RESEARCH GALA 2023

DEPARTMENT OF BIOMEDICAL SCIENCES

9TH MAY

CityU Department of Biomedical Sciences
香港城市大學
City University of Hong Kong

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Acknowledgements

We gratefully acknowledge the generous support of the following organisations to the 2023 Research Gala. *(by alphabetical order)*

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Special thanks to our BMS General Office, Technical Office colleagues for providing the logistic support; Ms WANG Meijun (PhD student) for being the MC, and other PhD student helpers.

Welcome Message from Head (BMS)

Welcome to the 2023 BMS Research Gala held on 9 May 2023. This is a highly anticipated event that celebrates the achievements and research efforts of our talented researchers and students right after the Covid-19 pandemic. This Research Gala is also a testament to the hard work and dedication of everyone in the Department, from our students and researchers, to the faculty members who have provided guidance and support every step of the way.

Our students, researchers and faculty members have been working tirelessly to unravel the mysteries of the human body and to develop innovative solutions to some of the most pressing health challenges of our time. Their passion and commitment to advancing the field of biomedical sciences has resulted in groundbreaking discoveries, which are truly commendable.

Today, we come together to celebrate their accomplishments and honor their hard work and dedication. We hope that this annual event will inspire and encourage all of us to continue pushing the boundaries of biomedical research, and to work together towards a brighter and healthier future.

To put a research gala of this magnitude together is not a small task. To that ends, I want to express my sincere gratitude to the generous sponsors, who have supported us in making this event even more successful. I would also like to recognize the tremendous efforts put forth by our Organizing Committee, students helpers, MC and staff from General Office and Technical Office. They have worked effectively to ensure that every aspect of this event runs smoothly and make the event memorable.

Lastly, I would like to thank all participants for their contributions to the success of this research gala. Wish all of you a very enjoyable and fruitful day!

Prof HUANG Yu

Programme Rundown

09:00 – 09:15	Welcoming Speech by Prof HUANG Yu, Head and Chair Professor
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10:25 – 10:40	Break
10:40 – 11:15	Oral Presentation 2
11:15 – 12:30	Poster Presentation 1
12:30 – 14:00	Lunch Break
14:00 – 15:40	Oral Presentation 3
15:40 – 17:00	Poster Presentation 2
17:00 – 17:30	Break
17:30 – 18:00	Award Presentation Concluding Remarks

List of Oral Presenters

Cancer Biology & Therapy

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1	HE Qingling	Dr. CHIN Rebecca Y M
2	HU Shiman	Dr. CHAN Kui Ming
3	LIM King Hoo	Dr. CHOW Kwan Ting
4	YANG Zihan	Prof. YANG Mengsu, Michael

Genomics & Bioinformatics

Abstract No.	Presenter	Supervisor(s)
5	HE Qian	Dr. CHAN Kei Hang Katie
6	YI Wenkai	Dr. YAN Jian

Infectious Diseases & Immunity

Abstract No.	Presenter	Supervisor(s)
7	AWASTHI Pragati	Dr. ZHANG Liang
8	WANG Xuejiao	Dr. YAO Xi
9	WANG Yiran	Prof. HE Mingliang
10	ZOU Yuling	Dr. YUAN Hsiang-Yu Sean

Vascular, Metabolic & Regenerative Biology

Abstract No.	Presenter	Supervisor(s)
11	CHENG Chak Kwong	Prof HUANG Yu
12	PU Aoyang	Dr. BAN Kiwon
13	ZHANG Xuebing	Dr. KIM Jin Young

Oral Presentation - Abstract 1

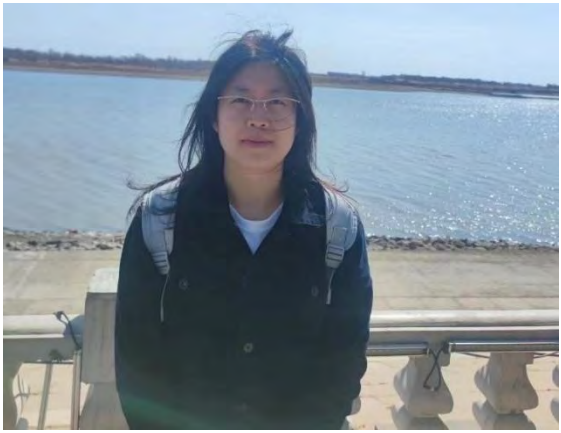
FOSL1 is a key regulator of super-enhancer driving TCOF1 expression in triple-negative breast cancer

Qingling HE [1], Jianyang HU [1], Hao HUANG[1], Tan WU[1], Saravanan RAMAKRISHNAN[1], Yilin PAN[1], Kui Ming CHAN[1], Liang ZHANG[1], Xin WANG[2], Y Rebecca CHIN[1]

[1] Department of Biomedical Sciences, City University of Hong Kong, Hong Kong.
[2] Department of Surgery, The Chinese University of Hong Kong, Shatin, Hong Kong.

Triple-negative breast cancer (TNBC), an aggressive subtype of breast cancer with limited targeted therapeutics. By performing multiomic profiling, we revealed different super-enhancer (SE) patterns among various subtypes of breast cancer and one of TNBC-specific SEs specifically drives the expression of a novel oncogene TCOF1 (we name it TCOF1-SE). However, the exact upstream mechanisms that control TCOF1 expression by TCOF1-SE remain to be elucidated. Here, by combining DNA pull-down assay and bioinformatic prediction, we identified several potential transcription factors which bind to TCOF1-SE. FOSL1 was found to be one of the top genes in transcription factor DNA motif analysis. FOSL1 is often detected in the more aggressive and highly malignant subtypes of breast cancer, such as TNBC. Hence, the transcription factor FOSL1 was chosen for further study. FOSL1 depletion inhibits TCOF1 mRNA and protein levels. Furthermore, by performing dual-luciferase reporter assay and CHIP-qPCR, we demonstrated that FOSL1/TCOF1-SE interplay promotes the transcription of TCOF1 in TNBC cells. Moreover, FOSL1 knockdown inhibits the growth of TNBC cells in 2D and 3D spheroids, as well as supresses stemness properties. This work identifies TCOF1 as a direct transcriptional target of FOSL1, and highlights the potential of targeting FOSL1-SE-TCOF1 transcriptional program for therapeutic treatment of TNBC.

Oral Presentation - Abstract 2



Mechanism of histone H2BE113K mutation in breast cancer

HU Shiman

Nucleosome is the basic unit of chromatin which composes of 147bp DNA wrapped around two copies of core histones. All the four core histone proteins exert essential functions for condensing the genomic DNA as well as regulating gene expression through epigenetic mechanisms[1]. In the past decade, dozens of cancer-associated histone mutations have been identified in various cancers and these 'oncohistones' have been shown implicated in cancer development. We have recently reported two oncohistones: H2BG53D and H2BE76K in pancreatic and breast cancers respectively[2-4]. In addition to the G53D and E76K mutations of H2B, we identified the H2BE113-to-K/Q mutations which had high incidence in breast and lung cancers[5, 6]. Considering the vital role of H2BE113 site for being a part of nucleosome acidic patch[7], here we show that H2BE113K mutation reduces the interaction between Imitation SWI/SNF (ISWI) core subunits and nucleosome. The primary function of ISWI is to regulate nucleosome sliding and we show that the decreased binding of ISWI to E113K mutant H2B affect the chromatin accessibility of multiple genes. In addition, through examining the transcriptome in H2BE113K mutant cells, our results reveal that H2BE113K alters cancer-related gene expression by affecting chromatin accessibility and subsequently contribute to tumorigenesis in breast cancer. Moreover, we identify *G3BP2* as a target gene of H2BE113K and its overexpression leading to enhanced colony formation phenotype, revealing the significance of H2BE113K in cancer development. In summary, our work reveals that the H2BE113K diminishes ISWI core subunit binding to nucleosome and affects *G3BP2* expression level by regulating chromatin accessibility, subsequently impacts breast cancer development.

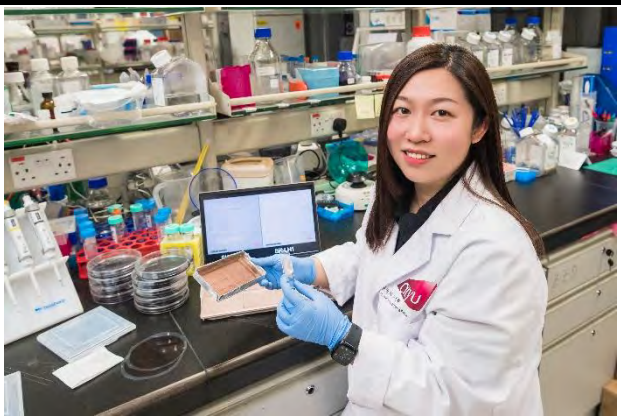
Oral Presentation - Abstract 3

Elucidating cell fusion mechanisms that lead to formation of osteoclast-breast tumor hybrids that can potentially facilitate bone metastatic disease progression

King Hoo LIM, Kwan Ting CHOW

Tumor hybrid cells in cancer biology have garnered greater attention with the discovery of circulating hybrid cells (CHC). CHCs are identified as the predominant cell type in circulating tumor cells (CTC) and they co-express leukocyte and tumor markers. Current studies indicate that tumor hybrids play an important role in tumor progression as they acquire genetic and phenotypic characteristics from parental cells and reportedly have enhanced motility and invasiveness, increased stem cell characteristics, and elevated resistance to chemo- and radiotherapies. It has been proposed that cellular fusion leads to the formation of these hybrids. Amongst leukocyte fusion partners, macrophages (osteoclast precursors) are identified as a frequent fusion partner with tumors. However, mechanisms and pathways that lead to fusion between macrophages and tumors are highly underexplored. To address this, we investigated macrophage fusion pathways that could be involved in regulating macrophage fusion with breast tumor. Here we report the finding of a novel hybrid cell type that is formed by in-vitro cellular fusion between osteoclast precursors/mature osteoclasts and breast tumor (hereby known as osteoclast hybrid) in the presence of osteoclastogenic cytokines. Fusion was observed at all stages of osteoclast differentiation with individual macrophages fusing with tumor cells, interfusion between osteoclast hybrids, and individual tumor cells fusing into mature osteoclast hybrids. Tumor nuclei in osteoclast hybrids were found to be transcriptionally active, which may infer that osteoclast hybrids are transcriptionally different compared to regular osteoclasts. Future directions will emphasize on understanding the mechanisms that lead to osteoclast hybrid formation and their potential roles in bone metastatic disease progression. These experiments will involve single cell sequencing on osteoclast hybrids and to evidence the formation of osteoclast hybrids in animal models.

Oral Presentation - Abstract 4



Lysyl hydroxylase LH1 promotes confined migration and metastasis of cancer cells by stabilizing septin2 to enhance actin network

YANG Zihan

Excessive extracellular matrix deposition and increased stiffness are typical features of solid tumors such as hepatocellular carcinoma (HCC) and pancreatic ductal adenocarcinoma (PDAC). These conditions create confined spaces for tumor cell migration and metastasis. Herein, we designed and fabricated microfluidic devices to reveal that lysyl hydroxylase 1 (LH1) promoted the confined migration of cancer cells at both collective and single cell levels. In addition, LH1 enhanced cell invasion in a 3D biomimetic model and spheroid formation in stiffer environments. High LH1 expression correlated with poor prognosis of both HCC and PDAC patients, while it also promoted in vivo metastasis. At the molecular level, LH1 bound and stabilized septin2 via the hydroxylase domain to enhance actin polymerization. Finally, the subpopulation with high expression of both LH1 and septin2 had the poorest prognosis. In summary, LH1 promotes the confined migration and metastasis of cancer cells by stabilizing SEPT2 and augmenting actin polymerization.

Oral Presentation - Abstract 5

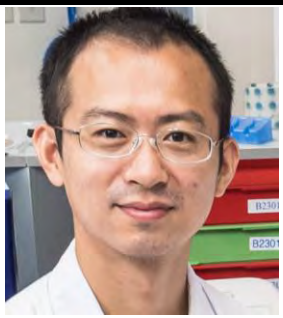
Nutritional Interventions for the Prevention of Cognitive Deterioration in Patients with Mild Cognitive Impairment and Alzheimer's Disease: A Network Meta-Analysis of Randomised Controlled Trials

[1]Qian HE, [1]Kevin Chun Hei WU, [1]Jia Yue ZHANG, [1]Shuyao TONG, [1]Adam N. BENNETT, [1,2,3]*Kei Hang Katie CHAN

1. Department of Biomedical Sciences, City University of Hong Kong, Hong Kong SAR, China
2. Department of Electrical Engineering, City University of Hong Kong, Hong Kong SAR, China
3. Department of Epidemiology, Centre for Global Cardiometabolic Health, Brown University, RI, USA

Background and objective: As ageing of the population accelerates, cognitive impairment, in the form of conditions such as Alzheimer's disease (AD) and mild cognitive impairment (MCI), is an important public health issue that has drawn a lot of attention. Nutritional intervention is a promising non-pharmacological therapy for cognitive dysfunction, but it is unclear which type of nutritional intervention is the best. This study involved a systematic review and network meta-analysis (NMA) to inform clinical practice by comparing different nutritional interventions. **Methods:** A pair-wise and network meta-analysis were adopted to analyse the intervention effectiveness according to direct and indirect evidence. In this study, 11 comparative nutritional interventions, which were: multi-ingredient (such as omega-3 fatty acid with antioxidant) nutrition, omega-3 fatty acid, vitamin B complex, vitamin E complex, Vitamin D, minerals, chemical compounds, dietary interventions, triglycerides, coenzyme Q, Chinese herbs, and a placebo group, were included. The mini mental state examination (MMSE) was the primary outcome and the cognitive subscale of the AD assessment scale (ADAS-cog) was the secondary outcome. **Results:** Fifty-one trials were included, in which 8,420 people took part in the study. For the primary outcome, 39 trials were eligible, which involved 6,698 participants. Our NMA analysis indicated that multi-ingredient nutrition (standardised mean difference (SMD) = 1.30; 95% confidence interval (CI) = 0.43, 2.30) was statistically superior to placebo, and this finding was confirmed through the application of pair-wise meta-analysis (SMD = 0.45, 95%CI = 0.14, 0.77). The subgroup analysis indicated that multi-ingredient nutrition (SMD = 1.30, 95%CI = 0.64, 2.0) was superior to placebo as measured by MMSE in the MCI group. In the AD group, no potentially promising intervention was identified. **Conclusion:** Our study concluded that multi-ingredient supplementation might be the most effective nutritional intervention to prevent cognitive decline among patients with cognitive impairment, especially those with MCI.

Oral Presentation - Abstract 6



The Molecular Mechanism of HERV-H RNA in Regulation of 3D Genome

YI Wenkai

The three-dimensional (3D) genome organization has been proven to play a critical role in gene expression through the loop extrusion model, which underlay topologically associating domains (TAD) formation via cohesin and other boundary elements. However, the molecular mechanism of chromatin remodel remains to be fully elucidated. Recently, transcriptionally active human endogenous retrovirus subgroup H (HERV-H) was reported to positively regulate TAD boundary formation to maintain the pluripotency of human embryonic stem cells (hESCs). To determine if HERV-H RNA was involved in 3D genome organization, we applied ChIRP-seq and newly developed CARPID 2.0 to dissect HERV-H RNA's genomic binding sites and binding proteins, respectively. With the HERV-H ChIRP-seq, we found that HERV-H RNA predominantly bound to HERV-H DNA loci, which are highly associated with TAD boundary formation. Only these HERV-H DNA loci with abundant HERV-H RNA binding were consistently enriched for cohesin subunits. Furthermore, numerous G-quadruplexes (G4s) were identified along HERV-H RNA binding loci and might be involved in regulating HERV-H RNA binding. To identify the binding proteins of HERV-H RNA, we developed CARPID 2.0 method, which owes high specificity and efficiency in living hESCs. We identified 250 HERV-H RNA associate proteins with CARPID 2.0. Among them, SAFA was successfully validated to interact with HERV-H RNA through orthogonal approaches, including RIP-qPCR and Immuno-FISH. Notably, the depletion of SAFA resulted in dramatically loss of HERV-H RNA binding along the HERV-H DNA loci. In this project, we found abundant HERV-H RNA was enriched at the transcriptionally active HERV-H DNA loci, which are highly associated with TAD boundary formation. Then, SAFA was identified to directly interact with HERV-H RNA and play an essential role in HERV-H RNA binding.

Oral Presentation - Abstract 7

Exploring the Potential of Dendrimer Encapsulated Silver Sulfide and Berbamine Nanoparticles as Broad-Spectrum Antiviral Agents: A Future Perspective

AWASTHI Pragati

Biomedical Engineering Department, City University of Hongkong, Hongkong

In the future, dendrimer encapsulated silver sulfide (Ag2S) and Berbamine (BBM) nanoparticles may be utilized as effective antiviral agents. This approach is based on the ability of Ag2S to induce reactive oxygen species (ROS) that can lead to viral inactivation, and the broad-spectrum antiviral activity of BBM. The nanoparticles can be synthesized through a simple and efficient method involving the use of polyamidoamine (PAMAM) dendrimers as a scaffold.

The antiviral potential of Ag2S and BBM nanoparticles has been studied extensively in recent years. Several studies have reported their ability to inhibit the replication of various viruses, including influenza, herpes simplex virus, and human immunodeficiency virus (HIV). The nanoparticles have also been shown to be effective against emerging viruses, such as the Zika virus.

The antiviral mechanism of Ag2S and BBM nanoparticles involves multiple pathways. Ag2S nanoparticles induce the production of ROS, which can damage the viral envelope and prevent viral entry into host cells. BBM, on the other hand, inhibits viral replication by targeting various stages of the viral life cycle. The nanoparticles can be further modified to enhance their antiviral properties, such as by incorporating other antiviral agents or by optimizing their size and surface charge.

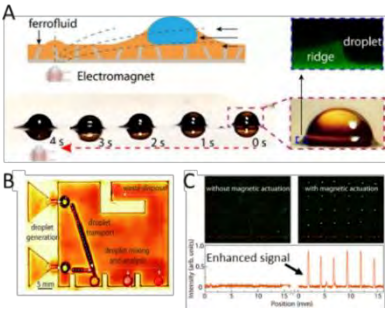
In conclusion, dendrimer encapsulated Ag2S and BBM nanoparticles have promising potential as antiviral agents in the future. The nanoparticles offer several advantages, such as high efficacy, broad-spectrum activity, and low toxicity. The use of these nanoparticles may provide an alternative approach to the treatment and prevention of viral infections, especially in the face of emerging viral threats.

