (0.0015 – 1.5 pmol) especially at the mid- and posterior gut. Tirzepatide affected the mid-gut at 0.015 pmol with increased retrograde velocity. **Conclusion**: A GI motility analytical protocol using zebrafish larvae was established and validated. The larvae models showed differential motility profiles confirming the existence of sub-types of GI complications in diabetes. The action of exendin-4 in the larvae were similar to the reported GI side-effects of GLP-1 receptor agonists in patients. Studies using tirzepatide revealed reduced gastric emptying which may also involve retroperistalsis of the mid-gut.

CBET7

Contribution of disrupted gastrointestinal myoelectrical activity to mechanisms of cisplatin-induced acute and delayed nausea and emesis

KHALID Aleena[1], LU Zengbing[1], NGAN Man Piu[1], RUDD John Anthony[1,2]

Supervisor: RUDD Anthony John

1School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, China. [2]Laboratory Animal Services Centre, The Chinese University of Hong Kong, Shatin, Hong Kong, China.

Background: Current anti-emetic guidelines for the treatment of chemotherapy-induced emesis include the use of 5-HT3 and NK1 receptor antagonists in combination with a glucocorticoid. Unfortunately, approximately 20% of patients remain unprotected. Our previous study revealed that cisplatin disrupted gastrointestinal slow wave signal shape (SWSS). In the present study, we evaluate if the NK1 receptor antagonist, netupitant and the glucocorticoid, dexamethasone, can prevent emesis and cisplatin-induced SWSS disruption in ferrets. **Method**: Ferrets were surgically implanted with radio-telemetric transmitters to record gastric myoelectric activity, blood pressure (BP) and core body temperature (CBT). Respiratory parameters were recorded by whole body plethysmography. 24h baseline recordings were obtained prior to the administration of netupitant (3 mg/kg, p.o.) or dexamethasone (1 mg/kg, i.p.) followed by cisplatin (5 mg/kg, i.p.). Recordings proceeded for a further 72-hour period. **Results**: Cisplatin induced acute and delayed emetic response and decreased body weight, food, and water intake. There was an associated increase in slow wave dominant frequency (DF), with a decrease in tachygastria and increase in normogastria. Cisplatin increased systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) with an increase in CBT during acute phase. In contrast, Dexamethasone prevented cisplatin-induced reduction in body weight, food and water intake and alleviated emesis. DF was reduced, tachygastria increased and normogastria decreased with overall increase in bradygastria. Dexamethasone elevated SBP and DBP, but reduced cisplatin-induced changes in HR. It antagonised cisplatin-induced increase in CBT. Netupitant failed to modify cisplatin-induced elevation in DF and normogastria, but significantly increased tachygastria during the acute phase. Conversely, netupitant prevented cisplatin-induced changes in SBP, DBP and HR in the delayed phase. Netupitant had no effect on cisplatin-induced increase in CBT but alleviated emesis in the delayed phase. **Conclusion**: This study showed that dexamethasone restored cisplatin-induced disruptions in SWSS. Conversely, netupitant was inactive. Nevertheless, both anti-emetics successfully antagonised cisplatin-induced emesis in the delayed phase. It is possible that the slow wave data may have relevance to mechanisms involved in nausea, which requires further investigation. The study was supported by RGC grant (14121520).

CBET8

Comparative mitochondrial genome and phylogenetic analysis of brain-eating amoeba: Balamuthia mandrillaris

LAW Cherie Tsz Yiu

Supervisor: TSUI Kwok Wing Stephen

B. mandrillaris is a free-living amoeba that causes granulomatous amoebic encephalitis (GAE), which is rare yet often fatal and has no available efficacious treatment. The diagnosis of this disease is usually delayed and postmortem due to its rarity and non-specific presentations. Only a few genomic data on B. mandrillaris are available and the data were mostly obtained from patients in geographic regions besides Asia. Genetic diversity of B. mandrillaris has been previously shown among strains uncovered from different geographic regions but the data is still limited. In this study, we isolated a new strain of B. mandrillaris named KM-20 from the brain tissue of a Thai patient diagnosed with GAE, the mitochondrial genome of KM-20 was de novo assembled and annotated. Phylogenetic analysis and syntenic comparisons of the linear chromosomal maps of KM-20 and nine other published strains were performed. The result showed the mitochondrial genomes of B. mandrillaris are significantly diverse among strains. We identified an array of protein tandem repeats in the ribosomal protein S3 (rps3) gene contributing to the diversification and the repeat number is polymorphic among strains. The KM-20 has the most variable and highest number of repeating units in rps3 among ten strains. The number of repeating units characterizes different strains and can be a target for clinical genotyping assay and strain detection. Moreover, two genotypes of rps3 in strain V039 were observed, and the difference arises from two extra repeating units, suggesting mitochondrial heteroplasmy in B. mandrillaris. This study unveils a range of mitochondrial genomic variations among B. mandrillaris strains, and the mitochondrial diversification mainly arises from rps3. The discovery of the protein tandem repeats in rps3 now paves the way to investigate the sequence variation in this region among strains and explore its functional role.

CBET9

Targeting BRAF mutations in head and neck cancer

LAW Chun Ho, CHAN Leung Franky, LUI Wai Yan Vivian

Supervisor: CHAN Leung Franky

Mutations of the mitogen-activating protein kinase (MAPK) pathway components are found in 18% of head and neck cancer (HNC). Among these, mutations of the serine/threonine-protein kinase B-Raf (BRAF gene) account for about 1.8% of HNC (based on TCGA-HNSCC cohort, USA). Serving as one of the key kinase members of the MAPK pathway, BRAF is considered as a precision drug target in various cancer types, including melanoma, thyroid cancer, and non-small cell lung cancer (NSCLC). While the significance of BRAF mutations remains underexplored in HNC, exceptionally favorable responses to BRAF and MEK inhibition had been reported in HNC patients carrying BRAF-mutations. Based on this, we hypothesize that BRAF mutations may confer drug sensitivity and serve as a predictive biomarker for personalized therapy in HNC. Here, we demonstrated that ectopic overexpression of mutated BRAF resulted in MAPK pathway activation in HNC cell models. We also showed that ectopic BRAFV600E could promote the in vitro growth of head and neck cancer cells in both 2D and 3D culture conditions, as well as enhance anoikis resistance, which is a prerequisite for metastasis. Further preliminary results showed that the BRAFV600E mutant could promote the invasiveness of HNC cells. In summary, our findings support that targeting the BRAF mutants could be a potential therapeutic approach or precision treatment for HNC.

CBET10

Tumor cell-derived lipids modulate macrophage activity to regulate immunotherapy resistance of hepatocellular carcinoma

Zhixian Liang[1], Jianquan Cao[1], Wenshu Tang[1], Haoran Wu[1], Yalin Tu[1], Zhewen Xiong[1], Lingyun Zhang[1], Jingying Zhou[1], Stephen L. Chan[2], Alfred S.L. Cheng[1]

Supervisor: CHENG Sze-Lok Alfred

[1]*School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, China.*

[2]*Department of Clinical Oncology, The Chinese University of Hong Kong, Hong Kong, China*

Immune-checkpoint blockade (ICB) therapies have transformed the treatment landscapes of hepatocellular carcinoma (HCC). However, the immunosuppressive tumor microenvironment (TME) constructed by tumor cells restricts the responsiveness of ICB therapies to a minority of patients. Triggering receptor expressed on myeloid cells-2 (TREM2) counteracts inflammation and maintains metabolic fitness in myeloid cells. Single-cell RNA-sequencing analysis of our pembrolizumab study (NCT03419481) identified a subset of tumor-associated macrophages over-expressing TREM2 in non-responders. Consistently, our syngeneic ICB-resistant HCC mouse models verified a group of Trem2+ myeloid cells, which were accumulated in the lipid-rich TME of ICB-resistant tumors. Compared to the parental ICB-sensitive tumor cells, the conditional medium (CM) of ICB-resistant tumor cells significantly increased Trem2 expression in macrophages, which was abolished when lipids in the CM were depleted. Notably, lentivirus-mediated Trem2 ablation reduced lipid accumulation in TME and overcame anti-PD-1 resistance with increased cytotoxic CD8+T cell infiltration. In conclusion, our study highlights TREM2 as an immuno-metabolic target to enhance HCC immunotherapy.

Acknowledgement

This study is supported by Collaborative Research Fund (C4045-18W) and Li Ka Shing Foundation. Z.L. is supported by Hong Kong PhD Fellowship Scheme.

CBET11

Toll-like receptor 4 is involved in morphine-induced nausea and emesis in Suncus murinus (House Musk Shrew)

Luping LIU[1], Zengbing LU[1], Yuen Hang Julia LIU[1], Man Piu NGAN[1], John A. RUDD[1]

Supervisor: RUDD Anthony John

[1]*School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, China*

Background: Opioids rank among the most potent analgesic drugs but have several side effects, including nausea and emesis, which limits their therapeutic utility. Some of the adverse effects of opioids are mediated via opioid receptors, but emerging evidence suggests that opioids also non-stereoselectively activate Toll-like receptor 4 (TLR4) and its signalling pathway. Previous studies concluded that morphine does not induce emesis in Suncus murinus. However, the previous studies used a wide-dose range and may have missed its emetic potential. In the present studies, we carefully evaluate the action of morphine to induce emesis and associated behavioural and physiological changes in animals implanted with radiotelemetry devices. **Methods**: Male Suncus murinus implanted with radiotelemetry devices. One week later, they were injected with morphine (0.01-10 mg/kg, s.c.) and placed into whole-body plethysmography chambers to permit a simultaneous recording of behaviour and respiratory function with gastric myoelectric activity (GMA) and temperature recordings using radiotelemetry. In other studies, isolated intestinal segments and electrical field stimulation (EFS) were used to characterize the potency of opioids at opioid receptors and/ or TLR4.

Results: The behavioural tests showed subcutaneous injection of morphine induced retching and emesis with a bell-shaped dose-response curve; the optimal dose was 0.3 mg/kg. In contrast, morphine dose-dependently elevated food intake and body temperature. Morphine (0.3-3 mg/kg) increased the dominant frequency (DF) of slow waves but did not change the percentage power of bradygastria, normogastria, or tachygastria. None of the doses of morphine affected respiratory function. The organ bath studies showed morphine inhibited EFS-induced contraction of isolated ileum and distal colon. The action of morphine on these tissues was antagonised by the opioid receptor antagonist naloxone and the TLR4 antagonist resatorvid.

Conclusion: Morphine induces emesis in Suncus murinus and causes behavioural modification consistent with some its known actions at opioid receptors/ TLR4. The in vitro studies provide evidence for an action of morphine at opioid receptors and TLR4.

CBET12

Molecular mechanism underlying KRAS regulation on STK31 expression in Pancreatic ductal adenocarcinoma

LIU Yuting

Supervisor: CHEN Yangchao

KRAS is the prevalent and central oncogenic driver in Pancreatic ductal adenocarcinoma (PDAC). Mutant KRAS caused activation of downstream signaling, thus contributing to tumor formation. Kinase inhibitors are ideal targeted therapeutics for mutant KRAS-driven cancer, so an esiRNA screening was prepared to identify some vital kinases that took part in KRAS mutant-driven pancreatic cancer. This screen identified that knockdown of STK31 significantly reduced cell viability in KRAS mutant PDAC cells except for KRAS wild-type PDAC cells. We found STK31 upregulated in KRAS mutant PDAC patients with poor survival rates in clinical data. Additionally, STK31 is also overexpressed in the KRAS G12D mutant PDAC cell lines. Gain and loss of function experiments revealed that STK31 is a relevant target in PDAC that KRAS positively regulates. Knockdown of STK31 in KRAS mutant cell lines significantly reduced PDAC cell proliferation, colony formation rate, and migration ability. C-Jun activates the transcription level of STK31 by binding to its promoter region. By analyzing RNA sequencing data, we validated that STK31 positively correlates with the expression of CCNB1, a cell cycle mediator that promotes cancer cell proliferation. In summary, our results show that STK31 promotes cancer cell proliferation under the control of the Kras/MAPK/c-Jun transcriptional axis in PDAC.

CBET13

Modulation of autophagy affects pyrrolizidine alkaloid-induced liver injury

PAN Yueyang

Supervisor: LIN Ge and ZHAO Hui

Pyrrolizidine alkaloids (PAs) are phytotoxins identified in over 6000 plant species. Humans are exposed to toxic PAs through the consumption of PA-contaminating foodstuffs, and/or PA-containing herbal medicine and supplements. PA intoxication is initiated by hepatic cytochrome P450 enzymes-mediated metabolic activation and the subsequent formation of pyrrole-protein adducts (PPAs), the primary culprit triggering cell mitochondrial damage and apoptosis in the liver. Many factors, such as diets, lifestyles, diseases states, etc., can affect hepatotoxic potency of PAs. Autophagy as a physiological response to nutritional deficiency or stress is capable of effectively removing cellular protein aggregates and damaged organelles, which are readily induced in daily life. However, effects of autophagy modulation on PA-induced hepatotoxicity are unexplored. The present study aims to investigate the potential and regulatory role of autophagy in PA-induced liver injury (PA-ILI). By using a series of histological and biochemical autophagic flux assays, we demonstrated for the first time that activation of autophagy by rapamycin, an autophagy inducer, attenuated PA-ILI in mice and HepaRG cells via removing PA-derived PPAs and damaged mitochondria, and thereby reducing apoptosis in the liver. On the other hand, the inhibition of autophagy by 3-methyladenine or chloroquine, the autophagy inhibitors, further exacerbated PA-ILI. The findings may facilitate better management of PA-ILI with a more rational regulation of PA exposure and potentially effective therapies, including pharmacological or diet/lifestyle intervention, in the future.

[Supported by Research Grants Council of Hong Kong Special Administrative Region (GRF Project No. 14106318)].

CBET14

The role of circular RNAs on pancreatic cancer cells ferroptosis

PENA-PALADINES Jessica Jazmin[1], WANG Shao Weng[1], WONG Chi Hin[1],

CHEN Yang Chao[1,2]

Supervisor: CHEN Yang Chao

[1]School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, NT, Hong Kong

[2]Shenzhen Research Institute, The Chinese University of Hong Kong, Shenzhen 518087, China

Despite the effort that scientists have put into advancing cancer therapeutics, the mortality rate remains high in pancreatic cancer patients. This is predominately due to the lack of diagnostic tools for early detection and poor response to currently available treatments. Therefore, investigating the oncogenic mechanisms of pancreatic cancer, as well as potential targets for treatment, becomes fundamental and necessary. Recent studies suggest cancer cells can be highly sensitive to a newly defined type of cell death — ferroptosis. On the other hand, circular RNAs (circRNAs), a type of non-coding RNA, have been involved in several cancer hallmarks, which has increased the interest in their role as potential effective targets for oncological treatments. Hence, this project is about elucidating the role of circRNAs on ferroptosis by analysing the cystine-depletion pathway, aiming to further investigate components and mechanisms in ferroptosis, as well as to provide specific circRNAs as effective targets for anticancer therapies. In this project, differentially expressed (DE) circRNAs were obtained by sequencing the circRNAs of pancreatic cancer cells that were induced to ferroptosis by treating them with a cystine-free medium. Three significantly downregulated circRNAs were validated and confirmed through RT-qPCR. The knockdown of these circRNAs affected the oncogenic features of PDAC cells such as viability, invasion, migration, and colony formation, which demonstrates their potential oncogenic role. Furthermore, the decreased expression of these circRNAs increased the levels of ferroptosis-related markers such as lipid peroxidation and intracellular iron levels in pancreatic cancer cells, which indicates their relationship with ferroptosis induction. Bioinformatic analysis of these DE circRNAs demonstrated their possible interaction with proteins and miRNAs with reported ferroptosis function, which relates them to ferroptosis. This supports the further investigation of the oncogenic and ferroptosis-related action mechanism of these DE circRNAs, which can potentially serve as cancer therapeutic targets.

CBET15

The whole genome sequencing and hybrid assembly of black carp (M.piceus) and bighead carp (H.nobilis) and phylogenomic analysis of Cyprinid fishes

Ling Shi[1], Kin-Wing Ng[1], Qing Xiong[1], Fu-Kiu Ao[1], Soo-Kyung Shin[1], Stephen Kwok-Wing Tsui[1]

Supervisor: TSUI Kwok-Wing Stephen

[1]*School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong*

Black carp (Mylopharyngodon piceus) and bighead carp (Hypophthalmichthys nobilis), together with grass carp (Ctenopharyngodon Idella) and silver carp (Hypophthalmichthys molitrix), are the four major carps in China. They are freshwater fish species from the family Cyprinidae, cultivated for thousands of years. Black carp and bighead carp can be consumed as food, utilized as biological control agents and involved in traditional Chinese medicine. However, they also have negative impacts that can induce food allergy and threaten the survival of native species when introduced to a new environment. Due to the lack of high quality and completely-annotated genomes, the in-depth investigation of black carp and bighead carp biology is limited and a comprehensive analysis of Asian carps is not available. In this study, we constructed the whole genome of black carp with 877 Mb in size and 97.6% completeness, and the whole genome of bighead carp with 860 Mb in size and 95.8% completeness. The total number of annotated protein-coding genes was 28,707 in black carp, and 29,583 in bighead carp. The evolutionary relationships and gene family comparison of black carp and bighead carp were analysed in the family Cyprinidae. Our results suggested that immunoglobulin-like genes were under rapid evolution in both black carp and bighead carp.

CBET16

Decoding cancer associated fibroblasts in immunotherapy outcomes of hepatocellular carcinoma patients by single-cell RNA sequencing

WONG Patrick Pak Chun[1], CAO Jian Quan[1], XIONG Zhe Wen[1], WU Hao Ran[1],

CHAN Lam Stephen[2], CHENG Sze Lok Alfred[1]

Supervisor: CHENG Sze Lok Alfred

[1]School of Biomedical Sciences,

[2]Department of Clinical Oncology, The Chinese University of Hong Kong, Hong Kong

Cancer immunotherapy, especially the immune checkpoint blockade (ICB) therapy has been widely used and investigated in hepatocellular carcinoma (HCC) in recent years. The ICB therapy strengthens our immune cells and facilitate them to target and eliminate the HCC cells. However, majority of HCC patients still not respond to the ICB therapy due to the immune evasion of HCC cells or HCC tumor microenvironment (TME) heterogeneity. In order to overcome the non-responsiveness to ICB therapy in HCC, we performed the single cell RNA (scRNA) sequencing with utilizing the tumor biopsy samples of HCC patients which had undergone ICB (anti-PD1) treatment to study potential dynamic changes of HCC TME before and after the treatment. We found that cancer-associated fibroblast (CAF) was significantly enriched in the tumor edge after treatment in non-responders (NR), compared to responders (R). Moreover, the subtypes of CAF, matrix CAF (mCAF) and vascular CAF (vCAF) show the distinct differential gene expressions on chemokine-related, extracellular matrix (ECM)-related genes and apolipoprotein-related genes respectively after the ICB treatment in NR. Further bioinformatics and experimental studies will be done to validate how the distinct differential gene expressions on specific CAF subtypes correlate to the ICB resistance due to ICB drug-induced alterations in HCC.