Oral Presentation - Abstract 8

Magnetic actuation of droplets for biosensing

WANG Xuejiao
Supervisor: Xi Yao

Flexible actuation of droplets is crucial for biomedical and industrial applications. Hence, various approaches using optical, electrical, and magnetic forces have been exploited to actuate droplets. For broad applicability, an ideal approach should be programmable and be able to actuate droplets of arbitrary size and composition. Here we present an "additive-free" magnetic actuation method to programmably manipulate droplets of water, organic, and biological fluids of arbitrary composition, as well as solid samples, on a ferrofluid-infused porous surface. We specifically exploit the spontaneously formed ferrofluid wetting ridges to actuate droplets using spatially varying magnetic fields. We demonstrate programmed processing and analysis of biological samples in individual drops as well as the collective actuation of large ensembles of micrometer-sized droplets. Such model respiratory droplets can be accumulated for improved quantitative and sensitive bioanalysis - an otherwise prohibitively difficult task that may be useful in tracking coronavirus.



Figure

(A) Demonstrate of magnetic actuation for droplet. (B) A proof-of-concept magnetic digital microfluidic chip. (C) Signal enhancement with magnetic actuation.

Oral Presentation - Abstract 9

Secreted LRPAP1 as a ligand binds and induces IFNAR1 degradation to facilitate virus evasion from innate immunity

WANG Yiran

The crucial role of interferon (IFN) signaling is well known in restriction or eradication of pathogen invasion and viruses take a variety of ways to target IFN-signaling intracellularly for decades. However, the way by virus to target IFN-signaling extracellularly has not been discovered. Infection by both SARS-CoV-2 and enterovirus 71 (EV-A71) can cause severe diseases such as neurological disorders and even death in children. Here, we show evidence that the protease of SARS-CoV-2 ($3CL^{pro}$) and EV71 ($2A^{pro}$) upregulates the expression and secretion of LDL-receptor-related protein-associated protein 1 (LRPAP1). As a ligand, the N-terminus of secreted LRPAP1 binds with the extracellular domain of IFNAR1 that triggers the receptor ubiquitination and degradation, and promotes virus infection both *in vitro, ex vivo* in the mice brain and *in vivo* in newborn mice baby. A small peptide derivated from the N-terminus of LRPAP1 effectively binds and causes IFNAR1 degradation that enhances both DNA and RNA viral infections, including herpesvirus HSV-1, hepatitis B virus (HBV), EV71, and beta-coronavirus HCoV-OC43; whereas α2M，a LRPAP1 inhibitor, arrests virus infections by stabilizing IFNAR1. Our study demonstrates a new mechanism used by viruses for evading host cell immunity, supporting a strategy for developing pan-antiviral drugs.

Oral Presentation - Abstract 10

Prediction of the second surge of Omicron spread in Hong Kong: A modeling study

ZOU Yuling

Background The Omicron subvariant BA.2 caused the start of the fifth epidemic wave in Hong Kong in early 2022, leading to a significant outbreak and triggering more people get immunized in a population with relatively low vaccine coverage. About half a year later, a second outbreak, largely dominated by BA.4 and BA.5 subvariants, began to spread and peaked within few months. We aimed at assessing the effect of the waning vaccine protection together with the immune escape properties and predicting the next surge of the Omicron outbreak.

Method We developed mathematical equations to formulate continuous change of vaccine waning after observing empirical serological data and incorporated them into a multi-strain discrete-time SEIR (Susceptible-Exposed-Infectious-Removed) model. Daily mobility index and temperature data were incorporated. The reported cases during the first outbreak between as the training set together with daily vaccination rates, population mobility and temperature,

Findings Using the reported cases during the first outbreak as the training set together with daily vaccination rates, population mobility index and daily average temperature, the model successfully predicted the second surge and the replacement by BA.4/5, leading to a cumulative number of cases about 543,600 (7.27% of total population) between 1st June 2022 and 31st October 2022. If vaccine protection maintained without decreasing, the number of predicted cumulative cases reduced 35%. Moderate level of social distancing (10% reduction in population mobility) reduced only 13.78% cases, which was not able to prevent the second surge.

Interpretation The results suggest future local outbreaks will remain as long as new immune escape happens with waning immunity, even if the moderate level of social distancing is present. Therefore, a more accurate model forecasting combined waning immunity remains needed in order to allow a better preparation of the next outbreaks.

Oral Presentation - Abstract 11

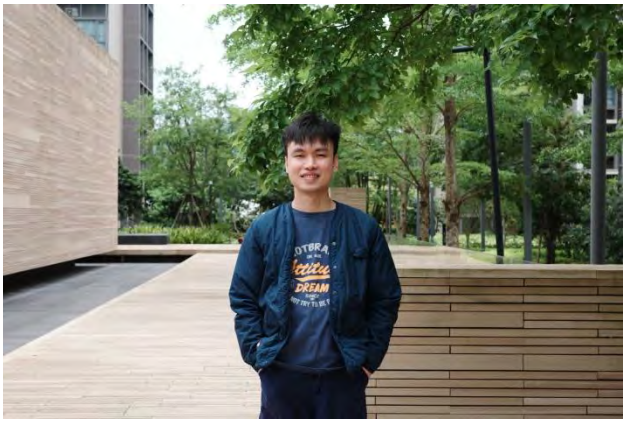
Exercise training activates KLF2/eNOS axis to ameliorate vascular dysfunction during diabetes

CHENG Chak Kwong[1], LUO Jiang-Yun[2], HE Lei[1], WANG Li[1], LAU Chi Wai[3], HUANG Yu[1,*]

[1]Department of Biomedical Sciences, City University of Hong Kong, Hong Kong SAR, China
[2]Institute for Developmental and Regenerative Cardiovascular Medicine, Xinhua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China
[3]School of Biomedical Sciences and Shenzhen Research Institute, Chinese University of Hong Kong, Hong Kong SAR, China

Diabetes mellitus is associated with endothelial dysfunction, where diminished activity of endothelial nitric oxide synthase (eNOS) promotes the development of cardiovascular diseases (CVDs) like diabetic cardiomyopathy and atherosclerotic CVDs. The current study aims to investigate potential strategies to restore eNOS activity and hence endothelial function upon diabetes. In the study, we showed that upregulation of Krüppel-like Factor 2 (KLF2), a shear stress-inducible transcription factor, can effectively improve endothelial function by enhancing nitric oxide bioavailability. Notably, KLF2 expression was inhibited in aortic endothelium of diabetic mice (db/db mice). Conversely, running exercise and simvastatin treatment both upregulated endothelial KLF2 levels in db/db mice. Adenovirus-induced KLF2 overexpression significantly improved endothelium-dependent relaxations and flow-mediated dilatations, and suppressed oxidative stress in diabetic mouse arteries. Mechanistically, KLF2 was shown to enhance eNOS phosphorylation at serine 1177 and eNOS dimerization. Further RNA-sequencing study suggested that KLF2 transcriptionally upregulated genes that are involved in insulin signaling pathway and cAMP signaling pathway, where these genes are believed to be upstream regulators of eNOS activity. In particular, KLF2 induction was found to activate both PI3K-Akt pathway and Hsp90 to promote eNOS activity. These findings implied that physiological approaches, particularly physical exercise, can induce KLF2/eNOS axis to protect against diabetic endothelial dysfunction, extending the mechanisms underlying exercise-mediated beneficial effects.

Oral Presentation - Abstract 12



Generation of autologous cardiac ECM patch for clinical grade of cell-based heart repair

PU AOYANG
(Dr.Ban Kiwon's Group)

Cardiac patch approach provides a novel platform to effectively deliver cells for heart repair. Natural materials such as extracellular matrix (ECM) conferring pivotal signals and exceptional biocompatibility have been proved as an ideal substance to construct cardiac patches. Due to limited donors, ECM from decellularized animal tissues can induce undesirable inflammation and immune reaction. Accordingly, we seek to fabricate ECM patch from human induced pluripotent stem cell-derived cardiac fibroblast (iPSC-CF) and hypothesize that this patient-tailored cardiac patch can be an ideal cell delivery vehicle to repair the damaged heart effectively and safely without the risk of unwanted immune responses. iPSC-CF firstly found uniquely expression cardiac factors such *GATA4* and *Hand2* compared with other fibroblast indicating CF-derived ECM exhibited heart-specificity. We secondly devised a stimulation cocktail of ascorbic acid and angiotensin II to enhance ECM production without toxicity. A novel "sandwich" technique was developed enabling sufficient ECM deposition in a biodegradable PLGA mesh within 10 days. Complete coverage of ECM such as collagen and fibronectin were confirmed by immunohistochemistry. Sprague Dawley (SD) rats were employed to investigate the autologous host response from epicardial implantation of ECM patch. Interestingly, ECM patches derived from SD rat-CF exhibited minimal host response as mesh controls showing significantly low quantity of total monocyte ($CD68^+$ cells), M1 ($CD68^+$ $iNOS^+$ cells) and M2 markers ($CD68^+$ $CD206^+$ cells) 3 and 7 days after implantation compared with patches generated by human primary CF and iPSC-derived CF. Mesenchymal stem cells (MSCs) encapsulated by cardiac patch were majorly viable after 15 days together with sufficient releasing beneficial cytokines suggesting the feasibility of CF-derived patch as a cell delivery vehicle for cardiac repair. Our approach of developing an autologous cardiac-specific patch will provide a novel platform for effective cell-based cardiac repair with safety and tissue compatibility.

Oral Presentation - Abstract 13

The circadian transcription factor BMAL1 in adult neural stem cells responds to neurodegeneration to replace damaged neurons

ZHANG Xuebing

Circadian clocks, endogenous oscillators generating 24h rhythms in physiological processes, are intrinsic in almost all cells, including various stem cells. It is known that circadian clocks are involved in embryonic stem proliferation and cell fate decision. However, how circadian clocks work in adult neural stem cells (aNSCs) has not been studied well. aNSCs can generate new neurons to replace damaged neurons in neurodegenerative conditions, but the neuronal regeneration is limited. Here, we show glutamate excitotoxicity, a common cause of neurodegeneration, reduces the circadian transcription factor BMAL1 levels in both neurons and aNSCs. Excitotoxicity induced by stereotaxic injection of NMDA into the hippocampus reduced BMAL1 levels in neurons in the CA1 and granular cell layer (GCL). Reduced BMAL1 in the CA1 was followed by neuronal death but not in the GCL. Interestingly, granule cells with reduced BMAL1 affect aNSCs in the subgranular zone (SGZ) to reduce NSC-BMAL1 subsequently. Reduced BMAL1 in aNSCs results in increased cell numbers, migration into the GCL, and differentiation into granular cells. These support that 1) neuronal clocks respond to neurodegenerative conditions and regulate its activities via BMAL1 levels; 2) altered neuronal clocks recruit aNSCs into lesions by regulating NSC-BMAL1 levels for neuronal repair. Thus, this study shows aNSC-BMAL1 as a potential target to regulate stem cell proliferation, migration, and differentiation to enhance limited neuronal repairs in the adult brain.

List of Poster Presenters (Cancer Biology &Therapy)

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1	ALAM Md Kowsar	Prof. YANG Mengsu, Michael
2	AU-YEUNG Allan Sung King	Prof. YANG Mengsu, Michael
3	BENNETT Adam Neil	Dr. CHAN Kei Hang Katie
4	DONG Chuanqiao	Dr. ZHANG Liang
5	DOTSE Eunice	Dr. CHOW Kwan Ting
6	GUNAWAN Renardi	Prof. YANG Mengsu, Michael
7	GUO Zhengjun	Prof. YANG Mengsu, Michael
8	LI Wenxiu	Prof. YANG Mengsu, Michael
9	LIAO Baoshan	Dr. XIONG Wenjun
10	NG Ka Ki	Prof. YANG Mengsu, Michael
11	PAN Yilin	Dr. ZHANG Liang
12	PENG Wang	Dr. CHAN Kui Ming / Dr. YUE Jianbo
13	QIAN Rui	Dr. ZHANG Liang
14	QIN Tiantian	Dr. CHAN Kui Ming
15	QIU Wenting	Prof. YANG Mengsu, Michael
16	SHEN Shuru	Dr. CHIN Rebecca Y M
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18	SO Chun Yan	Dr. CHOW Kwan Ting
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31	ZHANG Yuanfeng	Dr. LO Pui Chi
32	ZHENG Shixue	Dr. CHIN Rebecca Y M
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34	ZHOU Zhengdong	Prof. YANG Mengsu, Michael

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38	DING Yiqing	Dr. DENG Xin
39	FAN Ligang	Dr. YAN Jian
40	HAN Bing	Dr. CHAN Kui Ming
41	HAN Liangliang	Dr. DENG Xin
42	HE Lingli	Dr. ZHANG Liang / Dr. WANG Xin
43	JIANG Yuan	Dr. DENG Xin / Dr SHI Jiahai
44	JU Furong	Dr. YAN Jian
45	LI Jiang	Dr. CHIN Rebecca Y M / Dr. WANG Xin
46	LI Jingwei	Dr. DENG Xin
47	LI Tianmin	Dr. DENG Xin / Dr. WANG Xin
48	LIU Jingui	Dr. DENG Xin
49	LIU Shu	Prof. LI Ying
50	NI Ying	Prof. YANG Mengsu, Michael
51	SHI Yu	Dr. DENG Xin / Dr. SHI Jiahai
52	SUN Yue	Dr. DENG Xin
53	WANG Xiangeng	Dr. CHIN Rebecca Y M / Dr. WANG Xin
54	WU Tan	Dr. CHAN Kui Ming
55	XIE Qianwen	Dr. YAN Jian
56	YANG Fenglian	Dr. ZHANG Liang
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58	ZHANG Xianrui	Dr. DENG Xin / Dr. WANG Xin
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60	ZHU Zhongxu	Dr. ZHANG Liang / Dr. WANG Xin

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65	DING Zhaojun	Dr. YUAN Hsiang-Yu Sean
66	FANG Wenjie	Dr. WANG Li
67	FENG Yaxiu	Prof. HE Mingliang
68	GONG Jinhua	Dr. DENG Xin / Dr. SHI Jiahai
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75	NIE Qichang	Dr. LAU Chi Kong Terrence
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90	PU Yujie	Prof HUANG Yu
91	SI Xiaotong	Dr. CHAN Kui Ming / Dr. YUE Jianbo
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Poster Presentation - Abstract 1

Selective migration of single cancer cells into confined space depends on cell stiffness

Md Kowsar ALAM[a,b,c,§], Heng ZOU[a,b,c], Yu CHEN[a,b,c], Jiao ZHAI[a,b,c], Ying NI[a,b,c], Zemenu Mengistie SIMENEH[a,b,c], Xu TAO[a,b,c], Mengsu YANG[a,b,c*]

[a] Department of Biomedical Sciences, Tung Biomedical Sciences Centre, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon, Hong Kong SAR.
[b] Department of Precision Diagnostic and Therapeutic Technology, The City University of Hong Kong Shenzhen Futian Research Institute, Shenzhen, 518057, China.
[c] Key Laboratory of Biochip Technology, Biotech and Health Centre, Shenzhen Research Institutes of the City University of Hong Kong, Shenzhen, China.
[§] Department of Physics, University of Chittagong, Chittagong-4331, Bangladesh.
* To whom correspondence should be addressed. E-mail: bhmyang@cityu.edu.hk

Various intrinsic and extrinsic properties of cancer cells influence their migration into confined tracks or barriers of the metastatic cascade. Although many studies have focused on cell migration under confinement, the question of whether the migration of single cancer cells into spatially confined space is selected or random has remained unknown. Here, we developed a novel platform for spatially confined single cell migration and explored the effect of stiffness on single migrating cells. 40% of the trapping chambers of our developed platform were able to trap a single cell at the entrance of the migration channel. We then found a selective proportion of trapped single cancer cells with a lower stiffness level and less a dense assembly of vimentin intermediate filaments (i.e., lower vimentin expression) that demonstrated their migration into confined space. Further research disclosed that chemical stimulation (i.e., TGF-β1 treatment) and compressive pressure treatment reduced cells' stiffness level but increased their softness, which prompted the propensity of softer single cancer cells to migrate into confined spaces. Furthermore, the stiffness-related gene "VIM" was downregulated in its expression in single cancer cells that migrated into confined spaces compared to the control, unconfined ones. Collectively, these results suggest that the softer population of single lung cancer cells was selectively migrated into a spatially confined space.

Poster Presentation - Abstract 2

Device development on drug screening with intact tissues

AU YEUNG Allan Sung King

The need for patient-specific drug screening is vast yet often limited by the sample sizes collected in biopsy methods due to stress and availability on individuals. Hence there is a need to develop platforms that maximise each sampling opportunity. In our research, we have standardised uniformly sized microscale tissues and a microfluidic device to address such a question. Specifically, we have characterised and evaluated the viability of human glioma xenograft tumours and normal mouse liver tissues during incubation in the device. The proof-of-concept device consists of a hydrodynamic trap to align and fix each tissue in a desired position, enabling a specific drug delivery dosage. This paves the way for upscaling of both tissue sample generation and direct testing on tissues for higher throughput in tailored drug treatment and drug development.

Poster Presentation - Abstract 3

Drug repositioning for esophageal squamous cell carcinoma

BENNETT Adam Neil

Esophageal cancer (EC) remains a significant challenge globally, having the 8th highest incidence and 6th highest mortality worldwide. Esophageal squamous cell carcinoma (ESCC) is the most common form of EC in Asia, accounting for over 90% of cases in China. The high mortality rate of EC is due to the limited number of effective therapeutic options. To increase patient survival, novel therapeutic strategies for EC patients must be devised. Unfortunately, the development of novel drugs presents significant challenges as most novel drugs do not make it to market due to lack of efficacy or safety concerns. Drug repositioning is a more time- and cost-effective strategy as it allows repurposing existing drugs to treat EC. This can be achieved by comparing the gene expression profiles of disease-states with the effect on gene-expression by a given drug. In our analysis, we analysed previously published data and identified 167 differentially expressed genes (DEGs). Using weighted key driver analysis, 39 key driver genes were then identified. These driver genes were then used in Overlap Analysis and Network Analysis in Pharmomics. By extracting drugs common to both analyses, 24 drugs are predicted to demonstrate therapeutic effect in EC patients. Several of which have already been shown to demonstrate a therapeutic effect in EC, most notably Doxorubicin, which is commonly used to treat EC patients, and Ixazomib, which was recently shown to induce apoptosis and supress growth of EC cell lines. Additionally, our analysis predicts multiple psychiatric drugs, including Venlafaxine, as repositioned drugs. This is in line with recent research which suggests that psychiatric drugs should be investigated for use in gastrointestinal cancers such as EC. Our study shows that a drug repositioning approach is a feasible strategy for identifying novel ESCC therapies and can also improve the understanding of the mechanisms underlying the drug targets.

Poster Presentation - Abstract 4

Applying chemical proteomics to dissect the regulation of Glutaminase 1 (GLS1)

Chuanqiao DONG[1], Yusheng XIE[2], Liang ZHANG[1]

[1] Department of Biomedical Sciences, College of Veterinary Medicine and Life Sciences, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon 999077, Hong Kong China
[2] Department of Pharmacology, School of Basic Medical Sciences
Shandong University, Jinan 250012, China

Glutaminase 1 (GLS1) is a metabolic enzyme that catalyzes the lysis of the second principal growth-supporting substrate, glutamine, to generate glutamate and ammonia. GLS1 plays a vital role in metabolic reprogramming not only within many types of cancer but also in cell senescence and aging. However, it remains unclear about the molecular regulation of GLS1 in the reprogramming events. Here we developed photoaffinity labeling probes with enrichment handles designed for chemical proteomics profiling of GLS interactome based on click chemistry. We preliminarily evaluated the labeling efficacy of our probes to rHu-GLS1, and to native GLS1 both in cell lysates and live cells including normal cells and cancer cells. In-gel fluorescence assay showed that proteome including GLS1 was pulled down. This would help dissect the regulation of GLS1 in different biological processes including cancer and cell senescence. In addition, we expanded the utility of the probes to study the functional activity of GLS1 via cellular imaging. By using immunofluorescence and our probes, the distribution dynamics of GLS1 was visualized via co-localization analysis. Our results showed robust application of photoaffinity probes for profiling interactome and functional activity of GLS1 which also sheds light on drug development targeting GLS1.

Poster Presentation - Abstract 5

Chemoimmunotherapy of Paclitaxel with endosomal TLR 8 and 9 agonism promoted antitumor immunity in triple negative breast cancer

Eunice DOTSE [1], LIM King Hoo [1],WANG Meijun [1], TIU Cheuk Ying[1], CHOW Kwan Ting [1]

[1] Department of Biomedical Sciences, City University of Hong Kong, Hong Kong SAR.

Triple negative breast cancers (TNBCs) are characterized by lack of estrogen and progesterone receptors and HER2 overexpression. TNBCs tend to be more aggressive, with early onset and poor prognosis. Treatment is clinically challenging and often limited to chemotherapy which is toxic, short lived, and most patient's relapse. Immunotherapy represents a key area of promise for TNBC research. Application of immune checkpoint inhibitors (ICIs) with chemotherapy has shown significant clinical response in TNBC. However, very few patients benefit from ICIs alongside its immune toxicity. There is therefore an unmet need to explore other treatment regimens. TNBCs are more immunogenic, characterized by high number of intratumoral tumor infiltrating lymphocytes. These immune cells are known to express pathogen recognition receptors such as toll-like receptors (TLRs) and their engagement activate downstream pathways to elicit specific T-cell antitumor immunity. We therefore evaluated the combined efficacy of intratumoral administration of TLR7/8 (R848) and TLR9 (CPG-ODN-2395) agonists with paclitaxel or chemotherapy alone in syngeneic TNBC mouse model. Tumors were induced in Balb/c mice by orthotopic injection of 4T1 cells into the mammary fat pad. Mice were treated once a week from day 10. Tumor volume was monitored to access direct antitumor activity. Tumors were collected at experimental endpoint and analyzed for immune cell populations. Results showed combination therapy had significant tumor regression compared to chemotherapy alone. Paclitaxel/R848 treatment had better antitumor effect compared to Paclitaxel/CPG-ODN 2395. Paclitaxel/R848 treatment further altered the immune landscape of the tumor microenvironment by recruiting higher number of B-cells, pDCs, helper and cytotoxic T cells. Regulatory T cells (immunosuppressive cells) were significantly reduced in Paclitaxel/R848 treated mice compared to Paclitaxel/CPG-ODN 2395 and chemotherapy. Findings from this work suggest that combination therapy of TLR7/8 agonist (R848) and paclitaxel may represent a more effective treatment approach for TNBC.

Poster Presentation - Abstract 6

Detection and quantification of intracellular ions in single-cell resolution

Renardi GUNAWAN
Supervisor: Prof. Michael Yang

Intracellular ions are vital components in cells and are involved in multiple aspects of cellular mechanisms, such as in growth, modulation of cell cycles, and in pathological disorders. However, studies on these intracellular ions' effects on diseases are still limited and plagued by challenges to preserve cells' intracellular ions and prevent contamination from external ions. Moreover, most methods to analyze intracellular ions require tedious sample processing, specialized and often expensive instruments, harmful chemicals, and a bulk sample. Additionally, some sample preparation methods can alter intracellular ion concentration in the sample. Thus, a suitable method must be used for intracellular ion analysis.

In this work, we report a novel sample preparation method (FROZEN!) for intracellular ion analysis based on cell isolation (from the surrounding medium) through rapid de-ionized water cleaning, followed by flash freezing and freeze drying for preservation. The proposed sample preparation does not require chemical fixation and enables direct measurement by laser-induced breakdown spectroscopy (LIBS) and X-Ray analytical methods such as X-Ray Fluorescence (XRF) or Energy Dispersive X-Ray Fluorescence (EDS). Cell staining showed that the cells retained their content and location after preparation, which enables single-cell level analysis. Key electrolytes, such as sodium, potassium, chloride, magnesium, and lithium, were detectable at the single-cell level. Following quantification of intracellular Li and Cl, both were successfully detected in concentration as low as 0.5 mM and 0.9 mM in the cells, respectively. These results demonstrate the efficiency and simplicity of the proposed method in intracellular ion analysis, allowing quick and simple preparation to measure biological samples with high sensitivity for various intracellular ions, which may be applicable in the diagnosis of diseases.

Poster Presentation - Abstract 7

Thermal Penetrating Nanomissile targeting the Non-Small Cell Lung Cancer with combined chemotherapy and immunotherapy

GUO Zhengjun
Group of Prof. Michael Yang

For Non-small cell lung cancer, the WHO preferred therapeutic strategy is combined chemotherapy and immunotherapy. Therefore, a multifunctional mesoporous silica shell Fe3O4 core nanoparticle was constructed, carrying chemo drug and immune checkpoint antibodies against the lung cancer cells. After verification, the physical function and biomedical function were investigated. Results indicated that the novel nanoparticle showed great drug carrying/releasing efficiency and stableness. After internalization by cancer cells, it strongly inhibited the cell viability, invasion and metastasis of lung cancer cells *in vitro*. In mouse model, the combined therapy greatly suppressed tumor growth and metastasis, with minimal adverse events. Therefore, this novel multifunctional nanoparticle showed good performance on combined therapy and might be a promising therapeutic approach for patients.

Poster Presentation - Abstract 8

Fluid shear stress enhances the metastatic potential circulating tumour cells in vitro and in vivo

Wenxiu LI, Zhihang ZHOU, Bo GUO, Mengsu YANG

Fluid shear stress (FSS) is crucial in cancer cell survival and tumour development. Noteworthily, cancer cells are exposed to several degrees of FSS in the tumour microenvironment and during metastasis. However, it is unclear that how circulating tumour cells (CTCs) can survive various biological and mechanical insults during this journey and form metastatic tumour. To understand how CTCs withstand FSS, a microfluidic circulatory system with a wide range of physiological FSS was designed to study the cell survival and cell function characteristics of liver cancer cell line (HepG2 and SK-Hep-1) under shear stress treatment. We found that HepG2 exhibited higher cell viability than that of SK-Hep-1 under low and high shear stress. Interestingly, the migration of HepG2 was promoted by low shear stress. Furthermore, RNA sequencing analysis revealed that TLR4 and TPPP3 were overexpressed in shear stress-resistant HepG2 cells. After knockdown of there two genes, HepG2 cells demonstrated low cell viability, decreased colony formation, and poor migration under shear stress. Moreover, in vivo animal study also indicated that overexpression of TLR4 and TPPP3 in SK-Hep-1 cells significantly enhances the metastatic potential to liver and lung organs from the tail vein in mice. Overall, these data implicate TLR4 and TPPP3 may serve as new biomarkers for detecting and targeting metastatic circulating tumour cells.

Poster Presentation - Abstract 9

Mutation-independent gene knock-in therapy targeting 5'UTR for autosomal dominant retinitis pigmentosa

LIAO Baoshan

Despite the recent success of gene supplementation therapy for monogenic recessive diseases, therapeutic approaches to treat dominantly inherited diseases fall behind. Here, we present a new gene knock-in (KI) therapy which exploits AAV-Cas9-mediated homology-independent targeted integration (HITI) of the wild-type coding sequence (CDS) into the 5' untranslated region (UTR), more specifically immediately upstream of the Kozak sequence, of the disease gene. We tested this approach in the heterozygous $Rho^{P23H/wt}$ mice, which carry the most common dominant point mutation found in the autosomal dominant Retinitis Pigmentosa (adRP) patients. We show that HITI-AAVs can mediate highly efficient gene insertion in mouse Rho 5'UTR in vivo. NGS results showed 43% alleles with successful 5'UTR Rho KI, 44% alleles with 5'UTR INDELs, and 13% unmodified alleles in the purified AAV-transduced photoreceptors. The $Rho^{P23H/wt}$ mice had significantly prolonged photoreceptor survival and visual function following the 5'UTR gene KI treatment. In summary, we developed a mutation-independent gene KI approach that targets 5'UTR of the disease gene and demonstrated its therapeutic potential to treat dominant diseases.

Poster Presentation - Abstract 10

Using circulating miRNAs as a diagnosis biomarker for hepatocellular carcinoma

NG Ka Ki

Background:
Currently, there is no clinically recognized non-invasive biomarker for the diagnosis of Hepatocellular carcinoma (HCC). α-fetoprotein (AFP), although being the most widely used serological biomarker for HCC, only has low sensitivity for the diagnosis of HCC. Among multiple liquid biopsy options, we aim to use circulating miRNA in parallel with AFP for distinguish patients with HCC from its at risk population.

Method:
The study was conducted in two phases: a discovery phase (n=100) to screen miRNA candidates from all known human miRNAs using microarray, and a validation phase (n=329) to verify the miRNA candidates using qRT-PCR. Multivariate logistic regression analysis was used to develop a miRNA panel using 5-fold cross validation (repeated for 100 times). The diagnostic performance was evaluated using area under curve (AUC) analysis. 20ng/mL is used as a cutoff for AFP. The result will be further validated in independent cohort in future.

Result:
In the discovery phase, we identified 41 upregulated miRNAs in patients with HCC, which 9 of them have been reported as miRNA targets of HCC in previous studies. With another 14 reported miRNAs, a total of 55 miRNAs was included in the validation phase. Overexpression of 34 miRNAs was confirmed and all miRNAs with AUC>0.65 were used to construct a miRNA panel for the diagnosis of HCC. AUC of a 7-miRNAs panel with and without the use of AFP was 0.851 and 0.759 respectively, which both were superior to the diagnostic performance of using AFP alone (AUC = 0.710).

Conclusion:
The result of current study suggested that circulating miRNAs have superior detection capability than conventional serological marker. Most importantly, they can be used in parallel with AFP to improve the diagnostic performance for the detection of HCC.

Poster Presentation - Abstract 11

A Comparison Study of Two Nano-LC Performance in Proteomic Profiling of Human Serum EVs

Yilin PAN[1]

Mingchan LIANG[2], Yuhao AN[2], Rui WANG[2], Liang ZHANG[1]

[1] *Department of Biomedical Sciences, College of Veterinary Medicine and Life Sciences, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon, Hong Kong, China.*

[2] *Pingshan translational medicine centre, Shenzhen Bay Laboratory, Shenzhen, 518118, P. R. China.*

Cross-platform reproducibility represents an essential requirement for the application of liquid chromatography-tandem mass spectrometry (LC–MS/MS) in biomarkers discovery and clinical proteomics. In this study, we compared the performance of the Evosep ONE and the EASY nLC 1200 systems in comparative label-free MS/MS analysis of serum extracellular vesicles (EVs) from patients of malignant lung cancers or benign pulmonary nodules. The high-throughput Evosep workflow completed the analysis of 20 samples in 16 hours, substantially shorter than the 48 hours using the standard EASY nLC 1200 workflow. In contrast, the EASY nLC 1200 workflow had a better performance in depth and sensitivity, identifying 28% more proteins than the Evosep workflow, predominantly in the low-abundance category. Despite the difference in protein numbers, both workflows generated label-free quantitative datasets that distinguished EVs from patients of malignant lesions and benign nodules. Furthermore, both workflows shared a panel of differential proteins that are significantly upregulated in the serum EVs of lung cancer patients. Among these proteins, we confirmed ORM1 as a high-confidence EV proteins that stratifies malignant and benign pulmonary nodules, utilizing an independent cohort and ELISA assay validation. Our results support the Evosep workflow as a high-throughput and robust platform for biomarker discovery in clinical proteomics.

Poster Presentation - Abstract 12

1,3,5-triazine Derivatives Inhibit Endosome Trafficking Via Lipid Droplets-Endosome Contact

PENG Wang

Endosomal trafficking is an important cellular process for transporting cargoes, which plays vital role in cell function and hemostasis. Disorder of endosomal trafficking is closely related to various human diseases, like cancer, autoimmune diseases, and neurodegeneration. Thus, regulate the endosomal trafficking process could be a potential strategy for cancer therapy. Lipid droplets (LDs) are organelles responsible for lipid storage and metabolic energy. They are consisted by a hydrophobic neutral lipid core, which surrounded by a phospholipid monolayer membrane with special decorating proteins. Despite of energy providing, LDs could communicate with several organelles by formation of contact sites, including ER-LDs, mitochondria-LDs, lysosome-LDs contact, which is important for LDs biogenesis and regulation of other cellular functions. However, the contact between endosome and LDs has not been reported. Previously, we found 1,3,5-triazine compounds could inhibit endosomal trafficking, which might be related to LDs. To find a more potential endosomal trafficking inhibitor, we synthesized series of novel 1,3,5-triazine derivatives. We got AP1 after screening. AP1 showed good anti-cancer activity in vitro and in vivo. And it could inhibit endosomal trafficking via CapZB, and restrict the process at the “transition endosome” stage. Furthermore, we found AP1 inhibited endosomal trafficking via LDs-endosome contact by the designed probe AP1-Coumarin. AP1-comarin showed well colocalize with LDs and contact with early endosome and late endosome. The Mass spectra also indicated AP1 in isolated LDs. And AP1 failed to inhibit endosomal trafficking after inhibition of LDs. Collectively, our results indicated that the 1,3,5-triazine compound AP1 could serve as an anti-tumor agent by inhibition of endosomal trafficking via LDs-endosome contact.

Poster Presentation - Abstract 13

Precise Quantification of Proteins in Single Cells

Rui QIAN[1], Fenglian YANG[1], Liang ZHANG[1]

[1]Department of Biomedical Sciences, City University of Hong Kong, Hong Kong, China

Cell heterogeneity is the property of single cells, which accurately reflects the genetic, epigenetic and stochastic influences of individual cells. Precise quantification of protein abundance in single cells has profound implications for understanding biological models. Here, we have established a precise single-cell protein detection platform by combining proximity ligation assay (PLA) with digital PCR (dPCR). We compared the performance of PLA in both qPCR(Sybr Green and Taqman Probe) and digital PCR. Our results showed the detection sensitivity of dPLA is much higher than that of qPLA. qPLA can only obtain stable data at picomolar level but dPLA can reach femtomolar detection limit, a very necessary condition to detect single-cell protein abundance. We also compared the performance of dPLA and qPLA at the single-cell level using human epidermal growth factor receptor 2 (HER2) as the research object, which showed that dPLA can successfully detect HER2 at the single-cell level, the linear detection range up to 5 orders of magnitude, confirmed the better detection sensitivity of dPLA than qPLA at single-cell level. We proposed that this method can be widely used to detect any trace protein or protein complex, and also extend to the study of protein-protein interactions or protein-dna/rna interactions.

Poster Presentation - Abstract 14

Investigate the oncogenic mechanisms and therapeutics for H2BG53D mutant pancreatic ductal adenocarcinoma

Tiantian QIN, Kui Ming CHAN

Histones are nuclear proteins essential for genomic DNA packaging and epigenetic gene regulation. We have recently identified a novel H2BG53-to-D (H2BG53D) missense mutation in pancreatic ductal adenocarcinoma (PDAC)[1,2]. Our previous studies showed that the G53D mutant H2B are enriched at genes (e.g. *ANXA3*) involved in cell migration-related pathways and increased their expression by weakening the interaction between histone octamer and DNA[2,3]. However, the molecular mechanisms underlying the H2BG53D-specific localization and H2BG53D-driven oncogenic features remain unknown. Here, we show that H2BG53D mutation significantly increased the interaction between nucleosome and histone chaperone FACT. FACT knockdown impaired the increased expression of H2BG53D target gene *ANXA3* and abolished the enhanced migration ability of H2BG53D-PDAC cells. This data suggests FACT as an epigenetic modulator responsible for the H2BG53D induced gene regulation and subsequently aggressive phenotype of H2BG53D-PDAC cells. We will also perform CUT&RUN-seq to further decipher how FACT knockdown alter the enrichment of H2BG53D at genome. In an attempt to develop therapeutics for H2BG53D PDAC, our genome-wide CRISPR/Cas9 screening identified *RRM2* (Ribonucleotide Reductase Regulatory Subunit M2) as a synthetic lethal partner for H2BG53D mutant PDAC. The ribonucleotide reductase inhibitor exerts significant inhibitory effect on the growth of H2BG53D mutant cells than wild-type isogenic control cells. Interestingly, the combinatory treatment of ribonucleotide reductase inhibitor and the FACT inhibitor exhibits a synergistic cytotoxic effect on H2BG53D mutant cells with less toxicity to human pancreatic ductal epithelial cells. In addition, we have crossed the H2BG53D mice with KPC mice (a known mouse model for PDAC)[4] to decipher the *in vivo* role of H2BG53D on PDAC initiation and development. We will also use this KPC-G53D mouse model to examine the efficiency of treatment strategies identified from *in vitro* experiments, which will provide new therapeutics for H2BG53D mutant PDAC and other H2BG53D harboring cancers.

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Poster Presentation - Abstract 15

Automated single-cell loading and cell-cell interaction study of cancer immunotherapy using digital microfluidic chip

Wenting QIU, Wanqing WU and Mengsu YANG

Department of Biomedical Sciences, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong SAR, China

Immunotherapy has proven to be a cutting-edge cancer treatment approach in past decades. Adoptive cell transfer (ACT) exhibits a promising clinical response rate targeting breast cancer, melanoma, B cell leukemias, and lymphomas. Briefly, tumorreactive immune cells are isolated from the patient, expanded ex vivo and infused back to the patient to suppress the tumor progression. Growing evidence suggests that T cells extracted from the same patient can be various and result in distinct cancer treatment performances. Thus, precise evaluation of single T cell function such as cytokine secretion, cytotoxicity, and antigen specificity before infusion back to the patient. Besides, due to the large number of patient derivative T cells, it would be beneficial if the multiparameter analysis could be performed automatically and parallelly.

Herein, we present the first automated digital microfluidic (DMF) chip for cell-cell interaction study at single cell level. The precise single-cell loading and cell pairing are achieved by integration of hierarchical loading microwell structure into DMF chip. Multiple cells and corresponding functionalized beads can be manipulated, loaded and interacted on DMF chip based on their size difference. The multiple microwell array is integrated into the same DMF chip to realize in-situ multiparameter screening of T cell functions. To improve the cell loading efficiency hindered by the inherent hydrophobic interface of DMF, we further modified the localized cell loading area with bio-inspired polydopamine coating. The developed microwell-DMF platform shows promising potential for cell-cell interaction studies at single cell level.