CBET17

Decoding cancer immunotherapy resistance by single-cell multi-omics analysis

Haoran Wu[1], Stephen Chan[2], Kevin Yip[3], Alfred Cheng[1]

Supervisor: CHENG Sze Lok Alfred

[1]School of Biomedical Sciences,

[2]Department of Clinical Oncology, The Chinese University of Hong Kong, Hong Kong,

[3]Sanford Burnham Prebys Medical Discovery Institute, La Jolla, CA, USA

Tumor immunotherapy has been applied to liver cancer and made great clinical progress in the past few years, especially in the application of immune checkpoint blockade (ICB). However, in patients with hepatocellular carcinoma (HCC) treated with PD-1 inhibitors, the response rate is still limited to about 20%. It is crucial to identify a promising biomarker of response to ICB therapy. The aim of this study is to discover a novel biomarker that can predict the effective responses to ICB therapy. Here, we utilized single-cell RNA sequencing (scRNA-seq) and single-cell assay for transposase-accessible chromatin sequencing (scATAC-seq) to profile over 120,000 peripheral blood mononuclear cells (PBMC) from six hepatitis B virus (HBV) related HCC patients treated with pembrolizumab. Results show that non-responder patients have a significantly increased percentage of CD8+ effector memory T cells and distinctive transcriptional difference in the CD14+ monocytes cells. Furthermore, integrative analysis of HCC tumor-blood pairs revealed similar transcriptional profile of CD14+ monocytes between blood and tumor biopsy, which might be a potential linkage between the two sources. In the future, we will continue to study both the transcriptional and epigenetic differences in HCC patients, identifying the promising biomarker of response to ICB therapy. Collectively, this study describes the transcriptional and epigenetic landscape of PBMC in HCC patients, which may provide new insights for immunotherapy.

CBET18

Effect of centrally administered ghrelin on cisplatin-induced emesis in Suncus murinus

Lingqing Yang[1], Charmaine J.S. Kung[1], Zengbing Lu[1], M.P. Ngan[1], Julia Y.H. Liu[1], John A. Rudd[1]

Supervisor: RUDD Anthony John

Emesis Research Group, School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong

Purpose: Chemotherapy with cisplatin is associated with acute and delayed nausea and emesis. The recommended anti-emetics only protect approximately 80 % of patients. Our previous studies showed that centrally administrated ghrelin could antagonize cisplatin-induced acute emesis in ferrets. In the present studies, we investigate the effect of acyl-ghrelin (AG) and des-acyl-ghrelin (DAG) on the acute and delayed emetic response induced by cisplatin in Suncus murinus.

Methods: Male Suncus murinus were implanted with telemetry transmitters to record blood pressure, body temperature, and gastric myoelectric activity (GMA). Subsequently, the third ventricle was canulated, and AG (0.2, 1.0, 5.0 mg/kg/day) or DAG (0.2, 1.0, 5.0 mg/kg/day) or vehicle (Saline, 2 mg/kg) was delivered via osmotic minipumps four days before cisplatin (30 mg/kg, i.p.). After one-day pre-treatment with drug/vehicle, animals were transferred to whole-body plethysmography chambers to permit a recording of respiratory activity. Brain tissue was dissected at the end of the observation period, and samples were prepared for the assay of neurotransmitters through liquid chromatography-mass spectrometry (LCMS).

Results: AG and DAG did not modify food and water intake during the pre-treatment period. Cisplatin-induced emesis with 36.4±2.9 min of administration comprised 4.7±2.6 and 13.7±8.1 episodes during the acute (0-24) and delayed (24-72 h) periods, respectively. Cisplatin also decreased ($P<0.05$) the dominant frequency (DF) of gastric myoelectric activity from 13.9 ± 0.4 to 12.4 ± 0.3 cpm and decreased the dominant power (DP) during acute emesis; there was a reduction in the % power of tachygastria; food intake and body weight were reduced. DF decreased further during delayed emesis, where the % power of bradygastria increased. AG and DAG reduced cisplatin-induced delayed, but not acute emesis. AG and DAG differentially affected the timing of emetic events (as revealed by burst analysis), GMA, and changes in brain neurochemistry. **Conclusion**: The different profiles of AG and DAG may indicate that GHSR1A and perhaps an additional mechanism has been activated.

Acknowledgment: These studies were supported by the RGC (14119623).

CBET19

Deciphering the cellular and molecular determinants of immunotherapy resistance in NASH-associated hepatocellular carcinoma by single-cell analysis

ZHANG Lingyun[1], TANG wenshu[1], LIU Xiaoyu[1], XIONG Zhewen[1], LIANG Zhixian[1], Yang Weiqin[1], CHENG Sze Lok Alfred[1]

Supervisor: CHENG Sze Lok Alfred

[1]School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong SAR, China

Hepatocellular carcinoma (HCC) accounts for approximately 85% of liver cancer and is one of the leading causes of cancer deaths worldwide. In past years, non-alcoholic steatohepatitis (NASH) has become an important risk and the fastest growing cause of HCC. Although immune checkpoint blockade (ICB) therapy, such as anti-programmed cell death-ligand 1 (anti-PD-L1) has exhibited effect in subsets of HCC patients, recent studies unveiled that NASH limited anti-tumor surveillance in ICB-treated HCC. Here, we utilized orthotopic and spontaneous NASH-HCC mouse models to explore the mechanisms underlying ICB resistance in NASH-HCC.

Firstly, NASH was induced by feeding mice with methionine-and choline-deficient (MCD) diet for two weeks and determined by hepatic steatosis, inflammation and liver injury. Afterwards, orthotopic NASH-HCC was established by intrahepatic inoculation of liver cancer cell line RIL-175 in NASH mouse. NASH-HCC showed no response to anti-PD-L1 therapy. The results of multi-color flow cytometry implied that CD11b+F4/80+CD206+ M2 macrophages accumulated in liver during NASH development, and increased in liver and tumor upon anti-PD-L1 treatment in NASH-HCC. Interestingly, significantly positive correlations were identified among hepatic, intratumoral M2 macrophages and tumor weight. Besides, tumor-infiltrating PD-1-expressing CD8+ T cells positively correlated with tumor weight and intratumoral M2 macrophages, indicating potential interplay between M2 and PD1+CD8+ T cells underlying ICB resistance in NASH-HCC. Similar results were observed in choline-deficient, L-amino acid-defined, high-fat diet (CDAHFD)-based NASH-HCC mouse model. Furthermore, to delineate immune cell characterizations at single-cell resolution, tumor-infiltrating immune cells from pre- and post-treatment NASH-HCC will be isolated for single-cell RNA-sequencing analysis. Spontaneous NASH-HCC mouse models induced by long-term CDAHFD feeding will be also employed in future investigation. Our study will provide insight on mechanisms driving ICB-resistance and immunotherapeutic enhancement in NASH-HCC.

CBET20

Beta 2 adrenergic receptor (β2-AR): a potential target to inhibit liver metastasis by disrupting the immune suppressive microenvironment

ZHANG Yi Xuan[1]

Supervisor: ZHOU Jing Ying[1]

[1]*School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, China*

Liver metastasis is the most important cause of cancer-related death. We and others have demonstrated that the unique feature of liver immune microenvironment shaped by signals from primary tumors could facilitate the colonization and growth of metastatic tumor cells. Increasing evidence has pinpointed the potential immunomodulatory and tumor-supportive roles of neurotransmitter-receptor signals. We aimed to investigate if primary tumor could regulate neurotransmitter-receptor signals to facilitate the formation of metastatic-prone liver microenvironment. By analyzing published single cell RNA sequencing datasets from patients with primary colorectal cancer (CRC) and/or liver metastasis, we identified the ADRB2, which encodes beta2-adrenergic receptor (β2-AR), a receptor of norepinephrine as the top upregulated neurotransmitter receptor in both primary CRC and liver metastasis. Interestingly, by multi-color immunofluorescence (IF) staining, β2-AR is found selectively expressed by myeloid cells, which is abundant in primary CRC or metastatic liver, but not in adjacent liver from patients with hepatocellular carcinoma (HCC) or normal liver from healthy donors. To investigate if signals from primary CRC is essential for the upregulation of adrenergic-β2-AR signal in metastatic liver, we established a clinical-relevant spontaneous CRC liver metastasis mouse model by intracecal injection of a CRC murine cell line SL4. Multi-color IF and high-dimensional flow cytometry analysis demonstrated an abundance of adrenergic nerve fibers indicated by tyrosine hydroxylase (TH) and CD11b+β2-AR+ myeloid cells in the livers from spontaneously developed metastasis, similar to CRC tumors. Taken together, our preliminary data suggested that the adrenergic-β2-AR signal in metastatic livers may be activated by signals from primary CRC. Since pan β-AR inhibitors are widely used in clinical for cardiovascular and other diseases, we hypothesize that repurposing of β2-AR inhibitors for cancer therapy holds great potentials which is worthwhile for further investigation.

CBET21

Deletion of 11q curbs ETS1 expression to inhibit cell growth through destabilizing MYCN in neuroblastoma

ZHOU Liang Ji[1], ZHAO Hui[1], LIN Ge[1]

Supervisor: LIN Ge & ZHAO Hui

[1]School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong SAR, China

Neuroblastoma is the most frequent extracranial solid paediatric tumour caused by anomaly of neural crest development. Chromosome alterations, such as chromosome 1p LOH (loss of heterozygosity), 11q LOH, 17q gain, and MYCN amplification (MNA) are often found in neuroblastoma patients and linked with bad prognosis. Among the aforementioned chromosome alterations, the molecular mechanism of 11q deletion and its unfavourable outcomes in neuroblastoma (NB) have not been elucidated yet. Through bioinformatic analysis, we found that ETS1, located in 11q, is frequently downregulated in high-risk neuroblastoma and correlated with INSS stage 4. We further demonstrated that overexpression of ETS1 can inhibit the proliferation of NBL-s, a non-MYCN amplification NB cell lines harbouring 11q deletion, and reduce the protein level and stability of MYCN, a key oncogenic factor during tumorigenesis of neuroblastoma. Moreover, ETS1 can attenuate the phosphorylation of ERK and GSK-3β in NBL-s. Based on these data, we suggest that ETS1 exerts functions as a tumour suppressor in neuroblastoma.

SCHOOL OF BIOMEDICAL SCIENCES

Postgraduate Research Day 2022

Developmental and Regenerative Biology (DRB)

Developmental and Regenerative Biology

Title	Author	Number
Investigating the effects of a cytokine storm in a micro-physiological system of the microvasculature.	WONG Wing Tung	DRB1
Xeno- and serum-free manufacturing of a hypoxic culture enhanced mesenchymal stem cell (MSC)-derived extracellular matrix (ECM)-based biomaterial for therapeutic angiogenesis	LIT Kwok Keung	DRB2
Developing a liver-on-chip model for simulating liver inflammatory	CHAWLA Dhvanii	DRB3
Exosome-based therapy for liver regeneration	ZHANG Xuerao	DRB4
Explore SOX9 haploinsufficiency in neural stem cells	CHAN See Wing	DRB5
Identification of target genes and pathways involved in triple-negative breast cancer proliferation and stemness using genome-wide CRISPR screens	LI Tu	DRB6
Exploring roles of distal enhancers in SOX9 haploinsufficiency in sex determination	OU Fanghong	DRB7
Isolation and characterization of vagal neural crest cells for cell therapies	CHOI Seong Wang	DRB8
Schwann Cell-like Cells in Peripheral Nerve Repair	XIAO Dongmei	DRB9
Transcriptomic analyses on hair follicle-derived neural crest stem cells (HF-NCSCs) in the cell-based therapy for Hirschsprung's disease (HSCR)	YU Wai Him	DRB10
PI3K-Akt signaling in stem cell aging	TAM Hei Yin	DRB11
The studying of developmental defects in Bloom syndrome	YIU Tsz Ching	DRB12
Engineering hACE2 to develop therapeutic agents against SARS-CoV-2	LI Jiangchuan	DRB13
Development of Gene Therapy for β-Thalassemia through Therapeutic Genome Editing using Erythroleukemic Cell Model	URIP Brain Anugerah	DRB14
Investigation into the potency and safety of hACE2 decoy proteins in neutralizing SARS-CoV-2	WANG Jingyi	DRB15
CRISPR-Cascade: A Cascade Deep Learning Model to Accelerate CRISPR-Cas9 Off-target Site Detection	WEI Jun Kang	DRB16
Study of in vivo gene editing outcomes using AAV-CRISPR edited hemophilia B mice	ZHANG Siqi	DRB17
Potential Regulation of Spermatogonial Stem Cell Fate by Testicular Extracellular Vesicles Cargoes	ZHENG Ting Ting	DRB18
DHX9, the gene encoding the DExH-box helicase DHX9, underlies neurodevelopmental disorders and Charcot-Marie-Tooth disease	GUO Tianyu	DRB19

Functional characterization of recurrent truncating variant in UBAP1 associated with hereditary spastic paraplegia (HSP-SPG80)	HO Nicolas James	DRB20
Investigating the epigenetic role of extracellular matrix stiffness in OPC aging and remyelination capacity	PENG Shenyi	DRB21
Deciphering The Role of LGR5 in Oligodendrocyte Development and White Matter Repair	XU Dongke	DRB22
The Histone Demethylase KDM3A Controls Axon Growth by Its Dual Regulatory Role of Rac1	ZHANG Huan	DRB23
Development of a small molecular tool for regulating NGF/TrkA signaling in bone biology	LI Xiao	DRB24
Sucralose Improves Vascular Function and Reduces Oxidative Stress in Obese Mice	ZUO Yuanyuan	DRB25
The roles of Irx3 and Irx5 in mouse cochlear outer sulcus development	WENG Xin	DRB26
Impairing TRPML1 activity protects OA chondrocytes from apoptosis by attenuating mitochondrial dysfunction	YAO Hanyu	DRB27
An optimised hybrid hydrogel bioink generated by chemical crosslinking approach facilitates engineered cartilage formation	ZHOU Zheyuan	DRB28
Tendinopathy: Multiomics analysis of pathogenesis and development of a disease-targeting biomaterial for tendinopathy treatment	RAO Ying	DRB29
Targeting tendon biomimetic niche via a biochemically and biomechanically functionalized biomaterial for precision tendon regeneration	ZHANG Wan Qi	DRB30
Knockout of Thyroid Hormone Receptor Alpha a (thraa) Facilitates Heart Regeneration in Zebrafish	CHEUNG Man Yee	DRB31
Identification of Small Molecules That Promotes the Maturation of Human Embryonic Stem Cell-derived Beta-like Cells	DENG Jiani	DRB32
COILED COIL-DOMAIN CONTAINING PROTEIN-141 (CCDC141) IS ESSENTIAL FOR HEART DEVELOPMENT AND FUNCTION	DIO, Daniel Wuyang	DRB33
Identification of essential factors that promote pancreatic β cell differentiation using genome-wide CRISPR/Cas9 screening	TANG Qi	DRB34
Synthetic glucose–sensor gene circuit enhances blood-glucose homeostasis in type 1 diabetic mice	ZHANG Yuan	DRB35

DRB1

Investigating the effects of a cytokine storm in a micro-physiological system of the microvasculature

WONG Wing Tung

Supervisor: Prof. Anna BLOCKI and Prof. TUAN Sung Chi Rocky

The SARS-CoV-2 virus can infect a wide range of cells and systems in the body. In the current COVID-19 pandemic, it is noticed that one of the major causes of death is a systemic hyperinflammation caused by the induction of a cytokine. This hyperinflammatory response causes endothelial cell dysfunction, and the activation of endothelial cells induces proinflammatory gene expression, leading to localized hyper-permeability, inflammation via recruitment of inflammatory cells to injury sites and formation of microthrombi and microvascular damage.

We thus created a cytokine storm by allowing M1 activated macrophages to condition medium. The M1 secretome contained a majority of factors previously reported for COVID-19 related hyper-inflammation. Next, conditioned medium was introduced into an in vitro microvasculature-on-a-chip, followed by investigation of different essential biomarkers for endothelial functionality, such as basement membrane components, junctional proteins and endothelial activation markers.

We found that high concentrations of M1 conditioned medium resulted in partial disintegration of microvascular networks. At concentrations that allowed the majority of the network to persist, we observed a down-regulation of laminin α5 and a disintegration of VE-Cadherin cell borders, while von Willebrand factor (vWF) was being enriched within the cells.

These data suggest that the established cytokine storm promotes disintegration of vessels, increase in vascular permeability and endothelial activation. Future functional assays will focus on investigating vascular permeability, inflammatory cell adhesion and thrombus formation in the device. The here established model thus allows to study the effects of a cytokine storm on the microvasculature in high spatial-temporal resolution.

DRB2

Xeno- and serum-free manufacturing of a hypoxic culture enhanced mesenchymal stem cell (MSC)-derived extracellular matrix (ECM)-based biomaterial for therapeutic angiogenesis

Kwok Keung, LIT[1,2,3], Cheuk Kwan, LI[1], Sui Ki, CHIU[1]

Supervisor: Prof. Anna BLOCKI[1,2,3,4]

[1]*School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, Hong Kong Special Administrative Region of China*

[2]*Institute for Tissue Engineering and Regenerative Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, Hong Kong Special Administrative Region of China*

[3]*Center for Neuromusculoskeletal Restorative Medicine, The Chinese University of Hong Kong, Hong Kong SAR*

[4]*Department of Orthopaedics & Traumatology, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, Hong Kong Special Administrative Region of China*

Current therapeutic approaches to achieve revascularisation of ischaemic tissues, such as cell-based therapies and growth factors therapies have numerous limitations and drawbacks, prompting the development of a new biomaterial for therapeutic angiogenesis. We have recently engineered a MSCs-derived ECM-based biomaterial that was shown to exhibit superior pro-angiogenic and pro-healing properties in a skin wound model. Here we seek to employ a xeno/serum-free system for material manufacturing to pave the way towards clinical application, as well as exploit hypoxic culture to further enhance its pro-angiogenic properties. In brief, we evaluated 5 commercially available xeno- and/or serum-free media, by examining ECM yield and the pro-angiogenic properties of the resulting biomaterials. We also hypothesized that synthesis of this biomaterial under hypoxic culture conditions of MSCs, will further enhance their production of pro-angiogenic factors, which subsequently will be incorporated into the material. Results have shown that the use of all xeno- and/or serum-free media has promoted the deposition of ECM structural proteins (i.e. fibronectin and collagen I) as compared to serum supplemented basal medium, leading to an increased yield. Biomaterials produced using these media also showed augmented pro-angiogenic bioactivity. Finally, through the combinational use of xeno- and serum-free medium and hypoxic culture, the pro-angiogenic potential of the resulting ECM-based material was further enhanced. In conclusion, we have demonstrated that the use of xeno- and serum-free system combined with hypoxic culture can further enhance the pro-angiogenic potential of our ECM-based materials, while paving the way towards clinical application.

DRB3

Developing a liver-on-chip model for simulating liver inflammatory response

Dhvanii, CHAWLA[1,2]

Supervisor: Prof. CHAN Hon Fai Vivas[1,2] and Prof. TUAN Sung Chi Rocky[1,2]

[1]*School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, Hong Kong Special Administrative Region of China*

[2]*Institute for Tissue Engineering and Regenerative Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, Hong Kong Special Administrative Region of China*

Inflammation is a common response seen in liver diseases and is usually managed by immunosuppressive therapies. Uncontrolled inflammation can lead to irreversible damage and liver failure that may require liver transplantation. Since human-based in vitro models can mimic our biology more accurately than animal models, organ-on-chip platforms developed using cell and organoid culture have recently been employed to investigate disease mechanisms and perform drug testing. In this project, human embryonic stem cell (hESC) derived hepatocyte like cells (HLC) and HepG2 cells were cultured for modelling inflammatory conditions and screening potential therapeutic agents such as stem cells and stem cell-derived products. Successful HLC differentiation was shown by detecting the expression of hepatocyte marker genes including Alb, AFP, CYP34A. To model inflammation, THP-1 cells were differentiated into macrophages using PMA and further polarized and subjected to different concentrations of LPS and IFN gamma for obtaining conditioned media containing pro-inflammatory cytokines including IL-1β, TNF-α, and IL-6. HepG2 cells showed a significant drop in albumin production measured by ELISA after treatment with conditioned media. In conclusion, we have preliminarily demonstrated that inflammatory conditions can be recapitulated by administering conditioned media containing inflammatory cytokines to liver cells. This work has laid the foundation for developing more advanced hepatocyte cultures such as 3D organoid and organ-on-chip models to simulate inflammation in the liver, investigate disease mechanisms and screen potential therapeutic agents.

DRB4

Exosome-based therapy for liver regeneration

ZHANG Xuerao

Supervisor: Prof. CHAN Hon Fai Vivas and Prof. CHENG Sze Lok Alfred

Liver is the largest internal organ in our body, which performs many essential biological functions such as detoxifying toxic drugs and synthesizing proteins. Liver can normally regenerate after partial hepatectomy or following chemical and inflammatory injury. However, liver may fail to regenerate in cases such as during cirrhosis and acute liver injury. In the process of liver regeneration, in addition to the liver parenchyma, non-parenchymal cells, including immune cells, epithelial cells, fibroblasts, and stem cells, all play an essential role in regulating the liver regeneration process. Since cells will secrete exosomes, which are small extracellular vesicles (30-150 nm in diameter) that contain proteins, nucleic acids, and lipids with various functions such as regulating immune response, cell migration, cell differentiation etc, we hypothesize that exosomes collected from cell types such as macrophages could support liver regeneration. Our preliminary results showed that exosomes could be successfully isolated from medium collected from macrophage cell culture. In vitro studies showed that exosomes secreted by macrophage could promote angiogenesis in vitro. We also identified TNF-α, which is vital for the initiation of liver regeneration in the exosomes. Further studies will be performed to characterize the composition of exosome cargos and their effect in vitro and in vivo.

DRB5

Explore SOX9 haploinsufficiency in neural stem cells

CHAN See Wing

Supervisor: Prof. CHAN Wai-yee

Campomelic dysplasia (CD) is a rare congenital disease caused by SOX9 haploinsufficiency and only 5-10% of patients survived past infancy. Complications of CD patients include sex reversal, abnormal development of the skeletal system, and mild to moderate learning difficulties (Mansour, 2002). SOX9 is a relatively well-known gene in skeletal and gonadal development and has recently been associated with cancer as a metastasis marker. It also induces neural stem cells (NSC) and mediates neurogenic-to-gliogenic cell fate switch in the central nervous system (CNS). However, the underlying mechanism of the role of SOX9 role in the NSC induction remains unclear.

With CRISPR/Cas9 techniques, a SOX9 edited hiPS cell line of a CD patient was generated according to the medical literature. The cells were differentiated into NSC. Quantitative PCR results suggested that loss of SOX9 altered the expressions of key genes, such as SOX1, SOX2, Nestin, and PAX6, in iPSC-derived NSCs. Further analysis was performed by stranded-specific RNA sequencing. Based on the transcriptome profile, several pathways such as MAPK, p53, and Wnt signaling pathways were found to be altered in NSC. Further analyses including western blot, immunostaining, and qPCR analysis will be performed to validate the RNA sequencing results. NSC functions, such as neurogenesis and gliogenesis, and conductivity of the resulting neurons will also be tested by continuous differentiation and high-density microelectrode array analysis. Collectively, the current study aims to identify the role of SOX9 in NSC and the subsequent effect in the CNS to explain the learning difficulties reported in CD patients.

DRB6

Identification of target genes and pathways involved in triple-negative breast cancer proliferation and stemness using genome-wide CRISPR screens

LI Tu

Supervisor: Prof. CHAN Wai-yee

Breast cancer is the leading cause of cancer death among women. Unlike other subtypes of breast cancer, triple-negative breast cancer (TNBC) is acknowledged as one of the most aggressive and refractory subtypes of breast cancer that accounts for most of breast cancer-related mortality. For decades, the therapeutic strategies of TNBC were limited to several traditional treatments, which can only eliminate the bulk tumor population. However, the remaining core population - cancer stem cells (CSCs) can develop new tumor tissues with a few cells to initiate disease relapse and invade other organs that contribute to cancer metastasis. Advanced targeted strategies – immunotherapies provide exciting prospects in TNBC treatment. However, it is paramount to identify cancer-specific antigens in advance. Genome-wide CRISPR-Cas9 screens have proven to be an exquisitely powerful method for discovering and functional annotation of genetic or epigenetic elements in biological processes. This study used two CRISPR-Cas9 screening libraries to perform high-throughput pooled screens in TNBC cell lines. One is the human membrane protein activation library, which aims to identify candidate genes encoding antigens that can serve as tumor-specific targets in immunotherapy. The other is the human epigenetic knockout library, which we hope can reveal the epigenetic "drivers" or driver genes regulated by epigenetic factors in TNBC. After weeks of consecutive culture, gene-edited cells were collected, and skewed gRNA distribution in the population will indicate essential genes in cancer cell proliferation. Non-cancer stem cells and cancer stem cells (mesenchymal-like, CD44+/CD24-; epithelial-like, ALDH1+) were separated by FACS, genes critical to stemness transformation will also be screened out. Many known TNBC-specific or promoting genes, such as SUZ12, RAD51and PELP1, were among the top ranks in our screening results. These coincidences confirm the biological reliability of our findings. KEGG pathway analysis showed most candidate genes were involved in cell cycle, NF-κB, and thyroid hormone signaling pathways. The GEPIA database and Kaplan-Meier method also show that many top hits are tumor-specific and critical for patient survival. In the end, tissue

microarray will be used to determine the clinical significance of candidates. Overall, our results provide essential genetic and epigenetic targets for developing TNBC treatments.

DRB7

Exploring roles of distal enhancers in SOX9 haploinsufficiency in sex determination

OU Fanghong[1], SUEN Hoi-Ching[1], SUN Hailiang[2], WANG Zhangting[1], MIU Kai-Kei[1], SHI Peng[3], LIAO Jinyue[2], CHAN Wai-Yee[1]

Supervisor: Prof. CHAN Wai-Yee[1]

[1]*School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong SAR, China*

[2]*Department of Chemical Pathology, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong SAR, China*

[3]*Department of Biomedical Engineering, City University of Hong Kong, Kowloon, Hong Kong SAR, China*

Disorders of sex development (DSD) are congenital anomalies involving discordance between genotype to phenotype in gender identity. People with DSD suffer from ambiguous genitalia, impaired steroid hormone production, reduced or null fertility, and partial to complete sex reversal. Various mutations of the dosage-sensitive haploinsufficient SOX9 gene (sex-determining region Y-related high-mobility group box 9) were identified in male-to-female sex reversal patients. SOX9 regulates the production of the anti-Müllerian hormone (AMH) in Sertoli cells to inhibit the establishment of a female reproductive system in an XY embryo. A single copy of the wild-type allele of the heterozygous SOX9 is insufficient to maintain normal male reproductive tract development. Duplication and deletion of distal enhancers of SOX9 found in DSD patients suggest the potential roles of enhancers in SOX9 haploinsufficiency in sex determination. But the underlying genetic mechanism of haploinsufficiency is still largely unknown.

By integrating scATAC-Seq, complete stranded RNA-Seq, and published scRNA-Seq data, we have profiled temporal chromatin landscapes across gonadal somatic cell types and whole transcriptome during mouse sexual development and identified multiple putative cis-regulatory elements (CREs), transcription factors, and non-coding RNAs that may contribute to gonadal lineages specification. 3 putative cis-regulatory elements of Sox9 were identified from peak-to-gene linkages. Further validations will together provide a valuable prototype for studying haploinsufficiency from 3D chromatin topological interactions between distal enhancers, transcription factors, and non-coding RNAs.

DRB8

Isolation and characterization of vagal neural crest cells for cell therapies

CHOI Seong Wang

Supervisor: Prof. CHAN Wood Yee Woody

Vagal neural crest cells (VNCCs) from the neural tube adjacent to somites 1-7 during embryonic development form the majority of enteric neurons in the gastrointestinal tract. The abnormal development of VNCCs may therefore result in congenital enteric neuropathies such as Hirschsprung's disease with defects of enteric neurons. VNCCs, as neural progenitors of enteric neurons, can thus be a potential cell source for replacing abnormal or replenishing missing enteric neurons in patients with Hirschsprung's disease. In this study, VNCCs were isolated and characterized for investigations on cell transplantation to the gastrointestinal tract. First, neural tubes were explanted from mouse embryos at embryonic day E8.5-E9.5. Cells emigrated from the explants were collected 24 hours after the explants were cultured *in vitro*. Then, the collected cells were characterized with different biomedical methods. Results from immunofluorescence staining indicated that both emigrated cells and their daughter cells after passages expressed neural crest cell (NCC) markers. Fluorescence-activated cell sorting showed that 68.9% of the emigrated cells were positive to p75, a common NCC marker, whereas wound healing assays implicated that they possessed strong migration ability. When emigrated cells were cultured in different differentiation media, they were able to form neurons, glial cells and smooth muscle cells, indicating that they were multipotent. To prepare for upcoming transplantation studies, VNCCs were cultured on a non-adhesive surface, and spontaneously form spheres, the cells of which also expressed NCC markers. Our results demonstrated that the cells emigrated from the neural tube explants were likely to be VNCCs. They expressed NCC markers and possessed migration and differentiation abilities *in vitro*. They were also able to form spheres which were more suitable for subsequent cell transplantation studies.

The work was supported by the General Research Fund from the Research Grants Council of the Hong Kong Special Administrative Region, China (Ref. No.: CUHK14118818), and the research fund from Health@InnoHK program launched by Innovation and Technology Commission, the Government of Hong Kong Special Administrative Region, China.

DRB9

Schwann Cell-like Cells in Peripheral Nerve Repair

XIAO, Dongmei

Supervisor: Prof. CHAN Wood Yee Woody

Nerve fibres of the peripheral nervous system (PNS) have their intrinsic ability to regenerate after injury and this healing process is regulated by Schwann cells (SCs) owing to their unusual capacity to reprogram their genotype through dedifferentiation into repair SCs. The current treatment of severe peripheral nerve injury (PNI) is mostly based on the transplantation of autologous nerve graft. To avoid sacrifice of healthy donor nerves during transplantation, nerve guidance conduits (NGCs), considered as an alternative source of the transplant, are artificial nerve grafts composed of a biomaterial-based scaffold, seed cells and neurotrophic factors with the marked advantage of bridging the injured nerve gap to provide an ideal microenvironment for neuronal recovery. In the present study, we induced Schwann Cell-Like Cells (SCLCs) from Induced Pluripotent Stem Cells (iPSCs) via expandable intermediate cell types, namely Schwann cell precursors (SCPs), for seeding into the NGCs before transplanted to a PNI mouse model. Human iPSCs (hiPSCs) were utilized for iPSCs-SCPs-SCs induction. Primary cultures of mouse SCs isolated from postnatal day-3 mice were used as the positive control as they have already been shown to be able to assist in the regeneration of injured peripheral nerves. It was found that primary mouse SCs expressed Schwann cell markers, exhibited considerable capability of migration and were able to form myelin sheaths around neurites in vitro when co-cultured with spinal cord motor neurons. An induction protocol to induce SCLCs from hiPSCs for future transplantation was successfully established, and a surgical method to transplant iPSC-derived SCPs or SCs for regeneration studies was also developed. With this induction protocol, it is anticipated that nerve guidance conduits made up of novel biomaterials and filled with SCLCs and/or SCs, neurotrophic factors and extracellular matrix will be fabricated for therapeutic purposes.

This study was supported by the research fund from Health@InnoHK program launched by Innovation and Technology Commission, the Government of the Hong Kong Special Administrative Region of the People's Republic of China.

DRB10

Transcriptomic analyses on hair follicle-derived neural crest stem cells (HF-NCSCs) in the cell-based therapy for Hirschsprung's disease (HSCR)

YU Wai Him, ZHANG Yan, LIANG Yong Hao, Prof. TSUI Kwok Wing Stephen

Supervisor: Prof. CHAN Wood Yee Woody

Hirschsprung's disease (HSCR) is a congenital gastrointestinal motility disease characterized by the lack of functional neural network in parts of the intestinal tract. With difficulty in excreting their stool, patients with HSCR usually suffer from symptoms such as constipation and intestinal obstruction. The current surgical treatment of HSCR however frequently leads to post-operation complications, and therefore there is a need for an alternative therapy. Cell transplantation to re-establish the missing intestinal neural network has been proposed. One interesting candidate cell source is hair follicle-derived neural crest stem cells (HF-NCSCs). As more findings from biological studies on the transplantation of HF-NCSCs for therapeutic purposes are emerging, the bioinformatic analysis on the genome-wide expression profile of HF-NCSCs has become important for providing information on their molecular signature and also potential mechanisms underlying various biological processes in the cell therapy.

In the present study, the transcriptomic profile of HF-NCSCs was compared with that of enteric neural crest cells (ENCCs) which are the neural progenitors of the gastrointestinal tract. It was found that genes related to synaptic processes such as nicotinic acetylcholine receptors and neural transmitter releasing molecules were up-regulated in ENCCs. Up-regulations were also found in the genes involved in immune responses like interleukin receptors, whereas some NCC markers were down-regulated in HF-NCSCs. Further upstream regulators analyses reveal that key factors for neural crest development and neuronal differentiation like *kif1b*, *sox2* and *ascl1* were down-regulated in HF-NCSCs, suggesting HF-NCSCs may be less able to go through these developmental process.

The work was supported by a research grant from the Research Grants Council of Hong Kong Special Administrative Region, China (General Research Fund Ref. No.: CUHK14118818)

and the research fund from Health@InnoHK program launched by Innovation and Technology Commission, the Government of Hong Kong Special Administrative Region, China.

DRB11

PI3K-Akt signaling in stem cell aging

TAM Hei Yin, Cheung Hoi Hung Albert

Supervisor: Prof. CHEUNG Hoi Hung Albert

Stem cells are of paramount importance in maintaining and regenerating tissues throughout the lifespan of multicellular organisms. Decline in stem cell function upon aging results in loss of tissue homeostasis, which associated with increased likelihood of developing chronic disease, such as diabetes, cancer, osteoporosis, etc. Hence, identifying the intrinsic changes in stem cell aging will provide us a more comprehensive understanding of the aging mechanisms, and to provide novel strategic methods in combating aging and promote translational use of stem cells.

Werner syndrome (WS) is an autosomal recessive disorder characterized by premature aging due to RecQ helicase (WRN) deficiency. Clinical evidence and research suggest that WS stem cells are significantly impaired in terms of their self-renewal and regenerative potential. Our previous study demonstrated an inhibition of the PI3K-Akt-signaling pathway in WS mesenchymal stem cells (WS-MSC). WS-MSC fails to activate Akt signaling in response to growth factor stimulation. This finding further reveals the downregulation of hepatocyte growth factor (HGF), which results in reduced angiogenesis and cutaneous wound healing. However, the reason for this dysregulation remains elusive. Here, we have revealed enhanced expression of SHIP1 and SHIP2 in WS-MSC, which are the upstream targets of Akt. SHIP1 and SHIP2 are negative regulators of Akt, through hydrolyzing the 5'phosphate from phosphatidylinositol(3,4,5)-triphosphate PI(3,4,5)P3 to phosphatidylinositol-3,4-bisphosphate PI(3,4)P2. Reactivation of Akt-pathway using SHIP inhibitors results in increased HGF synthesis and proliferation in WS-MSCs. More importantly, the inhibitors can suppress senescence and senescence-associated secreted phenotype like p16 and IL6, which are the hallmarks of aging. Besides, we also revealed enhanced SHIP1 and SHIP2 expression in late passage MSCs from normal people, suggesting that target SHIP activity may be beneficial to reverse some of the aging phenotypes and improve stem cell function.