Poster Presentation - Abstract 16

The role and mechanisms of LINC01235 in the pathogenesis of triple-negative breast cancer

Shuru SHEN[1], Jiang LI[2], Xin WANG[2], Rebecca CHIN[1]

[1] Department of Biomedical Sciences, City University of Hong Kong, Hong Kong.
[2] Department of Surgery, The Chinese University of Hong Kong, Shatin, Hong Kong.

Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer lacking efficient molecular targeted therapeutics. Understanding the complex molecular mechanisms driving tumor initiation, progression and metastasis is critical for drug development. Long non-coding RNAs (lncRNAs), RNA transcripts with little protein-coding potential, are found to be closely associated with cancer. Using integrative network analysis with large-scale transcriptomic data, LINC01235 is identified as a master regulator of epithelial-mesenchymal transition (EMT) and invasion activation genes. Our preliminary experimental results support the hypothesis that LINC01235 is highly expressed in TNBC cell lines and is important for promoting EMT both in vitro and in vivo. Mechanistically, Sodium/potassium transporting ATPase interacting 1 (NKAIN1), predicted to be a tumor suppressor gene downregulated by LINC01235, is lowly expressed in TNBC cell lines compared with non-TNBC lines. Acting as molecular sponge is a common mechanism of cytoplasmic lncRNAs and miR-3127-5p is predicted to have interactions with both LINC01235 and NKAIN1 mRNA. Indeed, we observed increased levels of miR-3127-5p and NKAIN1 upon LINC01235 silencing. In addition, NKAIN1 knockdown partially restores the proliferation ability of LINC01235-depleted tumor cells. Work is currently in progress to elucidate how LINC01235 regulating EMT, whether miR-3127-5p and NKAIN1 are bona fide targets of LINC01235.

Poster Presentation - Abstract 17

Improving CTCs capture efficiency on Inertial focusing microfluidics by increasing CTCs size using microparticles

SI Tongxu

Inertial focusing chip is a high throughput CTC capture method from patient blood based on cell size. However, CTC size have overlap with normal blood cells. This limits the development of inertial focusing chip. To overcome this shortage, here we combined immune beads with inertial focusing method, and increase chip capture rate for small CTC. We use EpCAM-modified immune beads to specifically enlarge cell size of different cancer cells. We found that high concentration of immune beads can form cell & particle complex with EpCAM+ cancer cells. For cancer cell lines, especially small-size cancer cells, the capture rate of their clusters has an obvious increase than bare cells. For EpCAM- cell lines, such as WBC, the capture rate has no significant change. By adding immune beads into blood sample, we successfully capture more small-size cancer cells by inertial focusing chip than control. Our method combines the priority of CTC capture methods based on inertial focusing chip and immune magnetic beads. This provides a possibility for inertial focusing chip to capture small-size CTC from patient blood.

Poster Presentation - Abstract 18

The effect of Galectin-9 expression in triple negative breast cancer on plasmacytoid dendritic cells (pDCs) following Toll-like receptor (TLR) agonists activation

SO Chun Yan

Triple negative breast cancer (TNBC) is a special subtype of breast cancer carrying poor prognosis and treatment options are largely limited. The treatment response rate for TBNC is typically low. Immunotherapy in the treatment of cancer has gained significant attention over the past years with particular emphasis on inhibiting immune checkpoints to unleash the anticancer potential of immune cells. Among all immune cells, the function of plasmacytoid dendritic cells (pDCs) is rather mysterious but well known for their involvement in both innate and adaptive immunity via production of vast amount of type I interferon (IFN). There has been quite a number of attempts to make use of pDCs to fight cancer, in particular with the use of Toll-like receptor (TLR) agonists. However, despite promising preclinical data with regard to the antitumor effect of TLR agonists, they failed to translate into meaningful clinical efficacy, likely due to the immunosuppressive tumor microenvironment.

Galectin-9 (Gal-9) belongs to the lectin protein family that bears different functions intra- and extra-cellularly. It has recently been discovered that extracellular Gal-9 interacts with different types of immune cells to exert immunosuppressive effects. Nonetheless, little is known as to how Gal-9 interacts with pDCs and suppresses their production of type I IFN.

My research demonstrated that Gal-9 mRNA and protein levels express differentially among a panel of TNBC cell lines. CAL-1 cells (a human pDC cell line) exposed to the conditioned media of MDA-MB-468 and HCC70 (high Gal-9 expressors) following TLR agonist stimulation resulted in significantly lower level of Type I interferon production. More study is needed to define the role of secreted Gal-9 from TNBC cells on pDC.

Poster Presentation – Abstract 19

Organelle - targeting photocatalytic proximity labeling using iridium complexes

Tian TIAN [1], Peter Kam-Keung LEUNG [2], Kenneth Kam-Wing LO [2], Liang ZHANG [1]

[1] Department of Biomedical Sciences, City University of Hong Kong, Hong Kong, P. R. China
[2]Department of Chemistry, City University of Hong Kong, Hong Kong, P. R. China

Enzymic proximity labeling (PL) is widely used to study biomolecule interactions and cellular microenvironments. However, PL is always based on bioengineering. Organometallic transition metal complexes-based photocatalytic proximity labeling has become an alternative method because of its high spatiotemporal control over the labeling. Our study used cyclometallated iridium complexes to build an organelle-targeting photoactivable proximity labeling method. These complexes have a varied affinity to different cell compartments, such as ER and mitochondria, thus making it possible to do the organelle-targeting proteomics; The photocatalysis reaction is under 450nm light irradiation so that we can study the cell proteome in different cell states or time points. The cell tracker staining results showed that complexes could localize in different organelles. We also demonstrated that the iridium complexes-mediated photocatalytic labeling works on protein biotin labeling in vitro and in living cells. The immunofluorescence result confirmed that the photocatalytic biotinylation could colocalize with organelle markers. This method provides an alternative to enzyme-based proximity and paves the way toward more controllable photoactivatable proximity labeling. We will profile proteomics in ER while under transient stress to complete its application.

Poster Presentation - Abstract 20

Investigate the role of IRF5 in macrophages and myeloid cells in anti-tumor immunity

WANG Meijun, CHOW Kwan Ting

IRF5 is a crucial transcription factor that regulates the inflammatory immune response, which is a key driver of anti-tumor immunity. However, the pathways and factors that regulate IRF5 are not well-defined, especially in myeloid cells. IRF5 plays a crucial role in M1 vs. M2 macrophage polarization, and tumor-associated macrophages (TAMs) that display M2 phenotype are often associated with bad prognosis. Thus, we hypothesize that IRF5 activation within macrophages (Mφ) and myeloid cells is a key regulator of anti-tumor immunity. To reveal the role of IRF5 in anti-tumor immunity, we established the B16F10 melanoma tumor model in mice with a myeloid-specific deficiency of *Irf5* (*Irf5*-MKO) and wild-type (WT) mice. Our results indicated that as compared to WT, mice with myeloid-specific *Irf5* deficiency showed suppressed tumor growth. Tumors harvested from *Irf5*-MKO mice displayed increased leukocytes and M1 Mφ infiltration. These results suggest that *Irf5* activation in myeloid cells favors tumor progression in the melanoma mouse model. We therefore investigated the molecular pathways that regulate IRF5 activity in myeloid cells and Mφ. We found that contrary to plasmacytoid dendritic cells (pDCs), TLR7 and 9 stimulations failed to activate IRF5 in myeloid cells and Mφ. We hypothesize that a negative regulator is preventing the activation of IRF5 downstream of endosomal TLR stimulation. IRF5 is marginally activated when stimulated with high concentration of TLR8 agonist, while knocking out IRAK2 inhibited IRF5 activation. We are currently further characterizing the molecular basis of IRF5 activation in myeloid cells and investigating its role in immune modulation for the design of cancer immunotherapy.

Poster Presentation - Abstract 21

Novel Hsp27 inhibitor HK-2, a fluorine TDP derivative, is a potent anticancer agent

Xiong WANG, [1] Huangcan LI, [1,4] Bin ZHANG,[3] Wei-Hua HUANG,[3] Xiao ZHANG,[3] Yaxiu FENG,[1] Zhiqin DENG,[2,4] Shu CHEN,[2,4] Guangyu ZHU,[2,4] Gui LU,[3,*] Ming-liang HE,[1,4,*]

1 Department of Biomedical Sciences, City University of Hong Kong, Kowloon, Hong Kong SAR, P. R. China
2 Department of Chemistry, City University of Hong Kong, Kowloon, Hong Kong SAR, P. R. China
3 School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou, 510006, P. R. China
4 City University of Hong Kong Shenzhen Research Institute, Shenzhen 518057, P. R. China.

Hsp27 is widely accepted as an ideal drug target due to the association of its upregulation with many diseases, including human cancers, as well as an association with drug resistance. However, despite enormous efforts over the last decades, no clinical use for an Hsp27 inhibitor has yet been developed due its low specificity and significant side-effects. TDP, a natural compound isolated from a traditional Chinese herb, displays potent anticancer activity, but with significant side effects *in vivo*. We have designed and synthesized fluorine-TDP derivatives and tested their anticancer activities. Among them, HK-2 displayed high binding affinity with Hsp27, and induced Hsp27 degradation. Compared with TDP, the binding affinity was increased two-fold, and the IC_{50} required for curbing hepatocellular carcinoma cell growth decreased from 8 μM (TDP) to 4.5-211.5 nM (HK-2), while tumor to normal cell selectivity increased more than 53.4-fold. The target specificity of HK-2 was confirmed with the use of Hsp27 knockout cells. HK-2 also showed potency and efficacy in eliminating various cancers, arresting the epithelial to mesenchymal transition (EMT), and limiting cancer cell migration and invasion. The murine carcinoma xenograft model further confirms that HK-2 can effectively inhibit tumor growth and metastasis *in vivo* without notable side-effect or toxicity in immunocompetent mice. The results indicate a novel Hsp27 inhibitor with antitumor activity, and a new direction for developing small molecules targeting Hsp27.

Keywords: Hsp27, fluorine, Hsp27 inhibitor, anticancer, antimetastasis

Poster Presentation - Abstract 22

Endosomal trafficking in metastasis and anticancer immune response

WANG Xu

We demonstrated that 6J1 (a potent endosomal trafficking inhibitor synthesized by us) blocked the endosomal trafficking of PD-L to induce its accumulation at endocytic vesicles by activating Rab5. 6J1 not only rendered tumor cells more sensitive to the tumor-killing activity of co-cultured T cells in vitro, but also increased tumor-infiltrating cytotoxic T cells by inducing the secretion of chemokines in the tumor microenvironment in the syngeneic mouse model of mammary carcinoma, melanoma, or lung cancer. Moreover, the combination of 6J1 and the anti-PD-1 antibody significantly improved anticancer immune response when compared to either treatment alone. Taken together, our study indicates that manipulation of endosomal trafficking to change membrane PD-L1 and tumor microenvironment by 6J1 provides a promising means to promote the anticancer immune response in addition to the classical ICIs.

Poster Presentation - Abstract 23

Comparative proteomic profiling of the interactome and substrates of MEK1 and MEK2

Ying WANG[1,2], Haiying MA[1,2], Liang ZHANG[1,2*]

[1]Department of Biomedical Sciences, College of Veterinary Medicine and Life Sciences, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon, Hong Kong, China.
[2]Key Laboratory of Biochip Technology, Biotech and Health Centre, Shenzhen Research Institute of City University of Hong Kong, Shenzhen, 518057, China.

ERK signaling is a prominent pathway deregulated in cancer and is frequently represented as a linear RAS–RAF–MEK–ERK signaling cascade. Along this cascade, MEK1 and MEK2 have a high degree of homology and identical substrate specificity. Although studies have suggested that MEK1 and MEK2 have nonredundant roles, it remains unclear how MEK1 and MEK2 function distinctively in wider signaling networks. To address this, we developed the proximity-based phospho-interactome (Prob-Phl) platform to dissect the interactome and substrates of MEK1 and MEK2. We fused the MEK1 and MEK2 kinase with the engineered biotin ligase BASU. Upon biotin addition, BASU empowers the proximity labeling that captures stable and transient interactors, including the substrate. To cross-validate the substrates of MEK1 and MEK2 kinases, we used Trametinib to block the activity of MEK1 and MEK2 kinases and serve as the control. The biotinylated proteins were then captured and identified employing streptavidin pull-down. After trypsin digest and phosphor-peptides enrichment, the total interactome and substrates of MEK1 and MEK2 were analyzed by LC/MS/MS. Overall, we identified 392 and 473 potential interacting proteins of MEK1 and MEK2 using the Prob-Phl method. In addition, we also found 39 and 71 distinctive substrates of MEK1 and MEK2, respectively. Functional validation revealed that MEK2, but not MEK1, specifically interacts with and phosphorylates lysosome-associated membrane glycoprotein 3 (LAMP3 or DC-LAMP) and regulates autophagy. Overall, our study delineated the distinct interactome and substrates of MEK1 and MEK2. The robust performance of Prob-Phl could also be extended to functionally dissect other kinases.

Poster Presentation - Abstract 24

Aptamer-functionalized DNA Hydrogel Glue for Capturing and Releasing Circulating Tumor Cells

Yuan WANG,[a][b][c]# **Xiaoyu ZHOU,** [a][b][c]# **, and Mengsu YANG**[a][b][c]

[a] Department of Precision Diagnostic and Therapeutic Technology, The City University of Hong Kong Shenzhen Futian Research Institute, Shenzhen, Guangdong, China
[b] Department of Biomedical Sciences and Tung Biomedical Sciences Centre, City University of Hong Kong, Hong Kong, China
[c] Key Laboratory of Biochip Technology, Biotech and Health Centre, Shenzhen Research Institute of City University of Hong Kong, Shenzhen, Guangdong, China

# These authors contributed equally to this work.

Circulating tumor cells (CTCs) have attracted increasing attention in liquid biopsy. However, the enrichment of living CTCs for downstream analysis remains challenging. Herein, we present an aptamer-functionalized DNA hydrogel glue generated via rolling circle amplification (RCA) for capturing and releasing living CTCs. The DNA hydrogel glue demonstrates porous 3D nanoflower networks consisting of periodic repeats of epithelial cell adhesion molecule (EpCAM) aptamer units. Since most of the CTCs are of epithelial origin, the aptamer units contribute to high capture efficiency and sensitivity. Adding adenosine triphosphate (ATP) and the corresponding releasing primer, the encapsulated CTCs were released from the DNA hydrogel glue in 10 mins with minimal damage for downstream culturing and molecular analysis. In addition, the DNA hydrogel glue can be prepared in advance, simplifying the capturing process of CTCs. The high biocompatibility property of DNA hydrogel ensures cells maintain their viability and proliferation ability and can be cultured into tumor spheres after release. Furthermore, CTCs were successfully isolated from peripheral blood samples from patients with different cancers. The DNA hydrogel glue, with outstanding biostability and biocompatibility, provides a strategy for detecting CTCs for cancer diagnostics and prognosis in clinical settings.

Keywords: circulating tumor cells • rolling circle amplification • aptamer • DNA hydrogel • cell capture and release

Poster Presentation - Abstract 25

Efficient Enrichment and Detection of Small Extracellular Vesicles Using Immunoaffinity Cellulose Nanofibril Networks

WANG Zesheng

Extracellular vesicles (Evs) have shown great potential in diagnostic, therapeutic and drug delivery applications due to their various cargos and serving as significant messengers for cell communication. However, as its high heterogeneity and complexity, the traditional isolation methods suffer from low-yield, labor-intensive and bulky equipment required, making the efficient isolation of Evs remains a considerable challenge. Here, we show that high-surface-area TEMPO-oxidized cellulose nanofibril (TOCNF) networks with surface functionalization and excellent dispersion properties can significantly improve the enrichment effect of sEv. Specifically, we successfully fabricated TOCNF@CD63 nanomaterial for the enrichment of Evs, and the capture efficiency of the immunoaffinity material is 86.3%, which outperforms its counterparts. After enrichment, Ev-derived miRNA and proteins were detected and the performance of our method was further evaluated in a complex biological fluid (plasma). The results demonstrated that the method could achieve better relative yield than the conventional method. We expect this facile and efficient method for the enrichment and detection of sEv may have good potential for further biomedical application.

Keywords：Extracellular vesicles；cellulose nanofibril；immunoaffinity；biomedical application

Poster Presentation - Abstract 26

Activation of Photosensitizers via Bioorthogonal Decaging Reactions using Palladium(II)-containing Peptide for Targeted Photodynamic Therapy

Qianqian WU
Supervisor: Dr. Gigi P. C. Lo

It has been a challenging task to deliver the photosensitizer specifically and confine the photodynamic action precisely at the tumor site. Instead of conventional photosensitizers, activatable photosensitizers have attracted much attention recently. In this work, we utilized a novel bioorthogonal decaging reaction strategy for the targeted delivery and site-specific activation of a photosensitizer for photodynamic therapy. It involved the use of a propargyl-caged distyryl boron dipyrromethene (DSBDP)-based photosensitizer, labeled as Al-DSBDP. This compound is in the quenched state due to the capping of the ester substituent at the meso position of DSBDP, however, its photoactivity could be recovered through the bioorthogonal decaging reaction using a palladium-containing peptide, named HRGDH-Pd(II). This palladium-containing peptide could be internalized into $\alpha_v\beta_3$ integrin-overexpressed cancer cells selectively. Upon encountering the intrinsically quenched Al-DSBDP, HRGDH-Pd(II) could trigger the specific bond cleavage of propargyl substituent followed by self-immolation, thereby releasing the carboxy DSBDP 9 and restoring the photodynamic activity upon light irradiation, and hence causing cellular damage on cancer cells.

Poster Presentation - Abstract 27

Self-targeted magnetic nanoparticles for combined magnetothermal therapy and immunotherapy in cancer treatment

Xulin XIE, Mengsu YANG

Magnetic hyperthermia therapy (MHT) is non-invasive and features excellent tissue penetration for deep-seated tumors, but unfortunately, it suffers low therapeutic efficacy due to the insufficient intratumor accumulation of conventional intravenous-injected magnetic nanoparticles. Such a disadvantageous characteristic is expected to be solved by specially designed tumor-targeted magnetic nanoparticles. A kind of PEGylated iron oxide superparamagnetic nanoparticles with penetrating peptide modification has been synthesized which exhibits excellent and highly controllable magnetic hyperthermia performance for effective MHT at the local tumor site. The controllable mild MHT at 43−44 °C based on the tailored magnetic nanoparticles demonstrates almost complete inhibition of mammary cancer cell proliferation and tumor growth. More importantly, the mild MHT-treated mammary cancer cells are capable of activating natural killer (NK) cells. As a result, the growth of both xenograft mammary tumors was almost completely suppressed under mild MHT via induced NK-cell-related antitumor immunity *in vivo*. This work not only evidences the great potential of mild MHT but also reveals the underlying immunity activation mechanism in mammary cancer treatment by mild MHT.