DRB12

The studying of developmental defects in Bloom syndrome

YIU Tsz Ching

Supervisor: Prof. CHEUNG Hoi Hung Albert

RecQ DNA helicase family is conserved in evolution and plays fundamental functions in the maintenance of genomic stability and integrity. Among this family, the genetic mutation in BLM is known to cause Bloom syndrome (BS). Many pathogenic mutations result in the loss-of-function of the BLM helicase. Deficiency of BLM leads to genome instability in cell and various developmental defects in patients.

Patients with BS exhibit phenotypes in congenital short stature, growth retardation, skin photosensitivity, reduced fertility and predisposition to both carcinoma and hematologic cancer. BLM deficient cells show an exceptionally high level of sister chromatid exchange, which leads to homologous recombination-mediated genomic instability. The genomic instability illustrates the high incidence of cancers in BS patients.

Animal model using transgenic mice cannot, however, be used to study the pathogenesis of BS because of the embryonic lethality in Blm-/- mice. Therefore, we use zebrafish as an alternative animal model to study the developmental defects associated with the loss of BLM ortholog. Zebrafish model allows us to elucidate the phenomenon of developmental defects in BS. We have generated blm knockout zebrafish by CRISPR/Cas9. We observed that the body-mass index (BMI) of zebrafish is significantly different between blm-/- and blm+/+ genotypes. We also observed differences in the bone mineral density (BMD), radius neural arch angle and area. Interestingly, the knockout of blm helicase also causes developmental defect in the genital system, with all of the blm-/- zebrafish developed to male only.

DRB13

Engineering hACE2 to develop therapeutic agents against SARS-CoV-2

LI Jiangchuan, WANG Jingyi, LUO Bin, HE Xiangjun, WONG Hoi Ting, REN Jianwei, XUE Kwan Yuen Katherine, QIU Jiale, WEI Junkang, WANG Jue, FENG Bo

Supervisor: Prof. FENG Bo

School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China

ACE2 is the primary receptor that interacts with the spike protein of SARS-CoV-2 virus and mediates subsequent viral infection in human. At the same time, the infection of SARS-CoV-2 downregulates the expression of hACE2, leading to renin angiotensin system (RAS) imbalance and tissue injury. The extracellular domain of ACE2 is soluble and could be fused with immunoglobulin Fc domain (sACE2-Fc) to achieve excellent serum stability, which provides a therapeutic candidate for treating SARS-CoV-2 infection by acting as a decoy to neutralize infection or as a carboxypeptidase to suppress tissue injury. However, intravenous administration of excessive sACE2-Fc will also disturb RAS function and pose complicated cardiovascular risks. To investigate the potential of exploiting the two roles of sACE2-Fc separately in combating SARS-CoV-2, we engineered hACE2 by decoupling its spike binding affinity and enzyme activity to develop two distinct therapeutics against SARS-CoV-2. First, sACE2-Fc was mutated to enhance its binding to SARS-CoV-2 spike while abolishing the enzyme activity. Second, we engineered sACE2-Fc to reserve its enzyme activity while abolishing its binding affinity to spike. Our study found that spike-binding property and enzyme activity of human ACE2 could be decoupled to engineer ACE2 mutants executing a single function. Moreover, the engineered catalytically inactive sACE2-Fc could act as safe decoys to neutralize multiple SARS-CoV-2 pseudoviruses, including Delta and Omicron variants.

DRB14

Development of gene therapy for β-thalassemia through therapeutic genome editing using erythroleukemic cell model

Brian Anugerah URIP, Anson Kin Ching TSANG, Chenzi ZHANG, Zhenjie ZHANG

Supervisor: Prof. FENG Bo

School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China

β-thalassaemias are inherited blood disorders caused by deficiency or absence of the β-globin subunit of adult hemoglobin in red blood cells, which creates an imbalance between alpha- and beta-globin subunits. Patients with severe forms of β-thalassaemia (β-thalassaemia major) need to receive life-long regular blood transfusions, which might lead to several complications like iron overload and infections, posing serious burdens to the patients' health. Stem cells transplant can provide cure, but this method is restricted by the limited availability of donors and the risk of graft versus host diseases that might arise. Currently, gene therapies are being developed for this disease and they showed promising results. In this project, we are utilizing the CRISPR/Cas9 technology, combined with the clinically approved gene delivery vector, adeno-associated virus (AAV). The system is aimed to achieve a gene knock-in of a functional β-globin (HBB) gene into K562 cells, an erythroleukemic cell line that has been extensively used for globin gene expression study. We observed that CRISPR-based editing coupled with AAV6 vector delivering HDR-based donor generated an efficient knock-in in K562 cells, producing an active HBB expression in the two loci tested, GAPDH and HBA1 (hemoglobin subunit alpha 1). This work could serve as a foundation for a future application of AAV/CRISPR-based genome editing in developing an ex-vivo treatment for beta-thalassemia using autologous hematopoietic stem cells.

DRB15

Investigation into the potency and safety of hACE2 decoy proteins in neutralizing SARS-CoV-2

WANG Jingyi[1], LI Jiangchuan[1], CHIN Wing Hong Alex[2], LUO Bin[1], HE Xiangjun[1], WONG Hoi Ting[1], REN Jianwei[1], WEI Junkang[1], QIU Jiale[1], XUE Kwan Yuen Katherine[1], WANG Jue[1], ZHANG Siqi[1], POON Lit Man Leo[2], FENG Bo[1]

Supervisor: Prof. FENG Bo[1]

[1]School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China

[2]School of Public Health, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China

Emerging mutants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have resulted in frequent breakthrough infections during the coronavirus disease 2019 (COVID-19) pandemic, and many of them have been reported to escape from neutralization by convalescent and vaccine sera. Neutralization of SARS-CoV-2 with recombinant human angiotensin-converting enzyme 2 (hACE2) based on the evolutionarily conserved spike-hACE2 interaction is one of the therapeutic strategies under development. However, preliminary clinical studies using soluble hACE2 (sACE2) to treat COVID-19 showed little contribution of sACE2 to disease recovery. Here, we aim at generating sACE2 decoys with enhanced potency and safety. By fusing sACE2 with human IgG1 Fc (sACE2-Fc) and introducing selected mutations, we could achieve potent and broad-spectrum neutralization as well as abolish the enzymatic activity of sACE2-Fc as demonstrated in in vitro neutralization assays and activity assays. Long-term overexpression of sACE2-Fc candidates using adeno-associated viruses in mice showed low immunogenicity, minimal influence on the renin–angiotensin system homeostasis, and no signs of tissue damage. In K18-hACE2 transgenic mice inoculated with authentic SARS-CoV-2, single-dose intranasal delivery of engineered sACE2-Fc proteins as prophylaxis provided 100% protection from infections with no observable symptoms or body weight loss; and intravenous injections as either prophylaxis or therapeutics also improved the survival by 50% in respective groups. Our results demonstrate the potential of the receptor-based decoy design strategy for SARS-CoV-2 neutralization and show that potent and safe neutralization modalities can be generated by integrating structural modifications and mutagenesis.

DRB16

CRISPR-Cascade: A cascade deep learning model to accelerate CRISPR-Cas9 off-target site detection

WEI Jun Kang

Supervisor: Prof. FENG Bo

Off-target effect in CRISPR–Cas9 system can lead to inadvertent gene-editing outcomes and is a bottleneck in the development of gene editing therapy. However, genome-wide off-target site detection by high throughput sequencing is costly and has limited value in identifying optimal sgRNA with minimal off-target effect and providing guidance for prior sgRNA design. Thus, we proposed CRISPR-Cascade, a cascade and comprehensive deep learning framework for genome-wide off-target site detection. Data imbalance in off-target effect analysis is a challenging issue, and CRISPR-Cascade is the first computational approach to address this issue to predict the repair-directed off-target site. We demonstrated that CRISPR-Cascade can accurately predict repair-directed off-target sites in GUIDE-seq and sufficiently alleviate data imbalance issue using cascade strategy. Besides, CRISPR-Cascade can successfully capture the patterns of sgRNA-target site pair, using sgRNA secondary structure and genomic flanking sequence. The leave-one-sgRNA-out evaluations reveal that CRISPR-Cascade can efficiently identify the off-target sites (AUC-PR: 0.505, Recall: 0.976) and outperforms the DSB-directed model CRISPR-Net (AUC-PR: 0.452, Recall: 0.890). The future versions of CRISPR-Cascade will expand the species and cell lines coverage.

DRB17

Study of in vivo gene editing outcomes using AAV-CRISPR edited hemophilia B mice

ZHANG Siqi

Supervisor: Prof. FENG Bo

AAV-CRISPR mediated in vivo knock-in, via either HDR- or NHEJ-based strategies, has shown therapeutic efficacy in preclinical studies. NHEJ knock-in was demonstrated with greater flexibility but resulted in various editing byproducts. Therefore, it's necessary to thoroughly examine in vivo gene editing outcomes in a high-throughput manner, before next-step optimization and clinical translation.

In this study, we selected hemophilia B as a disease model and performed AAV-CRISPR NHEJ knock-in. Hemostasis correction was achieved in both adult and neonatal mice in a long term, and the minimal effective doses through liver-specific gene knock-in using the hyperactive hF9R338L variant were further determined. With the liver RNA collected from these mice, we performed RNA-seq and nanopore-seq analysis. The transcriptional levels of transgene hF9 were consistent with blood assay results. The integration of NHEJ donors at both forward and reverse orientations were quantitatively evaluated, which were found at comparable levels. AAV integration at targeting sites was also detected but at a much lower rate. Interestingly, indels in neonatal mice were observed at much lower rates than that in adult mice. Based on this analysis results, future work will be focused to optimize the current system, in terms of further lowering AAV doses, enhancing knock-in efficiency and reducing byproducts.

DRB18

Potential regulation of spermatogonial stem cell fate by testicular extracellular vesicles cargoes

Tingting Zheng, Kathleen Choy, Cyan Chan, Jing Jin, Tiffany Yu, Ellis Fok

Supervisor: Prof. FOK Kin Lam Ellis

Spermatogenesis, a highly complex and regulated process, relies on a delicate balance between self-renewal and differentiation of spermatogonial sperm cells (SSCs). Despite the identification of growth factors required for SSCs maintenance, much knowledge of SSC microenvironment remains unknown.

Extracellular vesicles (EVs) carry cargoes including DNAs, RNAs, proteins and lipids, which play roles in multiple biological processes. Recent studies reported that testicular EVs (tEVs) were found near the basement membrane of seminiferous tubules in the testes of some animal models, whose distribution is similar to that of SSCs. Together with the uptake of tEVs by spermatogonial stem cell line C18-4, thus we investigated the function of tEV in SSCs self-renewal and differentiation. tEVs were isolated from adult and postnatal day 7 mice testes, respectively. Exposing SSCs to tEVs induce proliferation and decrease expression of undifferentiated spermatogonia makers, as well as RET and LHX1 which regulate GDNF pathway. Proteomics analysis and small RNA sequencing were conducted to explore the potential cargoes which mediated SSCs fate. RT-qPCR indicated let-7b and let-7c were abundant in tEVs, as well as relatively highly expressed in SSCs exposed tEVs. Furthermore, transfection of let-7b and let-7c mimics reduced expression of undifferentiated spermatogonia makers. To study the cell source of the tEVs which cause loss of stemness in SSCs, EVs were isolated from various germ cell and somatic cell lines, including C18-4, GC-1, GC-2 and TM4. C18-4 and TM4 cell line-derived EVs could decrease expression of undifferentiated spermatogonia makers.

To further demonstrate whether let-7b and let-7c are miRNAs in EVs that reduces the stemness of SSCs, we plan to construct let-7b/c knockout C18-4 cell line to exposing SSCs to EVslet-7b/c KO.

DRB19

DHX9, the gene encoding the DExH-box helicase DHX9, underlies neurodevelopmental disorders and Charcot-Marie-Tooth disease

GUO Tianyu

Supervisor: Prof. GU Shen Linda

DHX9, a member of DExH-Box helicase family, is an enzyme that can catalyze the ATP-dependent unwinding of both RNA and DNA, e.g., the R-loop structure. It is a highly conserved protein with critical cellular functions in regulating transcription and maintaining genome instability. Through the Baylor College of Medicine Genomics Research Elucidates the Genetics of Rare disease (BCM-GREGoR) Consortium and GeneMatcher, we identified 16 individuals with neurodevelopmental disorder (NDD) or Charcot-Marie-Tooth (CMT) disease presenting predicted damaging missense or loss of function variants in DHX9. For individuals whose parental samples were available, all variants were de novo (11/16). Human Phenotype Ontology (HPO) analysis demonstrated that individuals possessing missense variants in the nuclear localization signal (NLS) domain showed severe NDD phenotypes while those with loss of function (LoF) variants were associated with mild NDD. To investigate why different variants in DHX9 caused distinct phenotypes (mild NDD, severe NDD or CMT) and establish the potential genotype-phenotype correlations, we performed in vitro studies on all DHX9 variants. While wild-type DHX9 was restricted to nucleus, NLS missense variants abnormally accumulated in the cytoplasm. CMT-associated missense changes caused nucleolar DHX9 accumulation and may induce cellular stress. The severe NDD-associated variant p. (Arg141Gln) did not impact DHX9 localization but instead increased R-loop levels and double-stranded DNA breaks. Since DHX9 is a helicase that requires the energy from ATP hydrolysis to unwind nucleic acids, we designed ATPase assay and helicase assay to test which variants could potentially impact these functions. We observed one missense variant located in the helicase core region with significantly reduced ATPase activity, while the LoF variants completely abolished such activities. Ongoing experiments on helicase assay are being performed to further demonstrate the potentially deleterious effects of the DHX9 variants

DRB20

Functional characterization of recurrent truncating variant in UBAP1 associated with hereditary spastic paraplegia (HSP-SPG80)

Nicolas James Ho[1], Chun Wa Ho[1], Yi Jyun Lin[1], Xiaocheng Li[1], Shen Gu[1]

Supervisor: Prof. GU Shen Linda[1]

[1]School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China

Ubiquitin-associated-protein-1 (UBAP1) associated hereditary spastic paraplegia (HSP-SPG80) is a juvenile-onset genetic motor neuron disease (MND) resulting from the length-dependent axonal degeneration of corticospinal upper motor neurons (CSMNs). Despite identification of more than 70 subtypes and on-going research, knowledge in HSP physiopathology, and hence efficient management and treatment strategies are still lacking. Patients are primarily managed by symptomatic treatment with no cure currently available. SPG80 has been hypothesized to develop due to a dominant negative effect of a truncated UBAP1 protein on its normal function. In this present study, we aimed to mechanistically characterize a most common recurrent truncating c.426_427delGA variant in UBAP1 associated SPG80. Human embryonic stem cells (hESCs) with the delGA variant were generated by CRISPR-Cas9 genome editing system. Neural progenitor cells (NPCs) and organoids containing CSMNs were differentiated from edited and control hESCs. Apart from morphological comparison and fluorescent-labelling of marker genes, differential proteomic and ubiquitomic analysis between wildtype and mutant cells were conducted through liquid chromatography-mass spectrometry (LCMS). Besides in vitro studies, in vivo mouse model carrying the truncating delGA variant in Ubap1 was also generated. We observed motor defects in mutant mice older than six months through behavioral analysis. Subsequently, neuronal tracing experiment and single-nucleic RNA-seq of the motor cortex will be performed to investigate the defect neuronal lineage(s). Differential morphological, networking and transcriptomic analysis between wildtype and mutant mice will be conducted. Given that UBAP1 is a ubiquitously expressed protein, the reason why a specific population of neurons in the central nervous system is selectively vulnerable would provide insights in the motor neuron axonopathy mechanism. In vitro and in vivo morphologic, proteomic, ubiquitomic, and transcriptomic analysis together will shed light not only on SPG80 pathology, but also potential common pathological mechanisms of other HSP subtypes, as well as other MNDs.

DRB21

Investigating the epigenetic role of extracellular matrix stiffness in OPC aging and remyelination capacity

Shenyi PENG

Supervisor: Prof. JIANG Xiaohua

Ageing causes deterioration of tissue regeneration capacity due to declined function of stem cells and progenitor cells. In the brain, one important example is the oligodendrocyte progenitor cells (OPC), which produces myelin-generating oligodendrocytes (OL) to facilitate saltatory signal transduction. With age, OPC gradually loses its proliferation and differentiation capacities, resulting in accelerated myelin loss and cognitive decline which are associated with the development of neurodegenerative diseases. The OPC resides in extracellular matrix (ECM), which spatially assembles in the brain extracellular space and binds to the cell-surface adhesion molecules to support cell proliferation, differentiation, and migration. Interestingly, the mechanical properties of ECM in the brain change with age which contribute to the dysfunction of brain cells.

Recently, research showed that age-related increase of brain stiffness is sufficient to cause OPC deterioration. However, the question of how stiffness regulates OPC functions remains largely unknown. Mechanical force regulates a variety of cellular functions through inducing modulations in nuclear chromatin structures. In addition, epigenetic modification is crucial in regulating cell phenotypic changes and cell fate alteration through gene activation/deactivation. Specifically, emerging studies have demonstrated various epigenetic modulators can regulate OPC functions in aging; however, it is still unknown how mechanical and epigenetic modifications couple together to regulate molecular and cellular functions of OPC.

To address this gap, we have established a hydrogel model to recapitulate the age-related mechanical change of brain ECM. We have also performed preliminary histone modifying enzyme (HME) screening to identify potential HME targets that are critical for OPC differentiation. Using the current resources, we aim to elucidate the role of target HME in mediating the regulatory effects of ECM on OPC functions in the context of brain ageing.