Poster Presentation - Abstract 28

Combining O2 Economizer with Photodynamic Therapy to Combat Tumor Hypoxia Resistance

XU Feijie

Tumor hypoxia, as a notorious phenomenon involved in tumor growth, has proven to be the major obstacle of many anticancer therapies, especially the oxygen-dependent type II photodynamic therapy (PDT). Instead of conventional O2 supply methods, herein we employ an innovative "O2-economizer" strategy, which enhances O2 concentration by decreasing cellular O2 consumption of cell respiration, to overcome the hypoxia barrier. The organic nitrate, as a well-known nitric oxide (NO) donor responsible for respiration inhibition and a photosensitizer zinc(II) phthalocyanine (ZnPc) for photodynamic therapy (PDT), are combined via the click reaction to get the ZnPc-specific O2-economizer ZnPc-NO. Compared to ZnPc, compound ZnPc-NO shows excellent photocytotoxicity and tumor regression under hypoxic conditions. More importantly, ZnPc-NO could induce immunogenic cell death (ICD), with the release of danger-associated molecular patterns. We demonstrate that O2-economizer strategy offers a novel approach to address the issue of hypoxia-induced tumor resistance to PDT.

Keywords: PDT, Hypoxia, Nitric oxide, ZnPc, O2-economizer, ICD

Poster Presentation - Abstract 29

Development of Self-Assembled Nanoagents for Multimodal Cancer Therapy

YANG Lin

A facile preparation method was applied to fabricate a series of multifunctional nanoagents. The Cu^{2+} ion could assemble with Fmoc-Leu-OH to form nanoparticles, labeled as Fmoc-Leu/Cu. With the addition of zinc(II) phthalocyanine-based photosensitizer (ZnPc) and glutathione (GSH) biosynthesis inhibitor sorafenib, the corresponding nanoparticles, namely Fmoc-Leu/Cu@Pc and Fmoc-Leu/Cu@sorafenib/Pc could be obtained. These nanoagents exhibited robust stability in physiological conditions, while they could disassemble upon internalization into HT29 cancer cells. The released Cu^{2+} could be reduced to Cu^{+} by GSH to catalyze the transformation of H_2O_2 enriched in cancer cells to hydroxyl radicals in HT29 cells, thereby inducing a significant chemodynamic effect. Fmoc-Leu/Cu@sorafenib/Pc exhibited the highest cytotoxicity on HT29 cells upon light irradiation, demonstrating the synergistic anti-cancer effects of Cu^{2+}, ZnPc, and sorafenib.

Poster Presentation - Abstract 30

Tumor-accumulating *Salmonella* reinvigorates tissue-resident memory (TRM)-like CD8$^+$ T cells against solid tumors through interleukin-10

ZANG Zhongsheng

The failure to elicit potent immune response in the solid tumor microenvironment (TME) present a major limitation to the application of state-of-the-art immunotherapy, such as immune checkpoint inhibitors and adoptive cell transfer. The condensed tissue structure and irregular blood vessel of solid tumors resist the infiltration of therapeutic agents. And the immunosuppressive TME further renders these agents in a dysfunctional state. We have programmed a *Salmonella enterica* strain to exclusively thrive in solid tumors but were quickly depleted in normal organs as administrated intravenously. On solid tumor models these bacteria robustly showed therapeutic efficacy and long-term immune memory against tumor recurrence and metastasis. The therapeutic effects primarily stem from the local reinvigoration and expansion of the pre-existing exhausted tumor tissue-resident memory (TRM)-like CD8$^+$ T cells in TME. Our analysis revealed that the revitalization of exhausted TRM-like CD8$^+$ T cells was mediated by interleukin-10 (IL-10)/IL-10 receptor (IL-10R) signaling. The IL-10 was predominantly produced by tumor-associated macrophages (TAM) in response to the bacterial colonization of solid tumors. Further, we found that the intratumoral preexistence of IL-10R not only primed TRM-like CD8$^+$ T cells for reinvigoration, but also promoted TAM for producing IL-10 and facilitated bacterial evasion from tumor-associated neutrophil phagocytosis. These results reveal the unsolved mechanism underlying bacterial cancer therapy, suggesting IL-10R expression as a new predictor for immunotherapy responses, and pave a way toward new therapeutic approaches for solid tumor non-invasive treatments by bacterially intratumoral immunomodulation.

Poster Presentation - Abstract 31

Zinc (II) phthalocyanine And Rhenium(I) Tricarbonyl conjugates for enhanced photodynamic therapy and chemotherapy

ZHANG Yuanfeng

Photodynamic therapy (PDT) is an attractive non-invasive precision treatment technique for cancer. PDT has been used clinically for the treatment of a range of cancers and noncancerous conditions. Through the interactions of photosensitizers (PSs), the light of appropriate wavelength, and oxygen, various reactive oxygen species (ROS), particularly singlet oxygen, are generated that can cause cellular and tissue damage. Because of the unique and distinct mechanism, PDT can circumvent the disadvantages of conventional anticancer methods. Its therapeutic efficacy depends on the tumor selectivity and ROS generation efficiency of the PSs. Recently, there has been considerable interest in combining PDT with chemotherapy, which could induce different cytotoxic pathways resulting in enhanced therapeutic efficacy. The zinc phthalocyanine with an α-substituted amino group (**Pc-NH_2**), glutathione (GSH) cleavable disulfide linker (ss), and $Re(CO)_3$(dmphen)(p-suc-lCN)]$^+$ (**TRIP-suc**) was successfully conjugated to offer the dual therapeutic compound (**Pc-ss-TRIP**). The photophysical properties, ROS generation, cellular uptake, and cytotoxicity of **Pc-TRIP** were examined. We have found that **Pc-TRIP** exhibited the most significant anti-tumor effect.

Poster Presentation - Abstract 32

Akt3-specific Signaling Networks in Triple-negative Breast Cancer

Shixue ZHENG[1], Tan WU[1], Liang ZHANG[1], Xin WANG[1], Y. Rebecca CHIN[1]

1 Department of Biomedical Sciences, City University of Hong Kong, Kowloon, Hong Kong.

As the first identified PI3K downstream effector, AKT (also called PKB) has been widely studied in biology and medicine research fields. PI3K-AKT signaling pathway plays an essential role in cell function and genes of this pathway are frequently dysregulated in cancer, making it a promising therapeutic target. Different AKT isoforms have distinct functions in cells. For instance, depleting AKT3 inhibits growth of triple-negative breast cancer (TNBC), indicating it as a potential therapeutic target for this aggressive subtype of breast cancer, where limited treatment strategy is available due to the lack of hormone receptors. Among all three Akt isoforms, Akt1 has the most substrates being identified, whereas Akt3 isoform specificity has not been well-studied. Exploring the downstream effectors of Akt3 would be crucial for depicting and understanding the PI3K-Akt signaling pathway. Based on our mass spectrometry-based proteomic results, several Akt3 substrate candidates have been identified. In this study, experiments are performed to validate the candidates as novel Akt3 substrates as well as identify the functional outcome of their phosphorylation in TNBC.

Poster Presentation - Abstract 33

Screening of Liver and Pancreatic cancer metastasis and regulatory genes and mechanisms in vivo with CRISPR/Cas9 library

ZHOU Li

Liver cancer and pancreatic cancer are the most common malignancies worldwide. Metastasis is the main causes of death from cancer, and circulating tumor cells (CTCs) are the key link. However, the difficulty in obtaining advanced cancer metastatic tissues and CTCs in clinical limits our in-depth understanding of the mechanism of cancer metastasis. However, orthotopic transplantation tumor model of liver and pancreas in mice can better simulate the process of metastasis in vivo. We used human CRISPR/Cas9 whole gene knockout library to construct metastasis models of mice, and isolated CTCs in peripheral venous blood of mice using self-developed inertial focusing principle microfluidic chip to screen cancer metastasis relevant genes, which is helpful to fully reveal the mechanism of cancer metastasis of liver and pancreatic cancer.

Poster Presentation - Abstract 34

CTC in vitro culture methods and application

ZHOU Zhengdong

Circulating tumor cells (CTCs) show promising clinical values in cancer diagnosis, therapeutic effect monitoring, and prognosis. The extreme rarity of CTCs in blood samples presents major challenges for further in-depth study. In vitro CTCs amplification makes up for the deficiency of CTCs quantity insufficiency, which provides consistent materials for a comprehensive understanding of the function and molecular characteristics of CTCs and the establishment of CTC-derived organoid or CTC-derived xenograft (CDX) models for personalized treatment. Over the past two decades, a growing number of CTC in vitro culture research has been reported. However, the existing culture strategies are also characterized by a considerable variety in culture methods, successful rate, and culture time. This review paper presents the development of CTC in vitro culture research. We focus on comparing the similarities and differences of these culture strategies applied in different works, mainly incorporating several crucial factors, including sample collection, CTC enrichment strategy, incubation condition, and medium ingredient. In addition, the ongoing applications of the CTC in vitro culture research are also reviewed. This work aims to systematically analyze these key nodes during the culture process and provide new insight for developing new in vitro culture strategies.

Poster Presentation - Abstract 35

Improving CRISPR Gene Editing Efficiency Through High-Throughput Screening of SaCas9 gRNA Structures Using Single-Cell Sequencing

Al Limei

CRISPR technology has revolutionized gene therapy and has shown immense potential for clinical applications. However, limitations such as low editing efficiency and off-target effects continue to impede its widespread use. One approach to improve the performance of CRISPR gene editing tools is to modify the secondary structure of the CRISPR guide RNA (gRNA) scaffold and identify gRNA structures that exhibit high genome editing efficiency. Traditional methods for screening gRNA structures are time-consuming and inefficient for large-scale library screening. In this study, we designed and evaluated over 10,000 different SaCas9 gRNA secondary structures using a high-throughput screening technique based on single-cell sequencing. This method allows for the rapid and efficient evaluation of the editing efficiency of thousands of gRNA sequences in a single experiment. We identified sequences that exhibited significantly higher editing efficiency than the wild-type gRNA, demonstrating the utility of our screening method for identifying highly efficient gRNA structures. Our study contributes to the optimization of CRISPR gene editing tools for clinical applications and provides a novel and efficient high-throughput screening technique for identifying gRNA structures with high editing efficiency. We believe that the application of this technique will lead to significant advances in the field of clinical gene therapy.

Poster Presentation - Abstract 36

Flexible CRISPR-Cas9 possessing dual-proofreading ability with improved specificity

BAO Yufan

The clustered regularly interspaced short palindromic repeat (CRISPR)-Cas9 system has been widely deployed as a powerful tool for genome editing, but is hindered by off-target DNA cleavage. The development of Cas9 variants that improve enzymatic specificity or broaden its protospacer-adjacent motif (PAM) compatibility is of great importance. However, previous modifications to proteins have often sacrificed accuracy for greater editing efficiency. Here, we exploited this rationally-designed flexible CRISPR-Cas9 protein, referred to as Flex-SaCas9, which demonstrates substantial improvement over prior protein engineering and still retains high accuracy. Over all the tested human endogenous sites with the canonical NNGRRT protospacer adjacent motif (PAM), we observed minimal off-target activities and comparable on- target efficiencies of Flex-SaCas9 to those of wild-type SaCas9. Furthermore, Flex-SaCas9 identified harboring mutagenesis of residues in the contact with both the target DNA strand (TS) and non-target strand (NTS) possess the dual-proofreading ability, which exhibits superiority over the single-proofreading high fidelity variant (SaCas9-HF). We expect these results to improve the performance of CRISPR screening and enlighten future research on Cas9 engineering.

Poster Presentation - Abstract 37

Exploring the regulating function of *PSPPH_4638* in *Pseudomonas syringae*

CHEN Fang

Pseudomonas syringae is one of the most common plant pathogens. It can infect more than 50 major crops, which has caused serious economic losses in the world. *P.syringae* has evolved a variety of strategies to promote virulence, such as transporting effector proteins into plant cells through the type III secretory system (T3SS), producing extracellular polysaccharides (EPS) and plant toxins to damage plant cells. T3SS is a common pathogenic secretory system among Gram-negative bacteria and is also the most important pathogenic pathway of *P.syringae*. It is a multi-protein nano-complex similar to a syringe, which directly injects various effector proteins into the host cell since it contacts the membrane of host cells. Type VI secretion system(T6SS) is another well-conserved T4 bacteriophage tail-like weapon, it is a powerful weapon used by bacteria against eukaryotic and prokaryotic cells. Transcription factors (TFs) contribute to the regulation of various important pathways in bacteria, including metabolism and pathogenicity. In previous work, we obtained data from 186 TFs of *P.syringae* through ChIP-seq methods, after analysis and EMSA validation, we found the TF, PSPPH_4638, which is a LysR family transcriptional regulator, has peak in a variety kinds of pathways in *P.syringae* , like Bacterial chemotaxis, Two-component system, and metabolism. And the ChIP-seq result also showed that it can bind to the promoter region of many virulence-related genes, including genes that contribute to biofilm formation, T3SS, EPS formation, and T6SS, which indicates that it may regulate the virulence and competition ability of *P.syringae* against other species. In this work, we want to explore if PSPPH_4638 can regulate genes involved in some of these important pathways directly by binding to their promoters so that more new functions of this TF could be revealed.

Poster Presentation - Abstract 38

7-methylguanine in bacterial

DING Yiqing

RNA modifications play essential roles in gene expression regulation. We attempt to investigate the epigenetics of bacterial pathogenicity. Our previous study reveals crucial roles that rG4s play in the regulation of bacterial pathogenicity and metabolic pathways. However, 7-methylguanine (m7G) is another known RNA methylation modification with a positive charge. The presence and related biological functions of m7G deserve to be studied. Although m7G is present in the ribosomal RNA of bacteria, its occurrence in mRNA still remains elusive. Here, we used a method that avoids using traditional chemistry for RNA sequencing and instead takes use of the creation of abasic sites and subsequent aniline cleavage (AlkAniline-Seq) to identify the m7G sites in the two model bacterial species (Escherichia coli and Pseudomonas aeruginosa). In this article, we introduce a novel approach to RNA-seq library construction that relies on positive read enrichment based on chemistry to produce libraries with unheard-of signal-to-noise ratios. We totally identified 85 m7G sites in E. coli and 59 m7G sites in P. aeruginosa, suggesting the abundant occurrence of m7G modification in these two bacteria species. Our results revealed the distinct m7G distribution patterns in bacteria. We found more than half of the m7G sites located in rRNA and tRNA. The m7G sites showed different base and codon preference between E. coli and P. aeruginosa. More m7G sites are present in the second base in a codon compared with other positions. The Density results of E.coli and PAO1 group showed that the m7G sites were enriched at the end of the 5 Untranslated regions. Taken together, this study uncovered dozens of m7G sites in bacteria with potential important biological roles.

Poster Presentation - Abstract 39

Identify the lncRNAs that regulate the chromatin modification by Chrom-seq

Ligang FAN[1], Jian YAN[1]*

[1]Department of Biomedical Sciences, College of Veterinary Medicine and Life Sciences, City University of Hong Kong, 83 Tat Chee Avenue, Kowloon, Hong Kong, China.

Long noncoding RNAs (lncRNAs) play a vital role in a variety of biological functions. Many of biological functions are involved in chromatin modification processes regulated by lncRNAs. Therefore, it is particularly important to find lncRNAs that regulate chromatin modification and study their functions. Although several methods have been developed to identify RNA interacting with proteins in recent years, it is still limited to fully reveal the function of lncRNAs. Here, we developed a method for the efficient identification of lncRNAs that regulate specific chromatin modification. Previous reports have shown that CBX1 can specifically recognize H3K9me3 modifications, and CBX7 can specifically recognize H3K27me3 modifications. At the same time, it was reported that APEX2 could add biotin to the RNA. Based on these strategies, we used the suntag system to combine CBX1 or CBX7 with APEX2 to develop a new method for identifying lncRNAs that regulated specific chromatin modification, named Chrom-seq. In this study, we identified many lncRNAs that regulated chromatin modification, some of which had been reported, such as H19, MALATA1, NEAT1 involving chromatin modification. In addition, we also further studied the biological function of new lncRNAs identified by Chrom-seq.

Poster Presentation - Abstract 40

Investigate the function of AATF in X chromosomal inactivation and transcriptional gene regulation

Bing HAN, Yi Ching Esther WAN, Kui Ming CHAN

The X chromosome inactivation (XCI) is a classic epigenetic gene regulation process to inactivate one of the two X chromosomes in female mammals to compensate X-gene dosage between sexes [1]. X Inactivation Specific Transcript (*XIST*), a long non-coding RNA (lncRNA), is required for the initiation and maintenance of XCI [2]. However, the protein partners of *XIST* and the molecular mechanism of *XIST*-proteins mediated XCI remain largely unknown. In collaboration with colleagues in BMS department, we have previously characterized SNF2L and TAF15 as regulators of the X-linked genes [3]. Here we show that the Apoptosis Antagonizing Transcription Factor (AATF) exhibits promising regulatory effects on XCI maintenance. The knockdown of AATF elevated the mRNA and protein level of GFP transgene inserted to the inactivated X (Xi) in female immortalized mouse embryonic fibroblast cells (♀iMEFs). In addition, AATF depletion results in a global reactivation of Xi-linked genes in female hybrid MEF cells revealed by allele-specific RNA-seq. Moreover, by formaldehyde-assisted RNA-ImmunoPrecipitation (fRIP)-qPCR, we confirmed the binding capacity of AATF with *XIST*. However, the exact binding pattern remains to be deciphered. We will include a UV crosslinking strategy to elucidate whether the binding is direct or indirect and establish the truncated AATF constructs to map the responsible binding domain (domain of interest, doi) with *XIST*. AATF-doi protein purification followed by mass spectrometry (MS) will be conducted to identify the candidate proteins that serve as bridges or chaperones in the AATF-*XIST* complex. The further exploration of AATF-doi MS candidates could shed more light on the mechanism of *XIST* in XCI.

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Poster Presentation - Abstract 41

Characterization of virulence-related transcription factors network in *Pseudomonas aeruginosa* and their functions

HAN Liangliang

Sequence-specific DNA binding proteins known as transcription factors (TFs) can read the code by binding to *cis*- regulatory elements to activate or repress gene expression. Each gene receives instructions from multiple TFs, and each TF targets thousands of genes, forming a regulatory network (TFN), which controls almost all biological processes inside the cell. Hence, it is critical to understand the mechanism, architecture, and behavior as well as conservation and diversification of the regulatory network. In *P. aeruginosa*, TFs play essential roles in regulating its virulence, such as quorum sensing (QS) and protein secretion systems (TSS). Therefore, it is crucial to investigate the biological functions of TFs in virulence regulation networks. However, TF binding studies in pare too few to produce a general picture of this complex network. In this study, to globally study the potential roles of TFs we take advantage of large- scale chromatin immunoprecipitation combined with next-generation sequencing (ChIP-seq) technology to detect the possible biological functions of all regulators and further reveal the crosstalk of 135 TFs and train machine-learning models to predict TF binding and co-localization in *P. aeruginosa*.Taken together, our results give novel insight into resolving infection problems caused by *P. aeruginosa*, show the variability in regulatory mechanisms of TFs, and expand upon the complexity of bacterial transcriptomes.