DRB22

Deciphering the role of LGR5 in oligodendrocyte development and white matter repair

XU Dongke

Supervisor: Prof. JIANG Xiaohua

White matter (WM) injury, characterized by demyelination and loss of axonal integrity, is an important cause of long-term sensorimotor and cognitive deficits after brain damage. WM repair, including axonal regrowth, oligodendrogenesis and remyelination of demyelinated axons, is critical for rebuilding neuronal connectivity and reestablishing signal conduction. Unfortunately, the human brain has limited capacity for remyelination, at least in part due to the failure of differentiation of oligodendrocyte progenitor cells (OPCs) into mature, myelinating oligodendrocytes (OLs). Leucine-rich repeat-containing G-protein coupled receptor 5(Lgr5) is a co-receptor for Wnt signaling that marks highly proliferative stem and progenitor cells in many epithelial tissues. Surprisingly, while Lgr5 is the most recognized marker of adult stem cells, Lgr5 does not mark neural stem/progenitor cells. Instead, Lgr5 is expressed in a few subtypes of terminally differentiated neurons in mice. Despite limited reports on certain type of neurons, the role of LGR5 in glia cells is completely unknown. In this study, we reanalyzed the public database including bulk and single cell RNA sequencing data, and observed that Lgr5 was mainly expressed in OPCs. Using an Lgr5-EGFP reporter mouse line, we observed that Lgr5-EGFP+ cells were only restricted to the lesions following demyelination and well colocalized with OPC marker Ng2. Moreover, knockdown of Lgr5 in mouse OPCs suppressed cell proliferation. These results support an injury-evoked expression of Lgr5 in OPCs. We hypothesize that Lgr5 positive cells might represent a subpopulation of activated OPCs that are responsive to injury and play important role in remyelination and white matter injury repair.

DRB23

The Histone Demethylase KDM3A Controls Axon Growth by Its Dual Regulatory Role of Rac1

ZHANG Huan

Supervisor: Prof. JIANG Xiaohua

Axon growth is critical for the normal function of neurons. Defect in axon growth is associated with not only genetic diseases, but also neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD). Currently, most known molecular factors controlling axon development are cytosolic protein. However, a recent study provided the first set of evidence showing that axon specification and growth depend on basal histone 3 lysine 9 bimethylation (H3K9me2) nuclear levels. The molecular mechanisms by which axon growth is controlled are largely unknown. Recent studies have indicated that epigenetic regulation plays a key role in the neuronal polarization and axon growth. KDM3A is one of the histone demethylases that can specifically demethylate mono- and dimethyl-H3K9. However, the role of KDM3A in brain development and brain diseases is unknown. Our previous study shows that KDM3A is highly expressed in the hippocampus and cortex, and loss of Kdm3a leads to learning and memory deficit in mice. Thus, we aim to investigate the potential role of KDM3A in axon growth in this project. Here, we report that KDM3A is expressed in polarizing hippocampal and cortical neurons. Loss of Kdm3a leads to impaired neuronal polarization and axon extension. These results are confirmed in retinoic acid (RA)-induced neuroblastoma cell lines and neural stem cell-induced neurons as well. Mechanistically, we find that KDM3A co-localizes with Rac1 in both nucleus and cytoplasm. Moreover, in vivo BrdU injection assay and TBR2 labeling indicate that cortical neuron migration is hindered in the Kdm3a KO mice at E15-18. Taken together, the present findings suggest the important role of Kdm3a in neuronal polarization and axon growth during brain development.

DRB24

Development of a small molecular tool for regulating NGF/TrkA signaling in bone biology

LI Xiao[1]

Supervisors: Prof. Rocky. S. TUAN & Prof. JIANG Yangzi

[1]School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China

Nerve growth factor (NGF) signaling is essential for the survival and development of neurons. Tropomyosin receptor kinase A (TrkA) is the high-affinity receptor for NGF. Dysregulation of the NGF/TrkA pathway is associated with many diseases, including Alzheimer's disease, chronic pain, cancer development, and musculoskeletal disorder. As a novel therapeutic approach, small peptide drugs are introduced to regulate NGF/TrkA signaling. Computation simulation approaches have facilitated the design of peptides and promoted the study of peptides-receptor interaction. Because of clarification of NGF/TrkA crystal structures, NGF-derived peptides can be designed corresponding to the interface of NGF and TrkA. This study aims to develop small peptides with high binding specificity with TrkA receptors to regulate NGF/TrkA signaling. We reviewed the reported NGF-derived peptides with biological functions, and one NGF derived peptide was selected as the prototype peptide because of its specific binding with TrkA. After modification, we predicted the binding of peptides with TrkA through molecule docking platforms. Then, two peptides with high binding affinity were selected and synthesized. The biological function assay showed both peptides enhanced the calcification of human chondrocytes and human BMSC using alizarin red staining, indicating that NGF derived peptides can regulate the biofunction of bone system. The molecule mechanism will be studied in the future.

DRB25

Sucralose Improves Vascular Function and Reduces Oxidative Stress in Obese Mice

ZUO Yuanyuan

Supervisor: Prof. KO Wing Hung

Objectives: Artificial sweeteners are the food additives which provide intense sweet taste. As they contain significantly less calories as compared to sugar, they are widely used as sugar alternatives in foods and drinks. Artificial sweeteners contain little or no sugars, and hence lower energy intake, which is useful in managing blood glucose and body weight. However, the vascular effects of artificial sweeteners are basically unknown. The present study aims to evaluate the impact of chronic treatment with sucralose on endothelial function in diet-induced obese (DIO) mice and to understand the possible underlying mechanisms.

Methods: Eight-week-old male C57BL/6 mice were fed with high-fat diet for 10 weeks to induce diet-induced obesity, and then sucralose was administered via oral gavage in two dosages: 10 and 30 mg/kg/day, respectively, for 8 weeks. By the end of treatment, mice were sacrificed, plasma levels of lipids and glucagon-like peptide-1 (GLP-1) were measured. Acetylcholine (ACh)-induced endothelium-dependent relaxations (EDR) in mouse arteries were recorded on a multi-channel myograph system. Accumulation of reactive oxygen species (ROS) was measured in en face endothelial cells of mouse aortas and human endothelial cells using dihydroethidium (DHE) staining.

Results: Chronic treatment of sucralose improved ACh-induced relaxations in mesenteric arteries from DIO mice. En face DHE staining shows that sucralose significantly decreased DHE fluorescence intensity in DIO mouse aortas, indicating that sucralose is effective to lower the ROS level. The ex vivo study showed that acute treatment with sucralose can attenuate interleukin-1β-induced endothelial dysfunction and suppressed oxidative stress in DIO mouse aortas. In addition, sucralose treatment increased the ratio of p-eNOS/eNOS and p-Akt/Akt, elevated the production of nitric oxide, and reduced ROS levels in cultured human endothelial cells.

Conclusions: The present study shows that chronic consumption of sucralose is beneficial in improving endothelial function in diet-induced obese but not in normal lean mice. This

vasoprotective effect is attributed to reduced oxidative stress and elevated NO bioavailability (This study is supported by HMRF 06173956).

DRB26

The roles of *Irx3* and *Irx5* in mouse cochlear outer sulcus development

WENG Xin[1], LEUNG King Wing Bernice[2], SO Kan Hei Karl[1]

Supervisor: Prof. SHAM Mai Har[1]

[1]School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR

[2]School of Biomedical Sciences, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR

Ion homeostasis in the cochlea is essential for maintaining the endo-cochlea potential. In mammalian cochlea, this is achieved by the outer sulcus (OS) cells and stria vascularis (SV). Although physiological studies have shown the importance of these cells, knowledge of their development and maturation is inadequate.

Mutation in a transcription factor *IRX5* leads to sensorineural hearing loss in Hamamy syndrome patients. Our previous data suggested *Irx5* and its family member *Irx3* are broadly expressed in cochlear epithelium, and enriched in mouse OS cells. Studies revealed that *Irx3* and *Irx5* have redundant and specific functions in diverse systems. In this study, we use *Irx3* and *Irx5* mutant mice models to explore the development of cochlear epithelium, and the roles of *Irx* genes in it.

SV is comprised by three cell layers which are marginal cells (MC), intermediate cells (IC) and the basal cells (BC). In *Irx3/5*$^{-/-}$, distribution of *Otx2*$^{+}$ Reissner's membrane and *Kcnq1*$^{+}$ MC was abnormal. Misalignment of SV was indicated by restricted MC, a medially located *Cd44*$^{+}$ IC. OS develops from E14 and matures at postnatal stages. *Cxn26* expression in presumptive OS remained at E16.5. However, adjacent LER domain was abnormal, indicated by absent *Cd44* expression. At postnatal stages, OS cells differentiate to form root processes in WT. In *Irx5*$^{-/-}$ and *Irx3*$^{-/-}$, *Aqp5*$^{+}$ OS was observed with shorter root processes. In *Emx2*^{Cre} *Irx3/5*$^{-/-}$, the root processes were absent.

These observations suggested that the dorsal cochlear cell commitment and the alignment of the SV were affected by *Irx3* and *Irx5*. The OS domain could develop in *Irx3/5*$^{-/-}$, but its differentiation was abnormal in the absence of *Irx3* and *Irx5*. How the root processes were regulated by the *Irx3* and *Irx5* requires further investigation.

DRB27

Impairing TRPML1 activity protects OA chondrocytes from apoptosis by attenuating mitochondrial dysfunction

YAO Hanyu[1], ZHANG Fengjie[1]

Supervisor: Prof. WAN Chao[1]

[1]School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China

Osteoarthritis (OA) is the most common type of arthritis, characterized by chondrocyte apoptosis and extracellular matrix degradation, resulting in disability in its advanced stages. Various risk factors, including genetics, trauma, and aging, have been implicated in the pathogenesis of OA, but the causative molecular mechanisms remain elusive. Lysosomal dysfunction has been shown to trigger chondrocyte apoptosis in OA, in which alterations of lysosomal Ca2+ signaling and its consequences may involve in this pathology, but the underlying mechanisms are lacking. In the present study, an in vitro chondrocyte degeneration model was established in cultured chondrocytes by addition of interleukin-1β (IL-1β) and an in vivo OA model was constructed in mice by surgical destabilisation of medial meniscus (DMM). In chondrocytes, IL-1β treatment reduced lysosomal Ca2+ concentration while upregulating the expression of a lysosomal Ca2+ channel transient receptor potential mucin 1 (TRPML1). This suggests that IL-1β induces lysosomal Ca2+ efflux by enhancing TRPML1 activity. By applying TRPML1 antagonist, ML-SI1, chondrocytes apoptosis was reversed in IL-1β-treated chondrocytes. Interestingly, this phenotype was associated with the potential of ML-SI1 to attenuate structural and functional changes in mitochondria and prevent mitochondrial dysfunction. In the mouse OA model, intra-articular injection of ML-SI was shown to efficiently attenuate articular chondrocyte apoptosis. These results reveal a potential role of TRPML1 in regulating mitochondrial dysfunction and chondrocyte apoptosis. Our data suggest that targeting TRPML1 may serve as a promising therapy for OA, yet its detailed molecular mechanisms and therapeutic efficacy await further investigation.

DRB28

An optimised hybrid hydrogel bioink generated by chemical crosslinking approach facilitates engineered cartilage formation

ZHOU Zheyuan, ZHANG Fengjie, WAN Chao

Supervisor: Prof. WAN Chao

School of Biomedical Science, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China.

Osteoarthritis (OA) has been a major clinical challenge and public health issue worldwide. Being lack of nerves and vasculature, articular cartilage has very limited self-regeneration capacity. Tissue engineering has been considered as a promising approach to facilitate articular cartilage repair or regeneration. Among the various newly developed tissue engineering modalities, 3D bioprinting has emerged as a powerful technique that could accurately fabricate 3D bioscaffolds using defined bioinks to facilitate tissue engineering. In this study, a type of newly defined alginate-gelatin (A-G) hybrid hydrogel bioink was synthesized using the carbodiimide chemistry crosslinking method with different concentrations (A3G6 and A4G8). Fourier-transform infrared spectroscopy (FTIR) revealed the formation of covalent bonds between the alginate and gelatin molecular structures. Young's modulus of the hybrid hydrogel (A4G8) in the EDC/sulfo-NHS-treated group was significantly higher (29.5 ± 5.3 kPa) than that of pure alginate controls (13.5 ± 3.6 kPa). This was accompanied by significantly decreased degradation rate following 7 days incubation. The chondrocytes incorporated A-G hybrid bioinks exhibited satisfied printability. Real-time PCR analysis showed that following up to 21 days culture, mRNA levels of chondrogenic marker genes *Sox9*, *Col2α1* and *Comp* were upregulated in chondrocytes in the A-G hybrid bioscaffolds compared with that of the controls. This was accompanied by increased Sox9 positive chondrocyte numbers and production of Col II in the extracellular matrix of the A-G hybrid bioscaffolds. In the subcutaneous transplantation model in severe compromised immunodeficiency (SCID) mice, following up to 6 weeks incubation, chondrocytes in the A-G hybrid bioscaffolds produced more abundant of proteoglycan component and the formation of typical chondrocyte lacunae structure than that of the controls. Our results suggest that the newly generated carbodiimide chemistry crosslinked A-G hybrid bioink possesses advantages of printability, biocompatibility and chondrogenic potential for generating engineered cartilage tissue by 3D bioprinting.

DRB29

Tendinopathy: Multiomics analysis of pathogenesis and development of a disease-targeting biomaterial for tendinopathy treatment

Ying Rao[1,2]

Supervisors: Prof. WANG Dan Michelle [1,2] and Prof. TUAN Sung Chi Rocky [1,2]

[1]School of Biomedical Sciences, Faculty of Medicine,[2]Institute for Tissue Engineering and Regenerative Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China.

Tendinopathy describes a complex multifactorial pathology of the tendon, characterized by pain, functional decline, and reduced exercise tolerance. Clinical treatment for tendinopathy has variable outcomes, which may be partially due to its poorly characterized pathogenesis. Considering this, multiomics approach provides insight into differences in molecular and metabolic pathways during disease development, which can be used to better understand tendon pathology and regeneration. To enhance tendon healing, our laboratory has previously developed a soluble, DNA-free, urea-extracted ECM fraction from bovine tendons (tECM), which exhibited robust tenogenic bioactivity on human adipose-derived stem cells (hASCs) and high regenerative effects for the repair of large acute tendon defects in both rats and rabbits. Therefore, in this study, we would like to further understand the pathogenesis of tendinopathy through multiomics analysis and develop a disease-targeting biomaterial for treating tendinopathy. In our present work, we established a collagenase-induced rat patellar tendinopathy model, evidenced by gross morphology and histopathology. Furthermore, we developed a tECM-based hydrogel to investigate the tendinopathy healing capacity. Our data show that collagenase-induced rat patellar tendinopathy model exhibited a complete loss of architecture, abnormal tenocyte, chondrocyte-like cells which are indicated by round cells with lacunar space, irregular fibres, and neovascularization. Furthermore, we developed a tECM-containing, gelatin methacryloyl (GelMA) hydrogel (tECM gel). Our in vitro data shows that tECM gel enhanced hASC tenogenic differentiation based on immunostaining and qPCR of tenogenesis-associated markers. Our in vivo data indicated tECM gel groups still showed obviously increased cellularity and the presence of chondrocyte-like cells. In summary, our data suggested that a rat tendinopathy model was established with a chondrogenic phenotype as similarly reported while tECM gel cannot completely change the chondrogenic phenotype of collagenase-induced rat patellar tendinopathy. Therefore, our future study may focus on

gaining a comprehensive understanding of the tendinopathy disease process and improving precision tendinopathy treatments.

DRB30

Targeting tendon biomimetic niche via a biochemically and biomechanically functionalized biomaterial for prevision tendon regeneration

ZHANG Wan Qi

Supervisor: Prof. WANG Dan Michelle and Prof. TUAN Sung Chi Rocky

Due to overuse or age-related degeneration, tendon injuries have become a common clinical problem. Tendon healing is slow due to the lack of sufficient cells and vascular, often accompanied by fibrotic scarring and adhesion formation. Mesenchymal stem cells (MSCs) have been extensively studied and are considered a promising cell candidate for tendon repair and regeneration. However, issues such as acute cell death, low functional engraftment yields, and off-target tissue formation remain critical obstacles for clinical translation. Here, by combining biochemical and biomechanical stimulation, a biomimetic hydrogel (TenoGel) was developed to create a functional tenogenic niche for stem cell pre-conditioning and delivery. TenoGel possessed an interpenetrating polymer network with high toughness and elasticity, which can support the adhesion, elongation and fast proliferation of human adipose-derived stem cells (hASCs). After being stimulated by tendon-specific inductive factors and tensile loading, hASCs revealed organized cytoskeletal structure along the loading direction and enhanced expression of tenogenic markers in vitro. Furthermore, in a rat patellar tendon defect model, implantation of co-stimulated TenoGel with rat adipose-derived stem cells (rASCs) achieved enhanced tendon regeneration as evidenced by wavy, organized matrix structure and enhanced biomechanical features, similar to the uninjured control group. It is worth noting that rat ASCs delivered in hydrogel without biochemical and biomechanical co-stimulation induced cartilage-like tissue formation instead based on histological observation. Therefore, the developed TenoGel scaffold creates a tendon biomimetic niche to induce reliable tenogenic differentiation of stem cells, and can be a promising strategy for pre-conditioning and delivery of stem cells for augmented tendon repair, which will facilitate future clinical implementation for stem cell-based tendon therapy.

DRB31

Knockout of thyroid hormone receptor Alpha a (thraa) facilitates heart regeneration in zebrafish

CHEUNG Man Yee

Supervisor: Prof. ZHAO Hui

The thyroid hormone (TH) has been recognized as an essential factor for regulating metabolism as well as embryonic development. Recently, it has also been implicated in heart regeneration. The active form of TH, triiodothyronine (T3), binds to nuclear TH receptors (TRs), which primarily reside in the nucleus and function as a ligand-dependent transcription factor for transcriptional activation. In order to elucidate the function of the TH-TRa1 signaling axis during heart regeneration, we utilized the zebrafish thraa mutant as the animal model to study the mechanism during this process. The thraa 8-bp ins mutant fish harbours an 8-bp insertion at the thraa gene locus, leading to loss of T3-binding activity and transcriptional capacity. We have performed a time-course experiment by RNA-sequencing to study the dynamic changes of transcriptomes in thraa mutant fish. We found that thraa mutant fish has a profound inflammatory response after injury; however, the mutant then exhibits a higher capability in replenishing the loss of cardiomyocytes by inducing cell cycle progression, proliferation, and differentiation in the regenerative stage. Our study suggests a beneficial effect of loss of function in TH-TRa1 signaling in heart regeneration by modulating the immune response and promoting the proliferation of cardiomyocytes.