Poster Presentation - Abstract 42

Deep learning-based recovery of gene expression profiles from FFPE samples

HE Lingli

FFPE tissue-derived RNA-sequencing data cannot be comparably employed for cancer molecular analysis as FF-derived tissue. Reliable strategy has not been raised. To recover the distorted gene expression profile effectively, we analyzed 672 FFPE RNA-sequencing transcriptomes across seven cancer types and 28 types of FF RNA-sequencing transcriptomes with totally 9568 primary tumors from TCGA. A convolutional recovery network was constructed and proved to be robust and effective in recovering both simulated and real FFPE RNA-sequencing tumor samples. The network can be applied with most types of cancer and is robust with distinct library preparation procedures and sequencing platforms. The recovered gene expression profiles showed more reasonable gene expression levels, illustrated more meaningful biological properties, and gained better prognosis.

Poster Presentation - Abstract 43

Determine Mechanical Characterization of Chiloscyllium Plagiosum IgNAR Diversity from mRNA

JIANG Yuan

Department of Biomedical Sciences, City University of Hong Kong.

Cartilaginous fish (Chondrichthyes) are the phylogenetically oldest living jawed vertebrates. It is believed that the adaptive immune system first evolved in the ancestor mammals shared with cartilaginous fish. Three immunoglobulin including IgM, IgW and IgNAR were discovered in cartilaginous fish. IgM and IgW belong to classical antibodies, while IgNAR, an H-chain only antibody, is exclusively arranged in the cluster organization on the genome and shows unique characteristics compared with mammalian immunoglobulins. Therefore, investigating cartilaginous fish, especially IgNAR immunoglobulins, is of great importance for the study of adaptive immune system evolution.

Whitespotted bamboo shark (Chiloscyllium plagiosum) is a relatively small size shark with small light spots on flanks. Bamboo sharks are the most recently evolved species in cartilaginous fishes, lying at a pivotal point. Thus, whitespotted bamboo shark has great potential in studying the emergence and evolution of the adaptive immune system.

Here, we systematically study the Chiloscyllium plagiosum mRNA and IgNAR immunoglobulins V region for the first time to reveal novel characteristics of Chiloscyllium plagiosum. Besides, considering limited diversity introduced by the VDJ recombination, we investigate other diversity generation mechanisms in IgNAR formation process such as N-nucleotide insertion and somatic hypermutation to explain the high affinity and diversity of IgNAR and to reveal the relationship between Chiloscyllium plagiosum and other species and the evolution of adaptive immunity.

Poster Presentation - Abstract 44

Profiling RNA at open chromatin chromatin targets by a dual transposase–peroxidase tagmentation

Furong JU, Ligang FAN, Xiaofan GUO, Wenkai YI, Jian YAN #

Department of Biomedical Sciences, Tung Biomedical Sciences Centre, City University of Hong Kong, Hong Kong S.A.R., China

The architecture of chromatin was controlled by transcription factors and chromatin-associated RNAs. However, map chromatin-bound proteins are well developed, it has proven challenging to map the RNA which composition of these large and dynamic structures. Here we describe a dual transposase–peroxidase approach, which integrative DNA and RNAs tagging, detects both DNA and RNAs associated with accessible regions of chromatin. We fused a series of TP fusion probes consisting of APEX2 and Tn5 transposase for peroxidase-mediated biotin labeling RNA and determine the sites of accessible chromatin. Hyperactive Tn5 transposase fused either N or C terminal serve as an anchor for proximal labeling of RNA associated with open chromatin. We applied this approach to capture the noncoding RNA that regular the open chromatin in human cells. Here we generated ATAC-seq libraries of 293T cells using our in-house purified TP probes, and our ATAC-seq libraries exhibited high TSS enrichment scores, the fragment length distribution. The average transcription start site (TSS) enrichment were also comparable to bulk ATAC-seq libraries. Furthermore, we captured a lot of noncoding RNAs that regular the open chromatin. Our findings demonstrate the power of this dual transposase–peroxidase approach as a platform for studying the dynamic Active Chromatin States.

Poster Presentation - Abstract 45

Identification of master regulator long non-coding RNAs underlying the mesenchymal-like subtype of gastric cancer through integrative network analysis

LI Jiang

Gastric cancer (GC) remains a formidable challenge in oncology owing to its high mortality rate and limited treatment options. Early-stage detection and therapy are critical in managing this disease. However, the highly heterogeneous nature of GC poses a significant hurdle to achieving favorable clinical outcomes. Like other cancer types, several molecular subtyping systems have been introduced to GC based on transcriptome and genomic mutation. Recently, a study classified GC into four subtypes: MSS/EMT, MSI, MSS/TP53+ and MSS/TP53-, of which the MSS/EMT subtype associated with the worst prognosis and high frequency of recurrence, the regulatory mechanisms underlying the most aggressive subtype, MSS/EMT, remain elusive. Notably, long non-coding RNAs (lncRNAs) have emerged as critical regulators of cancer-related processes, including tumorigenesis, DNA methylation, and gene expression dysregulation. In this study, we employed a network-based approach that integrates lncRNA and mRNA gene expressions and identified the key master regulator *LINC00968*, a clinically relevant lncRNA associated with GC progression. Our analysis revealed that *LINC00968* played a pivotal role in regulating the EMT pathway by upregulating two potential target genes, *FGF1* and *PDGFRB*. Furthermore, we comprehensively performed the ceRNA analysis by integrating the miRNA expression profile, indicating that *LINC00968* may promote GC progression through the upregulation of *PDGFRB* by sponging miR-423-5p. Importantly, survival analyses in independent cohorts confirmed the clinical relevance of our findings, underscoring the significance of these identified lncRNAs and putative target genes as potential targets for personalized therapeutics. Overall, our study highlights the importance of integrative network-based approach in unraveling the molecular complexity of GC and provides novel insights into the regulatory mechanisms underlying the highly aggressive MSS/EMT subtype of GC, which may lead to the development of personalized therapeutic strategies to improve clinical outcomes for this deadly disease.

Poster Presentation - Abstract 46

The bacterial two-component systems network reveals function and variability in signal transduction

LI Jingwei

Two-component regulatory systems (TCSs) play key roles in sensing signals to sustain survival and virulence in bacteria. But for the genome-wide regulatory variability as well as conservation of TCS regulating network still lack lots of study, especially about response to external stimulus and inner signal transduction. To systematic study the TCS regulating pattern, we integrate 120 transcriptome sequencing datasets and 38 chromatin immunoprecipitation sequencing datasets of the model phytopathogen *Pseudomonas syringae* to study about plasticity function of bacterial TCS regulatory roles under different environments. We reveal conservation and variability of TCS function in bacterial gene regulations under different cultural condition and present a regulating network of TCS from *P. syringae*. This network containing 232 and 297 functional genes under King's B medium and minimal medium conditions, indicating a universal regulating function of the TCS network. 7 TCSs that regulating the type III secretion system, motility, or exopolysaccharide production were revealed. Overall, our study about TCS network not only provides new insights into the mechanism of plant infections caused by P. savastanoi, but also present a model study about plasticity of TCSs as a reference for exploring of TCS-containing organisms.

Keywords: *Pseudomonas syringae*, Two-component system, regulating network, plasticity, RNA-seq, ChIP-seq

Poster Presentation - Abstract 47

Genome-wide identification and characterization of novel bacterial small noncoding RNAs in Pseudomonas aeruginosa and Pseudomonas syringae

LI Tianmin

Bacterial small noncoding RNAs (sRNAs) are known to play important roles in the cellular process by posttranscriptional regulation. While specific function of sRNAs in virulence is not well investigated in the pathogenic bacteria Pseudomonas aeruginosa (P. a) and Pseudomonas syringae (P. s). In this study, we widely screened sRNAs from these two pathogens over the whole genome by RNA-sequencing (RNA-seq). We used the Sliding Window algorithm to screen for possible sRNA candidates from highly expressed transcripts obtained through deep sequencing data. Following, the predicted sRNA candidates were experimentally verified by northern blotting. The overexpression strains of sRNA genes were then constructed to investigate the virulence regulation function of these novel sRNAs. In total, 34 sRNA loci were predicted, and 12 and 13 transcripts from P. a and P. s were screened to be novel sRNA candidates which were unannotated previously. According to the northern blotting results, 19 out of 25 candidates were verified to be expressed sRNAs (11/12 of P. a, and 8/13 of P. s). To date, 18 sRNA overexpression strains have been successfully constructed, 10 of which have been prepared for the cDNA library and sent for transcriptome sequencing, followed by RNA-seq analysis. Selected differential expression genes of each overexpression strain were then verified by RT-qPCR. The overall verification rate confirms a consistency with RNA-Seq results. To further explore the sRNA function, the phenotypic comparison between sRNA overexpression and wild-type strains will be conducted. This work will reveal novel sRNAs in P. a and P. s that are related to virulence regulation and provide more information on pathogenesis mechanisms exploration.

Keywords
Pseudomonas aeruginosa, Pseudomonas syringae, small noncoding RNAs, RNA-seq, virulence

Poster Presentation - Abstract 48

Functional identification of hypothetical proteins in *Pseudomonas aeruginosa* PAO1

LIU Jingui

Pseudomonas aeruginosa is a species of rode-shape, Gram-negative opportunity pathogen that has multidrug resistance, causing nosocomial infections in patients with comprised immunity. *P. aeruginosa* has high adaptability to different environmental conditions because of its large genome. Its genome is about 6.3 Mbp long and has 5570 ORFs, among which more than 2000 ORFs are hypothetical proteins (HPs) that are predicted proteins without identified functions. In *P. aeruginosa*, these uncharacterized proteins are highly expressed and play a crucial role during its survival strategy to environmental stressors and host immunity. According to The Pseudomonas Genome Database, 2194 HPs distribute all over the genome in *P. aeruginosa* PAO1. Under normal conditions (LB medium), RNA-seq results showed many of HPs had high expression in the wildtype PAO1, indicating the vital role of HPs in PAO1. To identify the function of HPs, we yielded 602 deletion mutants of HPs, involving 698 uncharacterized ORFs. We performed phenotypic screening to find the virulence-related HPs. We found 14 HPs contributed to the growth in *P. aeruginosa*, which are essential for the PAO1. Quorum sensing (QS) system is reported to associate with pathogenicity in *Pseudomonas aeruginosa*. Among the mutants, ΔPA3016 and ΔPA2229-30 showed a remarkable decrease on the production of all three QS system signals. In addition, ΔPA1550, ΔPA4691-92 and ΔPA1788 reduced the production of *Pseudomonas* quinolone signal. Combined with the RNA-seq results, PA1550, PA4691-92 and PA1788 positively regulated *pqs* system. There are 4 HP-mutants that had loss of Swarming motility, including PA3350, PA4463-65, PA3623, PA3352. PA0862 and PA3016 inhibited the biofilm formation while PA3350 and PA3352 positively regulated that. 9 HP-mutants had an influence on the production of pyocyanin. All these HPs were associated with virulence. The subsequent animal experiments demonstrated that 5 deletions (PA2229-30, PA3016, PA3350, PA3352, PA4463-65) had attenuated virulence on the mice. The phenotypic screening found many virulence-associated HPs and further demonstrated the accuracy of the functional identification network. The study gave us an insight into discovery of novel therapeutic strategies targeted to HPs.

Poster Presentation - Abstract 49

Astrocytes in CA1 modulate schema establishment in the hippocampal-cortical neuron network

LIU Shu

Previous experience, is a framework of acquired knowledge within associative network structures as biological correlate, which allows new relevant information to be quickly assimilated by parallel cortical encoding in the hippocampus (HPC) and cortex. Previous work demonstrated that myelin generation in the anterior cingulate cortex (ACC) plays a critical role for dynamic paired association (PA) learning and consolidation, while astrocytes in ACC play a vital role in cognitive decision-making. However, circuit components and mechanism involving HPC-anterior cingulate cortex (ACC) during schema formation remain uncertain. Moreover, the correlation between HPC-ACC circuit and HPC astrocytic activity is unclear.

Results: Utilizing a paired association (PA) behavioral paradigm, we dynamically recorded calcium signals of CA1-ACC projection neurons and ACC neurons during schema formation. Depending on the characteristics of the calcium signals, three distinct stages of schema establishment process were identified. The recruitment of CA1-ACC network was investigated in each stage under CA1 astrocytes Gi pathway chemogenetic activation. Results showed that CA1-ACC projecting neurons excitation gradually decreased along with schema development, while ACC neurons revealed an excitation peak in the middle stage. CA1 astrocytic Gi pathway activation will disrupt memory schema development by reducing CA1-ACC projection neuron recruitment in the initial stage and prevent both CA1-ACC projection neurons and ACC neuron excitation in the middle stage. CA1 astrocytes Gi markedly suppress new PA assimilation into the established memory schema.

Conclusions: These results not only reveal the dynamic feature of CA1-ACC network during schema establishment, but also suggest CA1 astrocyte contribution in different stages of schema establishment.

Poster Presentation - Abstract 50

Whole genome DNA sequencing using Nanopore R10.4 promises best practice for single cell variation detection and methylation profiling

NI Ying

Third-generation sequencing can be used in human cancer genomics and epigenomic research. Oxford Nanopore Technologies (ONT) recently released R10.4 flow cell, which claimed an improved read accuracy compared to R9.4.1 flow cell. To evaluate the benefits and defects of R10.4 flow cell for cancer cell profiling on MinION devices, we used the human non-small-cell lung-carcinoma cell line HCC78 to construct libraries for both single-cell whole-genome amplification (scWGA) and whole-genome shotgun sequencing. The R10.4 and R9.4.1 reads were benchmarked in terms of read accuracy, variant detection, modification calling, genome recovery rate and compared with the next generation sequencing (NGS) reads. The results highlighted that the R10.4 outperforms R9.4.1 reads, achieving a higher read accuracy of over 99.1%, superior variation detection, lower false-discovery rate (FDR) in methylation calling, and comparable genome recovery rate. To achieve high yields scWGA sequencing in the ONT platform as NGS, we recommended multiple displacement amplification with a modified T7 endonuclease I cutting procedure as best practice. In addition, we provided a possible solution to filter the likely false positive sites among the whole genome region with R10.4 by using scWGA sequencing result as a negative control. Our study is the first systematic benchmark of whole genome single-cell sequencing using ONT R10.4 and R9.4.1 MinION flow cells by clarifying the capacity of genomic and epigenomic profiling within a single flow cell. The best practice for scWGA sequencing together with the methylation calling results can benefit researchers who work on cancer cell genomic and epigenomic profiling using third-generation sequencing.

Poster Presentation - Abstract 51

Specific ABO blood group shortage or surplus is associated with migration in Shenzhen

SHI Yu

Blood shortage is a global problem, especially in those remote areas. However, in metropolitans with mass migration, seasonal blood shortage (especially in group O) or outdated (especially in group AB) mainly take place instead of severe long-term shortage. In the current research, this phenomenon mainly arises due to the usage of group O red blood cells (RBCs). Shenzhen is a typical metropolitan city located in China without excessive use of O RBCs, however, the blood donation situation in this city is still unknown.

In this time series study, twenty-one years (1998 - 2018) of blood donation data were collected from Shenzhen. Blood donations per thousand were calculated to judge the basic blood supply situation and a dynamic model was built to judge short-term specific blood group shortage or surplus. Features of blood donors and prosocial rate were analyzed to reflect the association with migration in metropolitans.

Indicated by an overall normalized deviation coefficient of -0.08 (95% CI: 0.07 to -0.07), Group O showed a specific blood shortage despite the most significant donation percentage (40%). By contrast, group AB showed a specific blood surplus with an overall normalized deviation coefficient of 0.15 (95% CI: 0.07 to -0.07) and the smallest donation percentage (7%). Migrant donors from the north of the Qinling-Huaihe line in China have the most considerable prosocial rate (27%) with the smallest percentage of group O (33.22%), compared to donors from the south with the lowest prosocial rate (20.07%) and the most significant in group O (43.98%).

In summary, specific blood group shortage can be defined based on the blood supply coefficient, and analysis of donors' demographics shows that blood supply deviation is related to migration in metropolitans.

Poster Presentation - Abstract 52

Maintenance of tRNA and elongation factors supports T3SS proteins translational elongations in pathogenic bacteria during nutrient starvation

SUN Yue

Sufficient nutrition contributes to rapid translational elongation and protein synthesis in eukaryotic cells and prokaryotic bacteria. Fast synthesis and accumulation of type III secretion system (T3SS) proteins conduce to the invasion of pathogenic bacteria into the host cells. However, the translational elongation patterns of T3SS proteins in pathogenic bacteria under T3SS-inducing conditions remain unclear. Here, we report a mechanism of translational elongation of T3SS regulators, effectors and structural protein in four model pathogenic bacteria (*Pseudomonas syringae*, Pseudomonas aeruginosa, *Xanthomonas oryzae* and *Ralstonia solanacearum*) and a clinical isolate (*Pseudomonas aeruginosa* UCBPP-PA14) under nutrient-limiting conditions. We proposed a luminescence reporter system to quantitatively determine the translational elongation rates (ERs) of T3SS regulators, effectors and structural protein under different nutrient-limiting conditions and culture durations. The translational ERs of T3SS regulators, effectors and structural protein in these pathogenic bacteria were negatively regulated by the nutrient concentration and culture duration. The translational ERs in 0.5× T3SS-inducing medium were the highest of all tested media. In 1× T3SS-inducing medium, the translational ERs were highest at 0 min and then rapidly decreased. The translational ERs of T3SS regulators, effectors and structural protein were inhibited by tRNA degradation and by reduced levels of elongation factors (EFs). Numeric presentation of T3SS translation visually indicates the invasion of bacteria and provides new insights into T3SS expression that can be applied to other pathogenic bacteria.

Poster Presentation - Abstract 53

Hybrid sequence reveals widespread functional isoform diversity and drug sensitivity-related novel isoforms in pancreatic ductal adenocarcinoma

WANG Xiangeng

Pancreatic ductal adenocarcinoma (PDAC) is one of the most vicious gastrointestinal malignancies. Even for the patients well-suited for adjuvant chemotherapy, acquired drug resistance often makes such intervention futile. Alternative splicing (AS) is a crucial mechanism for acquired drug resistance and is highly involved in cancer biology. However, the cancer-related isoform landscape in pancreatic ductal adenocarcinoma (PDAC) remains poorly charted.