DRB32

Identification of small molecules that promotes the maturation of human embryonic stem cell-derived beta-like cells

DENG Jiani and ZHAO Hui

Supervisor: Prof. ZHAO Hui

School of Biomedical Sciences, The Chinese University of Hong Kong, HKSAR, China

Diabetes mellitus is one of the leading causes of mortality and reduces life expectancy worldwide. Its global prevalence is about 9.3%, affecting millions of people worldwide. Type I diabetes is an autoimmune disease that originates when the immune system attacks and destroys cells that make insulin (pancreatic ® cells). Islet transplantation holds great promise to treat type I diabetes, but this therapeutic approach is limited by the shortage in supply of organ donors and immunosuppression issues associated with transplantation. Human pluripotent stem cells (hPSCs) can give rise to all body cell types, providing unlimited resources for self-renewal. Generation of functional ® cells from hPSCs has emerged as an attractive therapeutic approach. However, hPSC-induced pancreatic cells are far from satisfactory, lacking the capacity of fast responding and regulating blood glucose concentration in vivo. To further improve the differentiation protocol in the early stage and generate the functional mature cells for clinic transplantation, our research focuses on the identification of small molecules that can promote the maturation of hPSCs-derived endocrine precursors cells. We confirmed that the $Insulin^{GFP/W}$ reporter hES cell line could work well on our platform, and we have modified the differentiation protocol by using a simplified chemical combination. Our revised differentiation protocol is better suited for chemical screening to be done next. Overall, this project will provide a simple and robust treatment strategy for type I diabetes.

DRB33

Coiled-coil domain-containing protein-141 (CCDC141) is essential for heart development and function

Daniel Wuyang Dio[1], Wenjuan Zhu[2] and Hui Zhao[1]

Supervisor: Prof. ZHAO Hui

[1]School of Biomedical Sciences, The Chinese University of Hong Kong

[2]Centre for Cardiovascular Genomics and Medicine, The Chinese University of Hong Kong

Coiled-coil domain-containing protein-141 (CCDC141), also known as the Coiled-coil protein Associated with Myosin-II Disc-I (CAMDI), is coded by the *CCDC141* gene on the q-arm of chromosome 2 in humans. CCDC141 has been reported to co-localize with DISC1 around the centrosome and plays a critical role in radial migration and function in neurons. Mutations in this gene have been implicated in hypogonadotropic hypogonadism. However, its role in heart development has not been reported.

Secondary analysis of RNA-seq data of normal adult human heart, Neonatal mouse heart, and cardiomyocytes of zebrafish embryo at 20 hours post fertilization (20 hpf) showed that *ccdc141* is abundantly expressed in the heart predominantly in cardiomyocytes, among other cardiac cell types. Morpholino-induced knockdown and overexpression of *ccdc141* in zebrafish embryos interfered with heart looping and induced pericardial oedema. Furthermore, *ccdc141* morphant zebrafish embryos showed decreased heart rate nearly dose-dependently. We conducted in situ hybridization using probes of key cardiac markers (*cmlc2* and *nkx2.5*) to visualize the effect of knockdown of *ccdc141* at the early stages of cardiogenesis in zebrafish. Intriguingly, morpholino-mediated *ccdc141* knockdown induced a reduction in *cmlc2* expression and a concomitant increase in *nkx2.5* expression in a dose-dependent manner during the early stages of heart development. *Ccdc141* disrupted zebrafish mutant was generated by CRISPR/Cas9 to further characterise ccdc141 during heart development. The Crispants recapitulated pericardial oedema as seen in the morphants. Homozygous Mutant embryos also showed reduced heart rates. Further characterization of the *ccdc 1*41 mutants is ongoing.

In conclusion, our results portrayed CCDC141 to play an essential role in heart development and function. However, further studies are required to elucidate the exact molecular function of the protein during heart development.

DRB34

Identification of essential factors that promote pancreatic β cell differentiation using genome-wide CRISPR/Cas9 screening

TANG Qi[1], GUO Yue[2], DENG Jiani[1], CHEUNG Man Yee[1]

Supervisor: Prof. ZHAO Hui[1]

[1] School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, P.R. China

[2] Department of Surgery, Faculty of Medicine, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, N.T., Hong Kong SAR, P.R. China

Type I diabetes (T1DM) is an autoimmune disease caused by damage to the pancreatic β cells. Patients suffering from T1DM have to inject exogenous insulin by syringe or pump all their life to maintain their blood sugar levels. But these methods are invasive and traumatic. Alternatively, pancreatic islet transplantation serves as an effective therapeutic approach; however, this approach is barriered by lacking donor islets. Human embryonic stem cells (hESCs) are now regarded as promising sources for producing pancreatic β cells. But compared to the endogenous islets, the capacity of glucose-stimulated insulin-secretion of the hESCs-induced β cells is far from satisfactory. Mitochondria is essential for β cell functional maturation during the differentiation from pancreatic progenitor cells to pancreatic β cells. The underlying mechanisms as to how mitochondria affect the insulin secretion of hESCs-derived-pancreatic β cells, however, are not fully understood. We plan to perform CRISPR/Cas9 screening to identify essential factors that can promote the insulin secretion of hESCs-derived β cells. We have successfully established the β cell differentiation platform and examined the marker gene expression at each differentiation stage. In addition, we checked the quality of the CRISPR library and successfully maintained the pancreatic progenitor cells in vitro. The screening process and the following bioinformatic analysis are ongoing.

DRB35

Synthetic glucose–sensor gene circuit enhances blood-glucose homeostasis in type 1 diabetic mice

Yuan ZHANG[1]

Supervisor: Prof. Hui ZHAO[1]

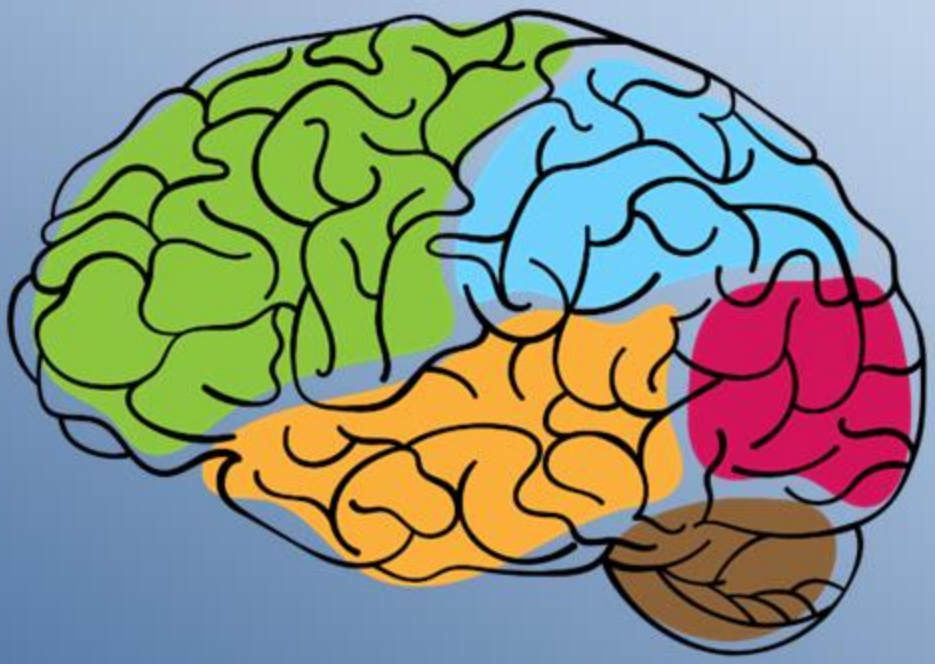
[1]School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong SAR, PR China

The abnormally high blood glucose level of type 1 diabetes (T1D) results from the autoimmune destruction of insulin-expressing islet beta cells. Periodic exogenous insulin delivery is the standard treatment for T1D. Although many formulations of insulin have been developed to stimulate endogenous insulin secretion, the risk of hypoglycemia secondary to insulin therapy still exists. Here, we show that a synthetic glucose sensor gene circuit can sense hyperglycemia and secrete insulin accordingly. This synthetic device consists of a yes-associated protein 1 (YAP1) fused with transcription factor. The fusion protein can be translocated into the nucleus in response to high glucose signals and triggers a dose-dependent insulin transcription via cooperating with a modified inducible Tet-Off expression system. The in vitro experiments proved that the glucose-sensor device could precisely sense the elevation of extracellular glucose levels and show broad applicability in mammalian cell lines. In vivo implantation of microencapsulated, glucose-sensor system-engineered cells could substantially improve blood-glucose homeostasis of T1D. A synthetic gene circuit designed to sense hyperglycemia and coordinate insulin expression bring insights to establish novel therapeutic approaches for diabetes mellitus.

SCHOOL OF BIOMEDICAL SCIENCES

Postgraduate Research Day 2022

Neural, Vascular, and Metabolic Biology (NVMB)



Neural, Vascular, and Metabolic Biology

Title	Author	Number
Neural representation of hindlimb muscle synergies in the mouse spinal cord revealed by spike-triggered averages of ontogenetically elicited electromyographic activities	HE Bo Rong	NVMB1
The mechanism and role of fidgety movements in infant motor development revealed by longitudinal comparisons of muscle synergies	LIN Jia Yin	NVMB2
LETM-domain containing 1 (LETMD1) as a key player in adipocyte energy dissipation: working mechanisms, therapeutic implications, and regulation of expression	LIU Jia Xing	NVMB3
The role of circHomer1 in dendritic spine maintenance and hippocampus-dependent spatial learning and memory	CAI Ying	NVMB4
Applications of two-photon in vivo imaging to dissect neural circuits in health and disease	YIP Hei Matthew	NVMB5
Cortical visual impairment in CDKL5 Deficiency Disorder (CDD)	YUAN Shi Yang	NVMB6
Modulating social transmission of affective states through competition	GOH Chen Wei	NVMB7
Roles of the prefrontal cortex in decision-making under situations of uncertainty	RONG Kang Lin	NVMB8
Empathy-Motivated Prosocial Behavior in Rats	ZHU Yun	NVMB9
Development of a novel therapeutic strategy for obesity and related diseases	LIN Zhi Qiang	NVMB10
The Effect of Arginine Depletion on Liver Cancer	ZHENG Lu Xi	NVMB11
Combination therapy of arginase and metformin on obesity and associated metabolic disorders	ZHENG Xiu Hua	NVMB12
The protective effect of statin against vascular toxicity induced by BCR-ABL tyrosine kinase inhibitors	HAN Yu Meng	NVMB13
The role of SOX4 in regulating vascular function during hypertension	WU Wei Yan Vivian	NVMB14
Mechanosensitive ion channel TRPV4 regulates cancer stemness in ovarian cancer	LEI Zhen Chuan	NVMB15
S-glutathionylation underpins the inhibitory effect of fusaric acid on banana K+ uptake MaAKT1 channels	ZHANG Jun	NVMB16
Neural circuits underlying thiamine perception	GAO Ming	NVMB17

Neural representation of hindlimb muscle synergies in the mouse spinal cord revealed by spike-triggered averages of ontogenetically elicited electromyographic activities

HE Bo Rong[1], SALMAS Paola[1], ZHANG Jing[2]

Supervisor: Prof. CHEUNG Chi Kwan Vincent[1]

[1]School of Biomedical Sciences, Gerald Choa Neuroscience Centre, The Chinese University of Hong Kong

[2]The James Franck Institute, University of Chicago, Chicago, IL, USA.

Movement execution requires the motor system to coordinate a large number of neuro-musculoskeletal elements. To simplify motor control, it has been proposed that spinal interneurons are organized into functional motor modules which activate different muscles concurrently as muscle synergies, and are recruited with distinct temporal activation profiles. Though previous studies using electrical micro-stimulation of the spinal cord delivered during electromyographic (EMG) and neural recordings have found that the spinal premotor interneurons underlie the muscle synergies, less is known about the specific interneuronal types that contribute to their organization. Here, we use optogenetics to selectively stimulate excitatory spinal interneurons in the anesthetized Thy1-ChR2 transgenic mice while simultaneously recording intramuscular EMG (6 hindlimb muscles) and neural spike trains of spinal interneurons. Neural activities were recorded using soft carbon nanotube (CNT) electrodes. Since their shapes are not rigid, CNT electrodes do not require any holder for mechanical support at the recording sites, thus leaving the exposed spinal cord surface free of obstacles. This in turn allowed us to stimulate the lumbosacral spinal cord comprehensively by moving the laser in steps of 200-μm increment along the spinal surface. Using spike-triggered averages of EMG, the post-spike effect of a spinal interneuron on the recorded muscles was quantified as a muscle field. We found that the muscle fields of the recorded interneurons matched the muscle synergies extracted from EMG by nonnegative matrix factorization; We argue that during locomotion, the hindlimb muscles are activated not randomly, but through recruitment of the muscle synergies with their temporal activities modified by the group of spinal interneurons. Furthermore, different synergies may result from different combinations of interneurons with distinct muscle fields. Nevertheless, the synergies' activation coefficients may be specified by a population of interneurons responsible for precise temporal control of the muscle synergies. Supported by CUHK Faculty Innovation Award (FIA/2016/A/04) to VCKC.

NVMB2

The mechanism and role of fidgety movements in infant motor development revealed by longitudinal comparisons of muscle synergies

Jiayin Lin[1], Kelvin Y. S. Lau[1], Richard Liu[1], Chao-Ying Chen[2]

Supervisor: Vincent C. K. Cheung[1]

[1]School of Biomedical Sciences, and The Gerald Choa Neuroscience Centre, The Chinese University of Hong Kong, Hong Kong, China

[2]School of Physical Therapy and Graduate Institute of Rehabilitation Science, College of Medicine, Chang Gung University, Taoyuan, Taiwan

Fidgety movements (FMs) appear at the age of 2-5 months of infants, with a significant predictive ability of later cerebral palsy (CP) with 98% accuracy. Considering the great prevalence of CP, 3 per 1000 births, and its leading harm to infant motor function, it is of importance to understand the functioning mechanism of FMs and explore its usages for early prevention and intervention. However, existing researches only proposed some behavioral features of FMs from kinematic data and failed to reveal how and why FMs could predict later behaviors. Muscle synergies (MS), within the framework of motor coordination, are capable of describing and quantifying the change of neuromotor control mechanisms under different scenarios. This project plans to collect multi-channel surface electromyographic (EMGs) on legs and extract out MS across three developmental stages for comparisons: FMs, supported walking (SPWalk) and independent walking (IPWalk). So far, for 5 healthy subjects, we have extracted out their MS of FMs, pointing out the exact way of muscle coordination behind FMs. Besides, we have compared MS of 4 types of behaviors at the first stage (FMs) with MS of the second stage (SPWalk) and found that MS of FMs among behaviors at the first stage are most similar to MS of SPWalk and best reconstruct EMGs of SPWalk. This high similarity of MS between FMs with SPWalk demonstrate that FMs may serve as the precursor of later behaviors, which might be a premise of FMs predicting CP. More analyses and comparisons are entailed to reveal the nature and function of FMs.

NVMB3

LETM-domain containing 1 (LETMD1) as a key player in adipocyte energy dissipation: working mechanisms, therapeutic implications, and regulation of expression

LIU Jia Xing

Supervisor: Prof. HUI Xiaoyan Hannah

Obesity is characterized by excessive accumulation of body fat and is now becoming a major public health issue. Beige adipocyte is a special type of adipocyte that is capable of burning energy in the form of heat and therefore has been a new therapeutic target for metabolic diseases. By Tandem Mass Tag-based quantitative proteomics analysis of the purified mitochondria from inguinal white adipose tissue (iWAT), we previously identified LETMD1 protein as a potential candidate contributing to energy dissipation in mouse beige adipocytes. However, the function of LETMD1 in human beige adipocytes as well as how the expression of LETMD1 is regulated remains to be elucidated. In this project, we cultured the human induced pluripotent stem cell (hiPSC)-derived beige adipocyte, and demonstrated that overexpression of LETMD1 enhanced mitochondrial respiration. Furthermore, such an effect was still present when uncoupling protein 1 (UCP1), the classical thermogenic gene was knocked-out, demonstrating that LETMD1 engages a non-classical energy dissipation pathway. Further, we established a fluorescence-based LETMD1 reporter hiPSC line, which can reflect endogenous LETMD1 expression in a high-throughput manner. This LETMD1 reporter cell line enabled us to screen a preclinical compound library in which several potential hits have been identified. An unbiased CRISPR-Cas9-based transcription factor knockout library screening will also be performed to identify key regulators and pathways controlling LETMD1 expression in human beige adipocytes. Meanwhile, we are currently also examining the anti-obese, anti-diabetes function of LETMD1 in the animal model. We will examine whether enhancing the expression of LETMD1 in beige adipocytes could improve whole-body metabolism. Collectively, the work of the current project will provide valuable insight into LETMD1-targeted approaches for the management of obesity, diabetes, and metabolic diseases.

NVMB4

The role of circHomer1 in dendritic spine maintenance and hippocampus-dependent spatial learning and memory

CAI Ying[1], XU Hao Yu[1], YUAN Shi Yang[1], ZHENG Zhong Yu[1], ZHU Yao[1]

Supervisor: Prof. IP Pak Kan Jacque[1]

[1]*School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, China*

Dendritic spines are structural basis of neural activity, but how the structure and synaptic protein expression are regulated is still unclear. Here we found circHomer1, a circular RNA derived from Homer1 gene and related to neural activity, regulates dendritic spine structure and density, as well as the expression of synaptic protein. To further understand the underlying mechanism, we explored the interacting partners of circHomer1. We validated that circHomer1 interacts with fragile X mental retardation protein (FMRP), a RNA binding protein involving in RNA transport, translation and stability on dendrites. In addition, circHomer1 knockdown affected FMRP dendritic distribution. To examine whether circHomer1 regulates neuronal function, we knockdown circHomer1 in vivo in the CA1 region of mouse hippocampus with adeno-associated virus, and performed object location test and reward T-maze to evaluate the spatial learning and memory of the circhomer1 knockdown mice, as compared to control mice. We found that knockdown of circHomer1 in CA1 region impaired the spatial learning and memory of mice. Taken together, our research indicates a crucial role of circHomer1 in regulation of dendritic spine structure and neuronal functions.

NVMB5

Applications of two-photon in vivo imaging to dissect neural circuits in health and disease

YIP Hei Matthew

Supervisor: Prof. IP Pak Kan Jacque

CDKL5 Deficiency Disorder (CDD) is identified as a severe X-linked neurodevelopmental disorder resulted from the catalytic activity loss of Cyclin-Dependent Kinase-Like 5 (CDKL5). In the deficit of this serine/threonine kinase, the CDD patients not only present variety of autistic features but also prominent cortical visual impairment. However, the cellular phenotypical change in the visual cortex is still unclear. In this project, two-photon in vivo microscopy was employed to study the neuronal activity of primary visual cortex (V1) under drifting grating visual stimulations. The calcium signals of the layer II/III neurons in the binocular zone of V1 were recorded in adult wildtype (WT) and CDKL5 knockout mice. Our results showed that populational orientation selectivity, the neuronal response towards specific orientation of visual stimulus, was impaired in the CDKL5 knockout mice. Moreover, the neuronal responses from contra- and ipsi-lateral eye in knockout model was poor correlated, as compared to WT animals. Additional analysis confirmed significant difference in preferred grating orientation of two eyes and suggested binocular matching, one of the neural circuit modification processes in critical period, was also impaired in the absence of CDKL5. In summary, our study using two-photon in vivo imaging visualized and revealed abnormal neuronal responses in the V1 of CDKL5 knockout mice. We will continue to investigate the underlying mechanisms in CDD pathophysiology.

NVMB6

Cortical visual impairment in CDKL5 Deficiency Disorder (CDD)

YUAN Shi Yang, ZHU Yao, YIP Hei Matthew, ZHENG Zhong Yu, CHAN See Wing Maggie, Siu Gavin

Supervisor: Prof. IP Pak Kan Jacque

Cdkl5 deficiency disorder (CDD) is an X-linked neurodevelopmental disorder caused by a genetic defect in a serine/threonine kinase, Cyclin-Dependent Kinase-Like 5(CDKL5). In addition to a range of neuropsychiatric features, patients with CDD consistently exhibit cortical visual impairments, and it is a distinct clinical symptom in at least 75% of CDD patients. However, it is still unclear how the mutated Cdkl5 gene leads to neuron circuit-specific sensory processing impairment and functional deficits. The present study shows alterations of spine number and morphology in excitatory neurons in layer II/III and layer V in the primary visual cortex (V1). Mechanistically, phopsho-proteomics screen identified Neuronal Elav (embryonic lethal, abnormal vision)-like (Elavl), which contains the Cdkl5 phosphorylation consensus motif and involved in RNA processing, splicing and is implicated in neurodevelopment disorders. Bulk RNA-sequencing showed Arc, Fos, and Npas4 are down-regulated, which are well-known activity-dependent genes in mammal brains. In summary, we have characterized the impacts of disruption of CDKL5 protein on visual functioning and investigated the mechanism of CDKL5 deficits in vivo.

NVMB7

Modulating social transmission of affective states through competition

GOH Chen Wei, YUNG Wing Ho, KE Ya

Supervisor: Prof. KE Ya

Empathy is an essential component of social communication that involves the transmission of affective states which in turn guides future behaviour. Previous studies have identified evolutionarily conserved processes of empathy in mice, which can manifest in the form of emotional contagion and prosocial affiliative behaviours such as consolation towards their conspecific-in-distress. However, the question of how social relationships, particularly the history of competition, modulate these empathic-like processes is rarely discussed. Here, we aim to provide evidence to support the role of competition in altering transmission of affective states between two conspecifics. By limiting the access to mating partner or food resources, pairs of socially interacting male C57BL/6 mice displayed increased inter-male aggression, suggesting the occurrence of a competition-like internal state. Contrary to expressing empathic behaviors towards a cagemate-in-distress, it was found that responses including vicarious freezing and consolation behavior were significantly attenuated between mice who had a history of competition, hence implied a lack of social transmission of negative affect. Utilizing a machine learning-based method for characterizing the underlying structure of mouse behavior, we revealed that mice would exhibit a specific pattern of actions which recapitulated active observation when an aversive stimulus was delivered to a competitor conspecific, but not towards a non-competitor cagemate. Moreover, the observation of a competitor experiencing misfortunes also triggered a positive emotional valence which manifested as conditioned place preference. Therefore, our results demonstrate that empathy is not a universal response to another distressed individual, but instead it is also strongly modulated by competition.

NVMB8

Roles of the prefrontal cortex in decision-making under situations of uncertainty

RONG Kang Lin, YUNG Wing Ho, KE Ya

Supervisor: Prof. KE Ya

Decision-making under situations of uncertainty is an issue that every organism frequently encounters. Under this condition, a good strategy is to rely on prior information from experience. In some diseases, e.g., Parkinson's disease, patients exhibit impaired use of prior information in conditions of sensory uncertainty. Given the role of the prefrontal cortex (PFC) in decision-making, this study explores the involvement of PFC in situations of uncertainly in which prior information may provide useful hint for decision-making. C57BL/6 mice were employed to develop an auditory decision-making behavioral paradigm. After one month of training, all mice were able to associate sounds of different frequencies (3kHz and 16kHz) paired with the left and right side of the experimental chamber respectively to get rewards, achieving a success rate over 80%. When new sounds were introduced (4.5 kHz, 7 kHz and 10.6 kHz) that were never learned, animals tended to choose the side closer in frequency to the learned sounds. The probability of choosing one side over the other generally exhibited an S-shape curve, and 10.6 kHz was most ambiguous to the animals. Furthermore, immunostaining results showed that compared with the control group, introducing an unequal number of low-frequency and high-frequency sounds increased c-fos expression in the orbitofrontal cortex of the prefrontal cortex. Therefore, in our preliminary study, we found that mice have the ability to make use of previously perceived information to make decisions under situations of uncertainty and that the prefrontal cortex may be involved in this process.

NVMB9

Empathy-Motivated Prosocial Behavior in Rats

ZHU Yun, YUNG Wing Ho, KE Ya

Supervisor: Prof. KE Ya

Empathy is an intrinsic ability to sense and share the feelings of others, which is evolutionarily conserved across mammals. This feature provides the foundation for humans and animals to develop diverse social behaviors and form a cohesive society. Among them, the behavior that is intended to benefit others is called prosocial behavior. Although emerging studies have been focused on empathy itself using various rodent models, the neural basis underlying empathy-motivated prosocial behavior remains elusive. Here, we studied the prosocial behavior in rats using a social instrumental task, where a helper rat needs to liberate a recipient rat from a distressed condition, namely water immersion. We found the rats could actively show prosocial behavior by rescuing their cagemates without any prior training experience and external rewards. Their skills to help others were honed through consecutive trials in a self-learning way. Importantly, we found this prosocial behavior was preferentially dedicated to conspecifics in aversive conditions and could be learned from the being helped experience. We also found the helper rats often performed intense self-grooming behavior before they initiated prosocial actions. Taking advantage of fiber photometry and calcium sensor, we monitored the neural dynamics in the anterior cingulate cortex (ACC) of freely-moving rats, a brain region associated with empathy and shared pain, and found the activity of ACC could be dramatically attenuated by self-grooming. In conclusion, our preliminary results suggest that prosocial behavior is an innate behavior that can be shown in animals like rats, and self-grooming could serve as an adaptive strategy to regulate the stress transferred from others to maintain emotional homeostasis and facilitate prosocial decision and action.

NVMB10

Development of a novel therapeutic strategy for obesity and related diseases

LIN Zhi Qiang[1]

Supervisor: Prof. SHUM Sau Wun Alisa[1]

[1]School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong SAR.

Introduction: Obesity is a chronic disease and is associated with various comorbidities such as non-alcoholic fatty liver disease (NAFLD), type 2 diabetes, cardiovascular diseases, and certain types of cancer. *Obesity* has become a serious global public health problem, with 40% of adults *worldwide* being classified as *overweight* or *obese.* Yet, safe and effective therapeutics against obesity are lacking. This project aims to investigate whether combined treatment of recombinant human arginase (rhArg) and all-trans retinoic acid (RA) can be an effective therapeutic strategy for obesity studied using a diet-induced obesity mouse model.

Methods: C57BL/6 male mice were fed a high-fat diet (HFD) for 12 weeks to induce obesity and then stratified by body-weight into 4 groups. For combination therapy, HFD-fed obese mice received an intraperitoneal injection once a week of rhArg dissolved in saline (vehicle 1) together with daily oral feeding of RA suspended in peanut oil (vehicle 2). For single therapy, HFD-fed obese mice received treatment with either rhArg together with vehicle 2, or RA together with vehicle 1. HFD-fed obese mice received treatment with vehicles (1 and 2) only served as the vehicle control. Age-matched C57BL/6 male mice fed a chow diet and received treatment with vehicles (1 and 2) served as the lean control. After 10 weeks of dosing period, organs were harvested for various analyses.

Results: Our results show that combined treatment of rhArg and RA could effectively reduce the body weight and fat mass of obese mice fed a HFD to levels similar to the lean control mice fed a chow diet. Moreover, it could markedly reverse hepatic steatosis, hyperglycemia, insulin resistance, and glucose intolerance. Further analyses of white adipose tissue reveal that expressions of several lipogenic markers were significantly decreased while lipolysis markers were significantly increased in the combined treatment group. Similarly, rhArg together with RA had potent effects on inhibiting adipocyte differentiation of the 3T3-L1 mouse cell line commonly employed for studying adipogenesis.

Conclusion: Combined treatment of rhArg and RA may be a promising therapeutic strategy for obesity and its associated metabolic diseases.

NVMB11

The Effect of Arginine Depletion on Liver Cancer

ZHENG Lu Xi[1]

Supervisor: Prof. SHUM Sau Wun Alisa[1]

[1]School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong SAR.

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer in adults. Common approaches for cancer therapy involve chemotherapy and surgery. However, cytotoxicity and non-selectivity of these approaches could damage normal cells. Therefore, there is an imperative need to develop novel drugs for the treatment of HCC. Amino acid deprivation has emerged as an effective therapeutic strategy for many amino acid auxotrophic tumors. Our focus is on arginine depletion. Arginine is a semi-essential amino acid. It becomes an essential amino acid in cancer cells. This project aims to develop a new therapy for HCC by a novel arginine-depleting agent, recombinant human arginase (rhArg), and to investigate the underlying mechanisms. Preliminary data show that rhArg significantly inhibited HCC cell proliferation and growth, which may be mediated via rhArg-induced G2/M phase cell cycle arrest. Furthermore, prolonged arginine starvation by exposure to rhArg had potent effects on inducing mitochondrial oxidative stress with impaired mitochondrial bioenergetics. Thus, our data provide novel insights into the mechanisms underlying the vulnerability of arginine auxotrophic liver cancer cells to arginine starvation.

NVMB12

Combination therapy of arginase and metformin on obesity and associated metabolic disorders

ZHENG Xiu Hua[1]

Supervisor: Prof. SHUM Sau Wun Alisa[1]

[1]School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong SAR.

Obesity is a chronic disease and is associated with a series of metabolic disorders, such as non-alcoholic fatty liver disease and type II diabetes. Patients with obesity are three times more likely to develop more serious symptoms of COVID-19. Currently, there are several anti-obesity pharmacotherapies approved by U.S. Food and Drug Administration. However, they have low efficacies and various side effects, especially on cardiovascular and nervous systems. Metformin is reported to have effects on inducing weight loss, while recent data from our laboratory show that recombinant human arginase (rhArg) can exert similar effects on obesity. This project aims to develop a new therapeutic strategy for obesity and associated metabolic disorders by combined treatment of rhArg and metformin, and to investigate the mechanism underlying the combination effect. C57BL/6J male mice were fed a high-fat diet (HFD) to establish an animal model of obesity. Liver tissue, white adipose tissue and serum were collected for histological, biochemical, and molecular analyses. Preliminary results show that combined treatment of rhArg and metformin had synergetic effects on reducing bodyweight, improving insulin sensitivity and glucose tolerance, reducing white fat mass and alleviating hepatic steatosis. Some of these effects could possibly be mediated via inhibiting the lipogenesis pathways, and activating lipolysis- and lipid oxidation-related pathways. In summary, combined treatment of rhArg and metformin show good synergistic effects on reducing obesity and various associated metabolic disorders.

NVMB13

The protective effect of statin against vascular toxicity induced by BCR-ABL tyrosine kinase inhibitors

HAN Yu Meng

Supervisor: Prof. TIAN Xiao Yu

Introduction: BCR-ABL tyrosine kinase inhibitors (TKI) therapy has revolutionized the treatment against chronic myelogenous leukemia (CML). However, severe vascular safety issues also arise in CML patients treated with TKI. Statins have been widely used among CML patients with hypercholesterolemia for the primary prevention against cardiovascular diseases. Particularly, statins also possess important anti-inflammatory properties in vascular endothelium. In this study, we investigate whether statins have protective effect against vascular endothelial dysfunction and vascular inflammation induced by BCR-ABL TKIs nilotinib and dasatinib.

Methods and Results: We studied the effect of three TKIs including imatinib, nilotinib and dasatinib, with or without statin treatment on human umbilical vein endothelial cells (HUVECs). Nilotinib and dasatinib both attenuated the viability and proliferation of HUVECs on a concentration-dependent manner, whereas imatinib showed no comparable effect. Nilotinib and dasatinib both increased the phosphorylation of myosin light chain 2 (MLC2), which was attenuated by simvastatin. Dasatinib altered F-actin organization, junctional proteins distribution and morphology of HUVECs. The effect of dasatinib on the re-distribution of VE-cadherin was attenuated by ROCK inhibitor Y27632. Moreover, simvastatin reduced the dasatinib-induced permeability increase of HUVECs to FITC-dextran leakage. By contrast, nilotinib did not show similar effect as dasatinib but increased the monocyte adhesion ability and the expression of adhesion molecules on HUVECs, which was inhibited by simvastatin. For in vivo studies, ApoE-deficient mice fed on high-cholesterol diet will be treated orally with imatinib, nilotinib, or dasatinib, to study whether TKIs promote atherosclerotic plaque formation and exacerbates vascular inflammation.

NVMB14

The role of SOX4 in regulating vascular function during hypertension

WU Wei Yan Vivian

Supervisor: Prof. TIAN Xiao Yu

Hypertension occurs with high incidence in Hong Kong, and it increases the risk of cardiovascular diseases including atherosclerosis, ventricular hypertrophy, and heart failure. As a member of the sex-determining region Y (SRY)-box (Sox) family, SOX4 is a transcription factor known to be involved in differentiation, proliferation, and survival. Despite it being the most highly expressed SOX family member in vascular cells, the role of SOX4 in cardiovascular disease has not yet been studied. Previous studies established the role of SOX4 as a phenotypic regulator of endothelial cells in atherosclerosis. In addition, EDN1, encoding the potent vasoconstrictor endothelin-1, is a known target gene of SOX4. Therefore, we hypothesize that SOX4 is involved in hypertension through the regulation of vascular cell phenotype and function. To determine the role and molecular mechanisms of SOX4 in the regulation of vascular cell function in hypertension, SOX4 expression was examined in vitro in human umbilical vein endothelial cells (HUVECs), human aortic smooth muscle cells (HASMCs), and in response to various stimuli including angiotensin II and TGFβ1, while an angiotensin II-induced hypertensive mouse model was used to study arterial SOX4 expression in vivo. Migrative and proliferative capabilities of HASMCs were also examined after SOX4 knockdown using small interfering RNA (siRNA). Our preliminary results showed that SOX4 is expressed in vascular cells and is upregulated in remodeled arteries from hypertensive mice. Additionally, migration and proliferation of HASMCs was attenuated after siRNA-mediated knockdown of SOX4. Together, these results suggest that SOX4 may be involved in vascular remodeling in hypertension.

NVMB15

Mechanosensitive ion channel TRPV4 regulates cancer stemness in ovarian cancer

LEI Zhen Chuan

Supervisor: Prof. YAO Xiao Qiang

Cancer stem cells (CSCs) are a small subpopulation of cancer cells which possess self-renewal ability, tumorigenicity ability, differential ability, remote metastasis ability and chemotherapy-resistant ability. Ovarian cancer is one of the most fatal gynecologic malignancies in which CSCs play a vital role in cancer progression. During the development of ovarian cancer, the cancer cells are exposed to different types of mechanical forces, including shear force, stretch, compression and matrix stiffness, etc. On the other hand, transient receptor potential vanilloid-type 4 (TRPV4) is a mechanical-sensitive cation ion channel that can sense diverse mechanical stimuli and is reported to play an important role in cancer cells. In our study, we found that TRPV4 expression is upregulated in ovarian cancer tissues, and its expression level is even higher in ovarian cancer CSCs. Furthermore, inhibition of TRPV4 decreases the self-renewal ability of ovarian cancer CSCs while activation of TRPV4 has opposite effect. Moreover, mechanical stimuli also activate TRPV4 in ovarian cancer cells and influence the stemness of CSCs.

NVMB16

S-glutathionylation underpins the inhibitory effect of fusaric acid on banana K+ uptake MaAKT1 channels

ZHANG Jun

Supervisor: Prof. YAO Xiao Qiang

Fusarium wilt is a serious disease that threatens the banana industry. The disease is caused by the infection of fungal Fusarium oxysporum f. sp. Cubense tropical race 4 (Foc TR4) on banana. During infection, Foc TR4 secretes a major toxin fusaric acid (FSA), which can cause tissue necrosis and kill host cells. To date, however, the detailed mechanism of how FSA aggravates the infection remains unknown. K+ is an essential macronutrient for plants. It is acquired by specific K+ uptake systems located in roots, mainly the inward-rectifier AKT1-like channel, to fulfill a broad range of physiological movements, such as growth, metabolism, and photosynthesis. In the present study, we found that banana AKT1 (MaAKT1) overexpressed in HEK293 cells could mediate inward rectifying current. Further, our results revealed that FSA could inhibit MaAKT1 current in a dose-dependent manner, which can be mimicked by oxidants, H2O2 and diamide, but prevented by ROS scavenger, TEMPOL. In whole-cell recording, washout of FSA could not restore the inhibited MaAKT1 current. Besides, in inside-out configuration, FSA, H2O2, and diamide could not inhibit MaAKT1 currents, indicating that FSA and the oxidants did not act on the channel directly, possibly by S-glutathionylation modification of MaAKT1 protein, which was evidenced by the inhibition of the channel by H2O2 and diamide plus glutathione (GSH), GSSG, and several thiol oxidants, but not by H2O2, diamide, or GSH alone. In addition, the inhibitory effect of S-glutathionylation inducer, GSSG, on MaAKT1 channels can be rescued by the reducing agent dithiothreitol (DTT). Collectively, the results here demonstrate that S-glutathionylation is responsible for the inhibitory effect of fusaric acid on MaAKT1 channels.