Short-read RNA sequencing (RNA-seq) is mostly applied for the characterization of AS events and novel isoforms. However widespread, the accuracy of the identification of novel isoforms and AS events are hindered by its short-read nature. Long-read sequencing (LR-seq), represented by Pacific Biosciences' (PacBio) single-molecule real-time (SMRT) sequencing, has been widely applied in multiple research areas, which is capable of precisely recapitulating isoforms in a full-length manner, excluding the necessity of transcript reconstruction tools. However, LR-seq cannot achieve satisfactory sequence depth with an acceptable budget. A hybrid sequencing paradigm, combining RNA-seq and LR-seq, should be adopted to obtain a broad and deep view of the transcriptome sophistication in malignant tumors.

Most of the pharmacogenomic studies merely examined the relationship between gene-level expression and drug vulnerability. However, it is the expression of a specific isoform of a gene that mediates drug resistance. Recently, several research has started to evaluate the potentials of isoforms as drug response biomarkers, they only consider known isoforms and/or just consider spliced isoforms in a case-by-case manner. Cancer cells can acquire drug resistance through the activation of novel isoforms. Therefore, cancer-related novel isoforms should be considered when finding drug-sensitivity biomarkers.

In this study, we have utilized the hybrid sequencing paradigm to characterize the transcriptome in PDAC. Our analysis built a reference PDAC transcriptome, revealed widespread potential functional consequences of novel isoforms, and found novel isoforms have potential in the prediction of drug response.

Poster Presentation - Abstract 54

Dissecting super-enhancer heterogeneity: time to re-examine cancer subtypes?

WU Tan

The heterogeneity of transcriptional regulations by super-enhancers (SEs) is poorly understood in human cancers. Herein, we summarize a bioinformatics workflow for genome-wide SE profiling and identification of subtype-specific SEs and regulatory networks. Dissecting SE heterogeneity provides new insights into cancer biology and alternative therapeutic strategies for cancer precision medicine.

Keywords: cancer subtype; heterogeneity; multi-omics; regulatory network; super-enhancer; transcription regulation.

Poster Presentation - Abstract 55

Transposable elements distortion during aging at single cell resolution

Qianwen XIE[1], Jilin ZHANG[1], Jian YAN[1]

1 Department of Biomedical Sciences , City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong

Mammal genomes contain a large number of transposable elements(TE), part of which are still active and able to transpose in the host genome. TE shown an important role during development, aging and disease.
Several studies have provided evidence that decreases of heterochromatin or heterochromatin-establishing factors contribute to increased TE activity with age. Genetic interventions that promote retrotransposon silencing can increase lifespan. However, the extent of TE activation in chronologically aged versus senescent cell types is not known. Since senescent cells comprise only a small fraction of cells in aged tissues and TE activation is found in diverse species and specific developmental stage. Hence, we are interested in mechanism of TE distortion during aging. Herein, we first drawn the TE landscapes at single cell resolution and correlation between TE and RNA binding protein(RBP) to find possibly related molecules. We show that the expression of TE is tissue and cell type specific . Then thought the different young and old tissue scRNA-seq data, we show that TE activation during aging may be associated with specific RBPs both in mouse and human. The target of RBP which highly related to activate TE will be further studied for a better understanding of mechanism of TE distortion during aging.

Poster Presentation - Abstract 56

Profiling Adipocyte Secretome in Vivo

Fenglian YANG[1]，Rui QIAN[1], Liang ZHANG[1]

[1]Department of Biomedical Sciences, City University of Hong Kong, Hong Kong, China

Organ functions are highly specialized and interdependent. Secreted factors regulate organ development and mediate homeostasis through serum transport and inter-organ communication. Current methods for identifying cell-and tissue-type-specific secreted proteins are largely limited to in vitro or ex vivo models, which often fail to recapitulate in vivo biology. There is growing interest in studying the secretome, the totality of molecules released by specific tissues in vivo.

Integrating with proteomics, enzyme-catalyzed proximity labeling is a powerful technique that enables the identification of proteins in specific cellular compartments. Here, we have established and utilized an ER-BioID2HA-IRES-Golgi-BioID2HA hybrid system to study the secretome in vivo. The system can biotinylate proteins that pass through the endoplasmic reticulum and Golgi secretory pathways. Subsequently, biotinylated proteins can be enriched and identified from distal organs using quantitative mass spectrometry. In preliminary studies, I have proved that the hybrid system is robust in labeling secreted proteins. In addition, I have developed a sample processing method that allows more reliable quantitative comparison of the enriched proteins. We specifically expressed the system in the adipose tissue of mice. This enabled us to trace the in vivo distribution of adipocyte-derived factors. We confirmed that the ER-BioID2HA-IRES-Golgi-BioID2HA can be a versatile and powerful tool to study the spatiotemporal dynamics of secreted proteins in vivo. These proteins will serve as a valuable class of biomarkers and therapeutic targets.

Poster Presentation - Abstract 57

Genome-wide characterization of virulence-related and antibiotic-resistance related hypothetical proteins in Pseudomonas aeruginosa

YAO Chunyan

P. aeruginosa is one of the most commonly-isolated nosocomial pathogen, accounting for 10% of all hospital-acquired infections worldwide. There are 2194 hypothetical proteins with 1701 operons (HPs, 38.5%) without almost any experimental data on their biological functions, making them dark matters in the genome. Recently, we have characterized that an HP (PA3880, AnvM) is involved in regulation of QS and host immune response. Most of HPs are well conserved in other bacterial species, suggesting they have similar functions. In this study, we constructed more than 1000 PA-HP mutants, using RNA-seq to perform a genome-wide characterization of these HPs. We will divide HPs into different functional clusters using machine learning models and map *pseudomonas aeruginosa* HPs regulatory networks in virulence-related, antibiotic-resistance-related, and metabolism-related. In addition, we identified 23 virulence-related HPs (VRHP) and 18 antibiotic-resistance related HPs (ARHP) phenotypic screening (bioassay of QS system, motility, biofilm formation, exopolysaccharide production, pyocyanin production proteolytic activity, rhamnolipid production and minimum inhibitory concentration). And 16 HPs influenced PAO1 pathogenicity in mouse infection experiment. We will further determine the function mechanism of key VRHPs and ARHPs. We performed pull-down assay for PA3016 and the interacting partners were identified. PA3016 may regulate virulence and antibiotic-resistance by interacting with FlgL and gyrB.

Our proposed final comprehensive regulatory networks of virulence and antibiotic resistance would provide an overall atlas of regulatory relationship among all genes in both pathways. This will also reveal the master regulators (including transcription factors and two-component systems), which will be ideal targets for future development of new antibiotics and novel anti-*P. aeruginosa* agents.

Poster Presentation - Abstract 58

Dynamic attention-based deep learning model to predict lymph node metastasis from primary tumor histology in papillary thyroid carcinoma

ZHANG Xianrui

Identifying lymph node metastasis (LNM) in patients with papillary thyroid cancer (PTC) is crucial for determining the appropriate treatment, such as total thyroidectomy and lymph node resection. The primary tumors of PTC patients contain micro-environmental information that may aid in predicting LNM status, but even experienced pathologists may struggle to accurately assess LNM status from primary tumor slide images. To address this issue, we aimed to develop a dynamic attention-based multi-instance learning (DA-MIL) model for LNM status prediction using routine H&E stained whole-slide images from primary tumors. Initially, we divided the entire slide image into thousands of smaller patches and used the pretrained ResNet50 model to convert them into representative features. Following this, we utilized a dynamic attention module to resample the patches and reduce the computational resources required for the subsequent multi-instance learning. Our DA-MIL model was then trained utilizing a discovery set of 306 samples from SMU. Next, the efficacy of the DA-MIL model was validated using two independent in-house cohorts (SCH: n = 158, SPH: n = 85) and a public TCGA dataset (n = 101). The DA-MIL model that we developed exhibited impressive predictive power, achieving an overall AUC of 0.864 on the internal validation set, and 0.789, 0.762, and 0.734 on the independent sets SCH, SPH, and TCGA, respectively. Our analysis of the attention scores for each cell type demonstrated distinct differences in the distribution of microenvironments between the LNM positive and negative groups, further underscoring the significant role that these factors play in predicting LNM status. We also performed immune and tumor purity analysis on RNA sequences data of TCGA, which yielded results consistent with our observed trends from pathological slide images. These findings offer important implications for understanding the pathogenesis of PTC and informing therapeutic decisions in clinical practice.

Poster Presentation - Abstract 59

Profiling in situ chromatin-associated RNA via proximity biotinylation

ZHOU Xiaomin

Inheritance of traits can be influenced by epigenetic changes that alter gene activity without altering the underlying genetic sequence. These changes can occur on DNA or the amino acid residues of histone proteins. Specific epigenetic modifications can play a role in regulating tissue-specific gene expression during development, as well as initiating and promoting certain types of cancer. Recent research has shown that both proteins and non-coding RNAs can participate in regulating epigenetic mechanisms by directly interacting with promoters and enhancers, or by recruiting chromatin-modifying complexes to modify chromatin structure. While there are established techniques for mapping chromatin-bound proteins, identifying chromatin-associated RNAs has remained a challenge. To address this issue, we have developed a novel method that uses an engineered ascorbate peroxidase (APEX2) fused with four different epigenetic modification readers (targeting DNA methylation or histone tri-methylation at H3K4, H3K9, and H3K27 residues) to label RNAs associated with specific epigenetic modifications. In the presence of biotin-aniline and H2O2 substrates, the proteins and RNAs in the vicinity are covalently labeled in situ, and subsequent affinity purification of biotinylated RNAs enables their identification by next-generation sequencing. This APEX2-mediated biotin labeling method is versatile and can be applied to primary cells or animal tissues without requiring the introduction of foreign plasmids. By identifying ncRNAs physically associated with specific chromatin modifications, we aim to predict their potential regulatory functions. We have successfully validated the enzyme activity of the recombinant protein and the proximity labeling method in vitro, and our next step is to process RNA sequencing to profile chromatin-associated RNAs.

Poster Presentation - Abstract 60

An Exosome-based Transcriptomic Signature for Noninvasive, Early Detection of Patients With Pancreatic Ductal Adenocarcinoma: A Multicenter Cohort Study

ZHU Zhongxu

BACKGROUND & AIMS: Pancreatic ductal adenocarcinoma (PDAC) incidence is rising worldwide, and most patients present with unresectable disease at initial diagnosis. Measurement of carbohydrate antigen 19-9 (CA19-9) levels lacks adequate sensitivity and specificity for early detection; hence, there is an unmet need to develop alternate molecular diagnostic biomarkers for PDAC. Emerging evidence suggests that tumor-derived exosomal cargo, particularly micro RNAs (miRNAs), offer an attractive platform for the development of cancer-specific biomarkers. Herein, genomewide profiling in blood specimens was performed to develop an exosome-based transcriptomic signature for noninvasive and early detection of PDAC. **METHODS**: Small RNA sequencing was undertaken in a cohort of 44 patients with an early-stage PDAC and 57 nondisease controls. Using machine-learning algorithms, a panel of cell-free (cf) and exosomal (exo) miRNAs were prioritized that discriminated patients with PDAC from control subjects. Subsequently, the performance of the biomarkers was trained and validated in independent cohorts (n = 191) using quantitative reverse transcription polymerase chain reaction (qRT-PCR) assays. RESULTS: The sequencing analysis initially identified a panel of 30 overexpressed miRNAs in PDAC. Subsequently using qRT-PCR assays, the panel was reduced to 13 markers (5 cfand 8 exo-miRNAs), which successfully identified patients with all stages of PDAC (area under the curve [AUC] = 0.98 training cohort; AUC = 0.93 validation cohort); but more importantly, was equally robust for the identification of early-stage PDAC (stages I and II; AUC = 0.93). Furthermore, this transcriptomic signature successfully identified CA19−9 negative cases (<37 U/mL; AUC = 0.96), when analyzed in combination with CA19− 9 levels, significantly improved the overall diagnostic accuracy (AUC = 0.99 vs AUC = 0.86 for CA19−9 alone). **CONCLUSIONS**: In this study, an exosome-based liquid biopsy signature for the noninvasive and robust detection of patients with PDAC was developed.

Poster Presentation – Abstract 61

Design and application of disposable fluidic module for "sample-in-result-out" molecular point of care test (mPOCT)

AYELE Bereket Workalemahu
Supervisor: Prof. Michael Yang

Undoubtedly, innovations in the real-time quantitative PCR (qPCR) has brought a fundamental transformation in the field of molecular diagnostics and has remained the standard method in a plethora of applications. This power of qPCR lies in the ability to quantify nucleic acids over an extraordinarily wide dynamic range and without the need for post-amplification manipulations. Although its proven and effective technology, its application remained in well structured laboratories operated by trained personnel due to a relatively complex technology requiring thermal blocks with precise temperature control for amplification, expensive optical components for detection and sophisticated design of amplification chemistry using fluorescent probes or extensive optimization when using DNA dyes. On the other hand, competitive chemistry to amplify nucleic acid and suitable for mPOCT application are widely available. These technologies primarily rely on constant temperature (isothermal) amplification obviating the need for complex instruments. However, true application in POCT remains challenging due to the lack of integrated sample and reagent handling module for simple operation by the end-user. Here, a device platform is designed for a simple operation of molecular tests by an untrained person that only involves putting the sample-in (e.g the whole nasal swab) and closing the modules for constant temperature incubation (e.g using simple heat block). Reading of the result can either be done visually based on color change (e.g. red to yellow) or using highly specific Lateral flow dipstick. The latter involves locking the whole module into a detection cassette for visual result reading based on the pattern of color band. The prototype device is successfully used to detect SARS-CoV-2 using simple user operational steps and based on an in-house developed dry LAMP-LFD chemistry. Since all necessary reagents to detect specific nucleic acid targets can be preloaded and kept dry inside the module, the system, coupled with competent chemistry, is useful to achieve a multi-parameter standard for an ideal POCT acronymized as "ASSURED" (Affordability, Sensitivity, Specificity, User-friendliness, Reliability, Equipment-free and Deliverability).

Poster Presentation - Abstract 62

Ultrastretchable Conductive Liquid Metal Composites Enabled by Adaptive Interfacial Polarization

CAO Chunyan

Gallium-based liquid metals (LMs) are emerging candidates for the development of metal/polymer-based flexible circuits in wearable electronics. However, the high surface energies of LMs make them easily depleted from polymer matrix and therefore substantially suppress the stretchability of the conductive composites. Here, we reveal that a dynamic interplay between LMs and a polyvinylidene fluoride (PVDF) copolymer can help address these issues. Weak and abundant interfacial polarization interactions between the PVDF copolymer and the oxide layer allow continuous and adaptive configuration of the compartmented LM channels, enabling ultra-stretchability of the composites. The conductive LM-polymer composites can maintain structural integrity with high surface conductivity and small resistance changes under large strains from 1000% to 10000%. Taking advantage of flexible processability in mild conditions and exceptional performances, our design strategy allows for scalable fabrication of conductive LM-polymer composites for a range of applications in wearable devices and sensors.

Poster Presentation - Abstract 63

ATP-derivative Hsp70s inhibitors suppressed EV-A71 infection through down-regulating hnRNP A1-mediated IRES activity

CHEN Cien

Enterovirus A71 (EV-A71), a positive-sense, single-strand RNA from the Picornaviridae family, is a causative factor for epidemics of hand, foot and mouth disease (HFMD) and severe neurological diseases in children. Up to now there is no specific treatment for EV-A71 infection. Heat shock proteins (HSPs) have shown multiple functions to facilitate infection process of multiple viruses including picornavirus, which are potential targets for designing antiviral drugs. In this study, we constructed a series of Hsp70s inhibitors and identified three active compounds, HSP-9, HSP-10 and HSP-13 that can protect RD cells from EV-A71 infection and reduce viral RNA production as well as viral protein expression level of EV-A71 in a dose-dependent manner. Compared with the commercial Hsp70s inhibitor Ver-155008, these three active compounds showed lower cytotoxicity while HSP-10 and HSP-13 showed stronger inhibition rates against EV-A71 infection and the selectivity indexes (SIs) of HSP-10 and HSP-13 against EV-A71 are 47.88 and 42.38, higher than that of Ver-155008 (11.98). Viral lifecycle assay showed that Hsp70s inhibitors suppressed viral replication in the post-entry steps. In mechanism, we found that Hsp70s inhibitors decreased the redistribution of heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) from nucleus to cytoplasm and thus suppressed the hnRNP A1-mediated internal ribosome entry site (IRES) activity of EV-A71 while HSP-10 and HSP-13 had stronger inhibitory effects to IRES activity than Ver-155008, which explained the stronger antiviral effects of HSP-10 and HSP-13. Altogether, these findings provide potential antiviral drugs against EV-A71 infection.

Poster Presentation - Abstract 64

Structure comparison and function implication of proteinase in rhinovirus

CHEN Sheng

Among three types (A, B, C) of rhinovirus, type C35 specifically can cause asthma while others can not. The factors contributing to the symptom and its mechanism are unclear. Proteinases 2A and 3C are critical factors for viral infection and replication. They are reported to help rhinovirus to evade the innate immune response. The difference in proteinase structure can partially explain how only several types of rhinovirus can cause asthma. By comparing the protein sequence of different types of rhinovirus, we identify the amino acid variance mainly located at the N- and C- terminals of 2A proteinase while throughout the whole sequence of 3C proteinase. They are mainly in the secondary protein structure like the alpha helix, beta strand, loop, or near the critical region related to viral infectivity. Those variances in N-terminal could imply different stability of 2A proteinase in different types of rhinovirus and enterovirus. The binding stability for 2A proteinase regulatory proteins that bind at N- or C- terminal could also differ. The unknown post-translation modification could happen at these sites, affecting 2A proteinase stability. Those amino acid variances could also affect the 2A proteinase binding capability with its substrates and thus affect the catalytic efficiency. The detailed mechanism needs to be further clarified. Observing the predicted structure of 2A proteinase of C35, A16, and EVA 71 constructed by alphafold, we found that 2A of C35 has a much more flexible structure than others. It hints that a less rigid 2A binding structure could contribute to high proteinase catalytic efficiency and the capability to bind to more substrates, which could cause more severe symptoms than other types of rhinovirus.

Poster Presentation - Abstract 65

The interaction between binding affinity, immune escape and fitness of SARS-CoV-2

DING Zhaojun

Background: Despite the abundance of experimental data measuring the virus binding affinity or the ability of immune escape for some possible mutations in the receptor binding domain (RBD) of SRAS-CoV-2 spike protein, the effect of these possible mutations with altered binding affinity and immune escape on the virus' fitness still needs to be evaluated.

Material and method: Sequence data from across the world since 1 January 2020 were downloaded from the GISAID database (https://www.gisaid.org/). After data cleaning, the multiple sequence alignment was performed using the Clusta Omega algorithm. Then, the binding affinity and ability of immune escape for mutations in the RBD region were calculated. Next, the fitness as a time-varying index of SARS-CoV-2 were calculated by the SEIR model. In the end, the Response Surface Methodology (RSM) was used to explore the interaction between binding affinity, immune escape and fitness of SARS-CoV-2

Preliminary results: the sequencing data processing for Italy was finished. There were 103,459 sequences from Jan-27, 2020 to Jun-15, 2022 in Italy included for further analysis. The binding affinity and ability of immune escape were calculated. The results indicated that the time-varying trend of binding affinity and immune escape of SARS-CoV-2 was significant different. The higher fitness of SARS-CoV-2 was under the lower binding and higher immune escape level.