NVMB17

Neural circuits underlying thiamine perception

GAO Ming[1], KE Ya[1], YUNG Wing Ho[1]

Supervisor: Prof. YUNG Wing Ho[1]

[1]School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong SAR.

Thiamine deficiency is a type of hidden hunger, or micronutrient deficiency, where an inadequate dietary intake of thiamine could lead to nerve, heart, and brain abnormalities. Understanding pathway of thiamine sensing is essential for us to comprehend the micronutrients-sensing mechanism and the associations between thiamine deficiency and neurocognitive disorders such as Wernicke-Korsakoff syndrome. However, the neural mechanism underlying thiamine perception is not clear. This project aims to investigate the effects of thiamine deprivation on feeding behaviour and unveil the neural mechanism of thiamine perception and regulation. Firstly, we established an animal model of thiamine deficiency by providing mice with thiamine-deprived food for three consecutive weeks. In this model, we observed the body weight of mice with thiamine deprivation started to drop significantly after 2 weeks, suggesting that the negative alterations may have occurred around this timepoint. To probe thiamine perception regulation in mice, we performed a food preference test, in which mice could choose between thiamine-deficient and thiamine-containing food for 30 minutes. In this test, we found that the thiamine-deprived mice would intake significantly more thiamine-containing food (0.01628g/g body weight) compared with thiamine-deficient food (0.0002843g/g body weight) (0.01600 ± 0.00272g/g body weight, $p<0.0001$, unpaired t test). To assess the importance of olfactory information in thiamine perception, we put the mice in an odour preference test, where they could only smell, but not eat the food. In this test, we found that thiamine-deprived mice displayed more investigation towards the odour of thiamine-containing food compared with thiamine-deficient food (investigation number difference: 51.20 ± 14.38 per 30 mins, $p<0.01$, unpaired t-test; investigation duration difference: 50.21 ± 10.49s, $p<0.05$, unpaired t-test). This suggests that the odour of thiamine-containing food is already sufficient to trigger its preference under a thiamine-deprived state. Next, we found that neurons in the lateral parabrachial nucleus (LPB) showed increased c-Fos expression after prolonged thiamine deprivation. Interestingly, activation of LPB neurons was also observed when normally fed mice were orally administered with thiamine solution, suggesting an association of LPB activity with excessive thiamine

intake. Hence, our findings suggest that mice could perceive the existence of thiamine at least through olfactory information, and abnormal levels of thiamine are reflected in the activity of LPB neurons.

Acknowledgements

The 2022 SBS Postgraduate Research Day Organizing Committee would like to express its sincere appreciation of the generous support of the following organizations:

Platinum Supporter:

BioArrow Technology Limited

Qiagen Hong Kong Pte Ltd

WeBio Co., Ltd

Tin Hang Technology Limited

Gene Co. Ltd

Molecular Devices (HK) Limited

Hong Kong Labware Co., Ltd.

Thermo Fisher Scientific (Hong Kong) Limited

AB Sciex (Hong Kong) Limited

ATCG Limited

Bio-Station Ltd

Medikonia Limited

BioArrow Technology Ltd.
寶爾衛生物科技有限公司

LET US BE YOUR
RESEARCH BOOSTER

NEW ENGLAND BioLabs Inc.

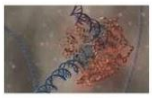

Restriction Endonucleases

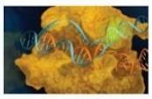

PCR & qPCR reagents


DNA assembly and Cloning


RNA reagents


Sample preaparation for NGS


DNA Modifying Enzymes


Genome Editing


Nucleic Acid Purification

TWIST BIOSCIENCE


Genes

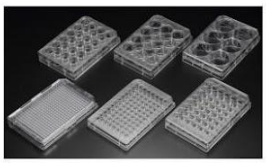

Libraries


Oligo Pools


NGS


Antibody Services

SPL LIFE SCIENCES



SARTORIUS

TECAN


Microplate Reader
Pipetting Workstation

logos biosystems


LUNA-II™ Automated Cell Counter

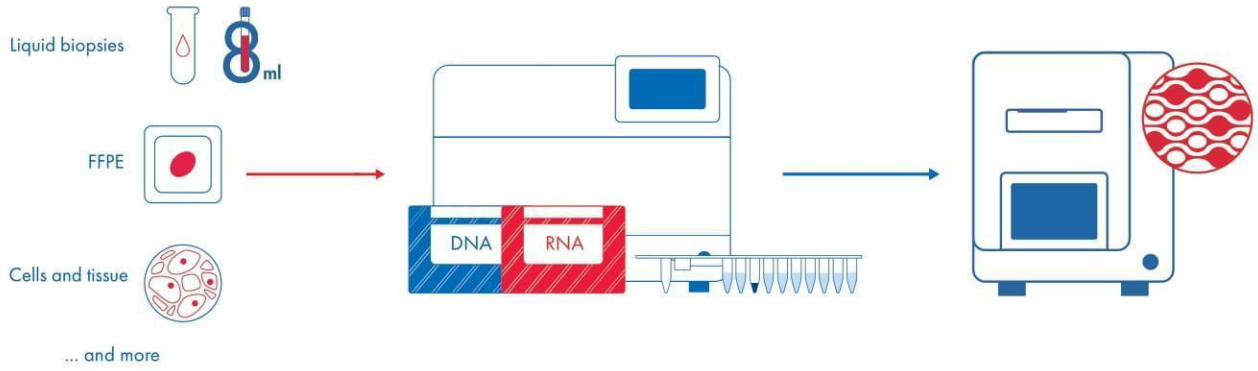
CONTACT US

BioArrow Technology Limited
sales@bioarrow.com
(852) 2179 7195

Two is better than One

Combine automated sample processing with nanoplate dPCR for mutational analysis from liquid biosy and FFPE samples

Wide kit portfolio supports many applications



Power up your workflow with the EZ2® Connect and QIAcuity®

EZ2 Connect

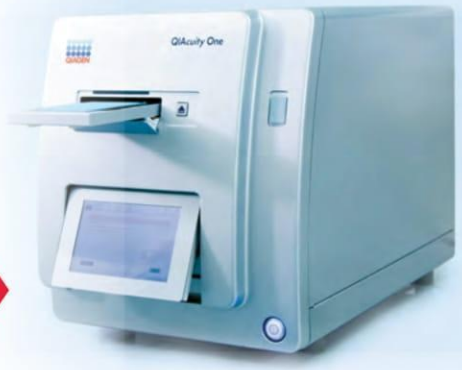
For end-to-end automation of nucleic acid extraction, from reagent setup to elution

- Magnetic bead-base extraction system
- Purify DNA and RNA from ≤24 samples as little as 20 mins
- Allow on-board heating and reagent pipetting
- Achieve high sensitivity with large volume (8ml) processing
- Boost reproducibility with prefilled reagent cartridges

QIAcuity Digital PCR System

For nanoplate-based digital PCR applications

- Fully integrated system
- Scalable format (1-, 4- and 8-plate instruments)
- Advanced multiplexing capabilities (up to 5 plex)
- Flexible sample throughput
- Comprehensive results in under 2 hours



reddot winner 2022

QIAGEN's EZ2 Connect wins prestigious Red Dot design award

Sample to Insight

WEBIO COMPANY LTD

WEBIO CO., LTD.
TEL : +852 3525 0164
FAX : +852 3525 1694
EMAIL : info@webioco.com
www.webioco.com

QIAGEN Hong Kong PTE. LTD
TEL : +852 3652 3652
FAX : +852 2730 2070
EMAIL : Orders-HK@qiagen.com

follow QIAGEN Life Science account on We Chat

TIN HANG TECHNOLOGY LIMITED
天恒科技有限公司

www.tinhangtech.com

One-Stop Laboratory Supplier

Analytical & Research Chemicals

Millipore Sigma, Avanti Polar Lipids Inc., Ehrenstorfer, Merck, LGC, Supelco, USP, CIL, Romer Labs, YMC, EuroProxima, American Elements, TRC, BOC Sciences

Life Sciences

Roche, Millipore Sigma, KAPA Biosystems, Merck, vivantis, ATCC, 斯帕法斯 SPF EGGS, ECACC, APHA Scientific, LASCO

Clinical Diagnostics

MRC Holland, MEDITE, BIOCARE MEDICAL, Trinity Biotech, CHROMSYSTEMS, EKF DIAGNOSTICS, DEVYSER RESULTS FOR LIFE, HARDY DIAGNOSTICS

Lab Equipment

IKA, ARCTIK, Drummond Scientific Company, ESCO, yakos, Motic More Than Microscopy, analytikjena, memmert, Agilent, Grant Scientific, SELECTA, Julabo, Hercuvan Lab Systems, HT high tech lab, Select BioProducts

Consumables

deltalab, VWR We Enable Science, ADVANTEC, DURAN WHEATON KIMBLE, SPL SPL LIFESCIENCES, Simport, Puritan Quality since 1919

Chemical & Bio-Material Import Services

Laboratory Furniture & Supply

SINCE 1996

Contact us at: Tel: 2817 2121 Email: sales@tinhangtech.com

19/F 340 Queen's Road Central, Hong Kong

MOLECULAR DEVICES

Tailored Drug Screening Solution
Answer your Biological Questions

Stunning images, Faster throughput, Powerful analysis

SpectraMax® Mini

Compact designed microplate reader with flexible detecting modes

ImageXpress® Pico

Automated Cell Imaging System for Personal lab

Contact us for more innovation solutions

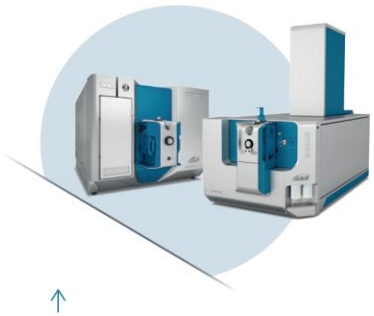
Hong Kong Labware
Info@labware.com.hk
www.labware.com.hk

www.moleculardevices.com
Info.twhk@moldev.com
Taiwan office: +886 2 2656-7585
Hong Kong office: +852 3971-3530

SCIEX

The Power of Precision

Strong portfolio of advanced and routine research workflows



High-performance mass spectrometry

Routine and standard workflows for research

Liquid chromatography

Reliable standard, microflow and nanoflow systems

Ion sources

Providing robust and high-performance ionization

SCIEX Now

Service, support and training, just a click away

Integrated LC-MS/MS

Mass spec workhorses, intelligently reengineered

Capillary electrophoresis

Helping biopharmaceutical labs take charge of their development pipelines

Powerful software

Intuitive, versatile, accurate and collaborative software

Methods and consumables

Verified reagents and pre-developed research methods

Advances in human wellness depend on the power of precise science

SCIEX delivers solutions for the precise detection and quantification of molecules, empowering our customers to help protect and advance the wellness and safety of all. We have led the field of mass spectrometry for more than 50 years. Since the launch of the first-ever commercially successful triple quadrupole in 1981, we have developed groundbreaking technologies and solutions that influence life-changing research and outcomes.

Today, we continue to pioneer robust solutions in mass spectrometry and capillary electrophoresis. Our customers are able to quickly respond to environmental hazards, bring relevant drugs to market faster and keep food healthier and safer.

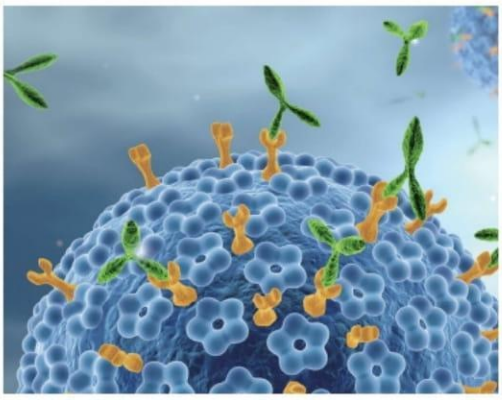
That's why thousands of life science experts around the world choose SCIEX to get answers they can trust to help them make critical decisions that positively impact lives.

AB SCIEX (HONG KONG) LIMITED
Address: Suite 1605, 16/F, Tower 6, China Hong Kong City, 33 Canton Road, Tsimshatsui, Kowloon, Hong Kong
Tel: +852 3102-3000

www.sciex.com

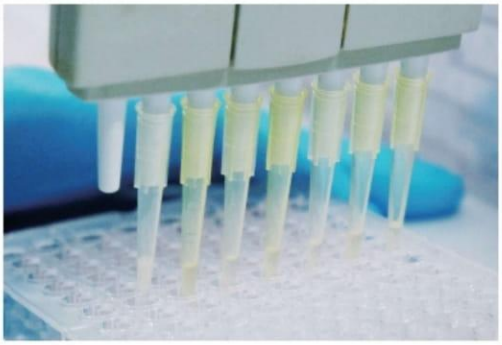
ABclonal Technology | Leader in Biomolecular Solutions for Life Science

ABclonal is aiming to provide reliable and cost-effective products and services for both basic and translational research in cutting-edge biomedical science. Innovation is in our DNA, with worldwide R&D centers focusing on technical innovation and product development for protein science and molecular biology.



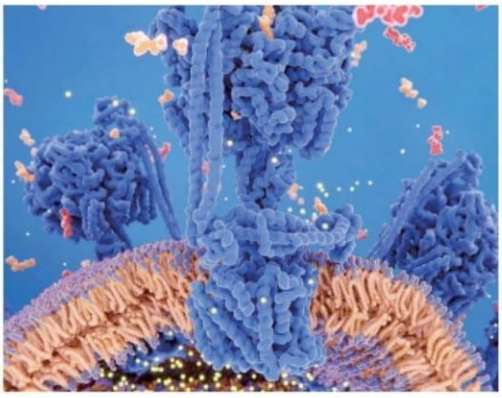
ANTIBODY

Over 15,000 monoclonal and polyclonal antibodies. A large collection of antibodies are available to make multiple choice in species and applications. Interactive and responsive new product development pipelines and custom antibody services will provide more choices for novel targets. Authentic in-house validation for multiple applications with a wide range of samples (including validation on KO cell models) ensure the reliable and stable performance of our antibodies in your experiments. Thousands of primary antibodies with IHC application for scientific research. Our antibodies have been cited on more than 10,000 publications including some high IF scientific journals-Nature, Science, and Cell.



ELISA

Current product series cover human, mouse, rat, and other conventional detection species, and 400 specific target ELISA kits. The categories include a variety of cytokines, chemokines, growth factors, proteases, and other molecules. We also provides customized services for the development of ELISA test kits and the screening of antibody pairs. Products are used for life sciences research, diagnostic testing, food testing, etc. Mainly used for serum plasma, Cell culture supernatant, Tissue homogenate supernatant Lysate.



RECOMBINANT PROTEIN

More than 1,200 high-quality recombinant proteins developed using advanced and mature protein expression technology, a complete protein expression platform, which can be used in many different cell biology research fields including immunology, neurobiology, stem cell research, cancer research, etc. We also provide high-quality target proteins required in the drug development process. More than 80% of our proteins are expressed by eukaryotic cells, which is more similar to the protein in native states and has better biological activity. The product has the features of high purity, high activity, low endotoxin and batch stability. Products are used for life sciences research, drug development, tissue engineering, diagnostic testing, etc.

CUSTOM ANTIBODY DEVELOPMENT

We provide customized services for application-specific polyclonal and monoclonal antibodies used in your research. A full service is available for all types of antibody production, including steps of antigen design, immunogen preparation, antibody development, antibody purification, modification, validation, and immunoassay development. We can develop antibodies for life sciences research, drug development, diagnostic testing, etc.

Our Featured Brands:

TaKaRa — Clontech TaKaRa cellartis

GUNSTER

Solarbio® LIFE SCIENCES

Acro BIOSYSTEMS

碧云天 Beyotime

ABclonal Technology®

DIAB

MCE® MedChemExpress

BIOSTATION 博士得有限公司

Tel: 3565 0223 Fax: 8161 8667
Email: sales@bio-station.net

MEDIKONIA

make research simply easier

Medikonia, based in Hong Kong, is a leading distribution company who is specialized in life science and biomedical sciences.

We are committed to make research and clinical diagnosis simply easier by offering innovative and superior value solution to scientists and medical doctors.

Please visit www.medikonia.com to know more about us.

Our Innovative Solutions

Ultrasensitive Biomarker measurement
Quanterix HD-X Analyzer

High Content Imaging
CellVoyager CV8000

***In vivo* Imaging**
NgihtOWL II LB

Full Spectrum Cytometry
Cytek Aurora

Bench-top Cell Sorter
Nanocellect WOLF

Single-cell Proteomics
Isoplexis Isospark

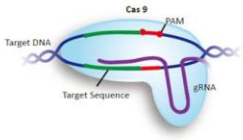
Binding Affinity
Monolith

Protein Structure Detection & HTS Screening
Prometheus Panta

Transfection
4D Nucleofector & Kits

Cell Therapy Manufacturing
Lonza Cocoon

Primary Cells & Culture Media
Lonza Primary & Stem Cells, BulletKit, Reagents



Non-viral Genome Editting
CRISPR-Cas9, sgRNA, dsDNA, ssDNA

Our Featured Brands

GenScript Make Research Easy · Lonza · TargetMol A DRUG SCREENING EXPERT · NORGEN BIOTEK CORP. · Vazyme

ARIMA GENOMICS · biorbyt Explore. Bioreagents. · Quantabio · RayBiotech Empowering your proteomics · TONBO A CYTEK BRAND

BERTHOLD · CYTEK TRANSCEND THE CONVENTIONAL · DeNovix · isoplexis · nanocellect Biomedical, Inc.

nanoTEMPER · Olink · Quanterix · UVITEC CAMBRIDGE · YOKOGAWA Co-innovating tomorrow™

T: 852 2866 8995 **F:** 852 2866 8069 **E:** cs@medikonia.com **Support:** support@medikonia.com

NOTE

NOTE

School of Biomedical Sciences

Organizing Committee of Postgraduate Research Day 2022

Executive Committee of SBS Postgraduate Student Association