Poster Presentation - Abstract 66

Engineered macrophages for viruses clearance

FANG Wenjie

The coronavirus disease 2019 (COVID19) pandemic caused by the sudden outbreak of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has an unprecedented impact on public health. Immunotherapies have advantages on more specificity with fewer side effects. The macrophage can ingest and degrade invading substances and orchestrate inflammatory processes. Chimeric antigen receptor (CAR) macrophages therapy, by genetically modifying autologous macrophages, achieves an astonishing effect in treating solid tumors and activating autoimmunity.

Macrophage C-type lectins (MCL) and Macrophage-inducible C-type lectin (Mincle) are the type II C-type lectin receptors (CLRs). CLRs are glycan-sensing receptors densely expressed in dendrites of antigen-presenting cells (APC), such as dendritic cells and macrophages and broadly involved in the immune response against pathogen infections. The novel chimeric adenoviral vector with different extracellular domains was synthesized based on MCL or Mincle. Surprisingly, non-professional phagocytes (293 cells) transfected with anti-GFP MCL illustrated the phagocytosis on green fluorescent protein (GFP). The antigen engulfment efficiency was evaluated to select the optimal transmembrane domain and intracellular domain of MCL or Mincle. We engineered macrophages with MCL-SPIKE, a chimeral receptor with nanobody fragment against COVID19 spike protein, to direct their phagocytic activity against SARS-CoV-2. CAR macrophages (CARMs) illustrated antigen-specific, viruses clearance, and protection against SARS-CoV-2 infection *in vitro*. Then we revealed how these CARMs processed the engulfed protein.

In the future, we will evaluate the T-cell activation induced by activated different CARMs and discuss whether CARMs impart a pro-inflammatory (M1) or anti- inflammatory (M2) phenotype. The CARM-induced B cell activation and antibody production will be investigated in mice. Since the antigen engulf and antigen degradation of overexpressed anti-GFP MCL 293 cells, we will further explore the potential application of C-type lectin chimeric antigen receptors in other cell types for anti-viral therapy.

Poster Presentation - Abstract 67

A novel dissolved Microneedle-based viral infection model to explore virus transmission pathway in vivo

FENG Yaxiu

An important prerequisite for virological research and treatment of related diseases is the successful establishment of the viral infection model. However, conventional viral infection methods not only cause pain and harm to experimental animals, but also require operators to undergo systematic training and strict assessment, bearing certain risks. Even if the animals are successfully infected, observing the infection pathway of the virus in vivo still confronts great challenges. Here, we report a novel dissolved microneedle-based (dMN) viral infection model for the painless injection of the Herpes simplex virus-1 (HSV-1) pioneeringly, achieving successful viral infection and fluorescent tracking in vivo assisted with quantum dots (QDs). The dMN is easily penetrated into the dermis without touching blood vessels and nerves, causing minimal and reversible damage to the skin. After loading the QDs-labeled HSV-1(QDs-HSV-1), the dMN model gradually released the virus as the hyaluronic acid (HA) needles encountered skin tissue fluid and dissolved, the HSV-1 signal and transmission pathway are detected and observed in vivo due to the fluorescent of QDs. Collectively, this QDs-assisted dMN virus infection model serves as a promising scientific research tool to realize painless viral infection, exhibiting great potential in alleviating injury to experimental animals and exploring the infection pathways of viruses.

Poster Presentation - Abstract 68

Shark vNAR-presenting Naïve Phage Library Panning Against Tuberculosis Antigens

GONG Jinhua

Tuberculosis (TB) is a respiratory disease caused by *Mycobacterium tuberculosis* (MTB) that creates the highest mortality from a single infectious disease before the SARS-CoVID-19 pandemic. Multiple TB diagnostic methods in the clinic could be divided into sputum-based and non-sputum-based methods. The sputum-based methods include sputum culture tests, acid-fast bacilli smear tests, and nucleic acid amplification tests (NAATs). The non-sputum-based methods incorporate interferon-gamma release assay (IGRA), tuberculin skin tests, CT scans, and MTB antigen detection. Although the mentioned diagnostic methods are commonly applied, they still have disadvantages. For example, the sputum culture tests consume weeks for the results, NAATs are expensive, and acid-fast bacilli smear tests are low in specificity and sensitivity. These sputum-based tests require saliva samples from patients, which cannot apply to those who lack the sample. The non-sputum-based tests overcome the defects of sputum-based tests, while the tuberculin skin tests and CT scans cannot distinguish the LTBI and aTB.
MTB antigen detection is a newly developed method for TB diagnosis, and it detects the free MTB antigens in the circular system to determine TB morbidity. Currently, proven MTB antigens for TB diagnosis include lipoarabinomannan (LAM), early secreted antigen 6(ESAT6) and10kDa culture filtrate antigen (CFP10) complex (E1C0), MPT64, and guanylate binding protein 5 (GBP5). Hence, we choose some MTB antigens to screen their specific antibodies against a white-spotted bamboo shark vNAR DNA-presenting phage library for POCT reagent development.

Poster Presentation - Abstract 69

Development of Antimicrobial-Nanodiamond as the Novel Antimicrobial Agents to Target Urinary Tract Infection

LAW Oi Kwan

Urinary tract infections (UTIs) are one of the most common infections in human that is the vast majority caused by uropathogenic Escherichia coli (UPEC), affecting over 150 million patients worldwide every year. Nowadays, no effective vaccine has been developed to prevent UTIs, and treatment of antibiotics is the only choice for UTI patients. However, the effectiveness of conventional antibiotic treatment and the delivery to the infection sites is decreasing. Apart from the reason of patient exposure to a higher level of antibiotics, leading to the development of multidrug-resistant bacteria. Another reason is the internalization of UPEC into the bladder epithelium where the intracellular reservoirs provide a shelter for them to evade the action of bladder immune response, and also antibiotics cannot reach them, which is prone to the recurrence of UTIs. Therefore, it is important to develop the novel antimicrobial agents that can target intracellular pathogens.

Nanodiamonds (NDs), a nanotechnology-based drug delivery system, serve as a tool for antibiotic delivery in this study and enhance their versatility as therapeutic agents. Nanosystems have been shown to offer several advantages over conventional formulations, including greater epithelium permeability and bioavailability, improved solubility and stability, and longer half-life of an antibiotic.

Here, we showed that the modified NDs, which exhibited high colloidal stability in various buffer systems, could efficiently penetrate the T24 human bladder cell. Moreover, this study demonstrated that by delivering tetracycline immobilized on NDs (NDs-Tet) into the T24 cell, the CFU of UPEC was reduced more than 50% compared with the antibiotic alone. In addition, minimum inhibitory concentration (MIC) assay proved that 80uM nanodiamonds did not exhibit antibacterial properties and did not significantly affect bacterial growth on their own.

As a whole, NDs-Tet could further enhance the killing efficiency against UPEC compared to Tet alone *in vitro*. Further studies and development of this nanomaterial-based approach to the delivery of antibiotics could provide validation of its potential usefulness for UTIs recurrence and therapy.

Poster Presentation - Abstract 70

Cellulose Nanofibril-based Multifunctional Robust Ionogel Enabled by Electrostatic Coacervation

LI Xin

Ionogels, as a promising substitute for hydrogels, have attractive wide interests in many fields, such as wearable electronics, sensors, theranostic patches, and energy storage devices, due to their excellent advantages. However, current ionogels suffer from low strength and poor ionic percolation. The polymeric matrix plays a vital role in the overall performance of ionogels. Therefore, inspired by mechanical reinforcement of natural biomacromolecules through noncovalent aggregates, a strategy to construct cellulose nanofibrils (CNFs)-based robust ionogel through electrostatic coaveration is proposed. CNF with outstanding mechanical properties and PIL with charged backbone are integrated as the matrix of the ionogel, simultaneously achieving high strength and a nanofluidic network structure. Moreover, the ionogels are high hygroscopic and moisture-adaptive, so they are applied to power generation from humidity gradient based on the ionic diffusion migration across the membrane. This strategy offers new perspectives for the fabrication of cellulose ionogels.

Poster Presentation - Abstract 71

Effects of vaccination on the spread of Omicron variant and hospitalization rate: An observational study during the first Omicron wave in the United States

Jingbo LIANG, Hsiang-Yu YUAN

Background
A higher population immunity may impact the spread of new immune-escape mutants. If these mutants have varying virulence levels from previous strains, the probability of infections requiring hospitalization could change. However, no evidence to support this at present. This population-based study assessed the impact of vaccine coverage on the adaptation of new variants and, therefore, the risk of hospitalization in the United States (US).

Methods
Daily state-level case hospitalization rate (CHR) and Omicron mutation proportion among infections were calculated based on the epidemiological and genomic sequential data from 50 states of the US and the District of Columbia during the first Omicron wave (between 11 December 2021 and 22 March 2022). Generalized linear mixed models (GLMM) combined with distributed lag nonlinear models (DLNM) were used to assess the impact of vaccination on CHR with confounders (i.e. meteorological data and hospital capacity data). We performed a mediation analysis to disentangle the indirect impact of vaccination on CHR through influencing the proportion of Omicron mutations from the direct impact of its protection.

Results
This study divided all states into two categories: those with high vaccine coverage and those with low vaccine coverage. Mean CHR in states with high vaccine coverage was significantly higher than states with low vaccine coverage. Cumulative incidence was similar between states with high and low vaccine coverage, but a higher vaccine coverage resulted in a peak about 10 days earlier. Higher vaccination coverage had both direct and indirect effects on reducing CHR. Directly, the increase from 45% to 70% in vaccine coverage decreased CHR with an odds ratio (OR) of 0.85. However, as the indirect effect, this change in vaccine coverage was associated with a 20% increase in the proportion of BA.1/BA.1.1-associated mutations, reducing CHR with an OR of 0.84.

Poster Presentation - Abstract 72

Development of bisbenzylisoquinoline alkaloid berbamine and its analogs to prevent and treat COVID-19 by compromising the endosomelysosome transport of ACE2

LIN Naixin

Although most coronaviruses are not dangerous, new coronaviruses have emerged over the past 50 years, and their infections can cause many human and animal diseases, ranging from gastrointestinal infections to upper respiratory tract infections. In response to the novel coronavirus (SARS-CoV-2)-infected pneumonia epidemic, global scientific research institutions and pharmaceutical companies are making every effort to develop therapeutic vaccines and antiviral drugs. Through in vitro cell experiments, we found that berbamine, an antiviral drug against viruses such as dengue virus, flavivirus and enterovirus, also has a good effect on fighting mouse coronavirus, and 5uM berbamine can inhibit mouse coronavirus infection. We found in a mouse model that berbamine was able to protect mice well against low doses of infection. We will further examine the difference in the protective ability of berbamine to mice under different doses of virus.

Poster Presentation - Abstract 73

A Novel sRNA Derived From Truncated Transposon Contributing to Improved Antibiotics Persistence

LIN Shuling

Bacterial small regulatory RNAs (sRNAs) play a major role in the regulation of various cellular functions. Here, we reported a novel multidrug resistance plasmids-carried small regulatory RNA, SttnpA, deriving from truncated transposon, contributing to improved antibiotics persistence. Antibiotic persistence is a phenomenon where a small subpopulation of bacteria survives exposure to high concentrations of antibiotics for an extended period of time, which is an important factor to the rising rates of antibiotic therapy failure and is responsible for generating recurrent and prolonged infections. However, the regulators and mechanisms that lead to this phenomenon remain poorly understood. By RNA pull-down assay and RNA co-immunoprecipitation, we show that SttnpA interacts with an ATP-binding component of a predicted ATP-binding cassette (ABC) superfamily efflux transporter which may take part in transport of antibiotics and lipids. According to our knowledge, SttnpA is the first plasmid-encoded sRNA that associated with antibiotics persistence. Taken together, our work indicates SttnpA as a novel regulator upon persisters formation, which may help the development of novel therapeutic strategies that overcome antibiotics persistance.

Poster Presentation - Abstract 74

Self-powered ionic skin with high elasticity

LYU Dong

Biological skins are stretchable and elastic, and achieve sensitive perception to external stimuli through ionic transport in reticulate sensory nerves. Artificial skins with such functionality are desirable for advanced robotics and wearable electronics. However, most existing component materials suffer from poor resilience, low stretchability and inferior ionic conductivity which highly limited the reliability and service life of the devices. Here, we report a highly elastic, ultrastretchable, highly reliable and environmental stable polyvinylidene fluoride copolymer-based ionogel enabled by microphase-separated bicontinuous structure that contains polymer-rich and ionic liquid (IL)-rich phases. The ionogel can sensitively respond to stimulus such as strain, pressure and temperature difference with output of ionic electric. We attribute this ionic current to the different cationic and anionic mobility which regulated by ion-polymer interaction.

Poster Presentation - Abstract 75

Comparative Analysis of Outer Membrane Vesicles from Uropathogenic Escherichia coli Reveal the Role of Aromatic Amino Acids Synthesis Proteins in Motility

NIE Qichang

Uropathogenic Escherichia coli (UPEC) is the causative agent that causes urinary tract infections (UTIs) and the recent emergence of multidrug resistance (MDR) of UPEC complicated the pathogenicity and increases the burden on the community. Recent studies of bacterial outer membrane vesicles (OMV) identified various factors including proteins, nucleic acids, and small molecules which provided inter-cellular communication within the bacterial population. However, the components of UPECspecific OMVs and their functional role remain unclear. Here, we systematically determined the proteomes of UPEC-OMVs and identified the specific components that provide functions to the recipient bacteria. Based on the functional network of OMVs' proteomes, a group of signaling peptides was found in all OMVs which provide communication among bacteria. Moreover, we demonstrated that treatment of UPEC OMVs affected the motility and biofilm formation of the recipient bacteria, and further identified aromatic amino acid (AAA) biosynthesis proteins as the key factors to provide their movement.

Poster Presentation – Abstract 76

PIM1 Facilitates β-Coronavirus Reproduction Through BTRC-Mediated IFNAR1 Degradation

WAN Qianya

In recent decades, three times outbreaks of human coronaviruses have made devastating impacts on the whole world. The outbreaks of severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) in 2003 and 2012, respectively, threatened us with their high fatality. The emergence of SARS-CoV-2 in late 2019 defected the world again with its high transmissibility and caused-severe diseases. The innate immunity of humans should be the first line to protect humans from being harmed by infected pathogens. The intact interferon induction and response were of great significance for anti-viral infection. However, numerous studies show that human coronaviruses have developed several strategies to counteract innate immunity. Here We found that human coronaviruses could induce interferon α/β receptor I (IFNAR1) degradation. IFNAR1 and IFNAR2 are the type I interferon co-receptors that start the interferon response. The level of IFNAR1 expression on the cell surface is essential for amplifying the interferon response. IFNAR1 expression level is delicately regulated both transcriptionally and post-transcriptionally. We discovered that human coronavirus infection increased the expression of PIM1. PIM1 played a great role in viral infection, immunological response, and malignancy. In my study, PIM1 promoted the degradation of IFNAR1. Mechanistically, PIM1 phosphorylated the E3 ligase of IFNAR1, BTRC. Phosphorylated BTRC at amino acid S82, accelerated the degradation of IFANR1 and improved the ability to interact with IFNAR1. We supposed that human coronaviruses may benefit from PIM1 to evade innate immunity and facilitate virus replication.

Poster Presentation - Abstract 77

Inhibition of Hsp27 Phosphorylation at Serine 78 Blocks hnRNP A1 Cytosol Relocation and Suppresses Enterovirus A71 Replication

WU Mandi

A positive-sense (+) single-stranded RNA (ssRNA) virus (such as enterovirus A-71, EV-A71) depends on viral polypeptide translation for initiation of virus replication after entry. Although EV-A71 hijacks Hsp27 to induce hnRNP A1 redistribution in the cytosol, the underlying mechanism is still elusive. Here we report that inhibition of Hsp27 phosphorylation with p38 kinase inhibitor SB203580 abolishes its nuclear translocation. Phosphorylation-deficient Hsp27-3A (serine to alanine mutation, $Hsp27^{S15/78/82A}$) and $Hsp27^{S78A}$ mutants fail to translocate into the nucleus and induce hnRNP A1 cytosol redistribution in Hsp27 knockout cells, while $Hsp27^{S15A}$ and $Hsp27^{S82A}$ mutants display similar effects to Hsp27 for nuclear translocation and induction of hnRNP A1 cytosol relocalization. Furthermore, we demonstrate that the viral 2A protease ($2A^{pro}$) activity is a key factor to regulate Hsp27 nuclear translocation and hnRNP A1 cytosol relocalization. $Hsp27^{S78A}$ almost eliminates the effects of Hsp27 on the IRES activity and viral replication, which are partially reduced by $Hsp27^{S82A}$. However, $Hsp27^{S15A}$ displays the same activity as the wild-type Hsp27. Taken together, we demonstrate the importance of Ser78 phosphorylation of Hsp27 regulated by virus in nuclear translocation, hnRNP A1 cytosol relocation, and viral replication, suggesting a new path for target-based antiviral strategy.

Poster Presentation - Abstract 78

How Pseudomonas syringae senses the endogenous phytohormone to express virulence by mediating host-pathogen interaction during the infection?

YUAN Jian

Bacteria employ two-component regulatory systems (TCSs) to quickly sense and respond to different environments and signal cues in their host organisms, especially in host–pathogen interaction. Pseudomonas syringae, a bacterial phytopathogen, can infect plant host cells through the stoma, and there are a few endogenous phytohormones in plant host cells so that plants can utilize these phytohormones to coordinate the immune system to combat pathogens. However, how the Pseudomonas syringae respond to these endogenous phytohormones and mediate the virulence for host–pathogen interaction is still elusive. Here, we used RNA sequencing to find differentially expressed genes under the treatment of phytohormones and deletion of TCS genes, then validate the sequencing results with RT-PCR and detect their promoters' activity. As a result， we found that histidine kinases genes PSPPH_2083 and PSPPH_3550 can sense salicylic acid （SA） and primarily regulate the alginate biosynthesis， which is an essential regulator for biofilm. The formation of biofilm is instrumental in expressing virulence factors during the infection because the alginate capsule surrounding the bacterium provides a protective barrier against antibiotics and host immune defenses. Alginate may also protect the bacterium from dehydration and facilitate adherence to the host cell. By clarifying the regulation mechanism, the potential control of pathogenicity with host cues via two-component systems presents a potential alternative to antimicrobials for new drug targets.

Poster Presentation - Abstract 79

Tough and weldable composites enabled by interfacial supramolecular assemblies for stretchable electronics

Al Liqing

Stretchable electronics are critical for soft robots, wearable technologies, and biomedical applications. Many flexible conductors can maintain electrical performances under control at large strains, however, the whole circuits may suffer from rapid failure at low strains because of the generated defects or depletion at the conduct of the rigid electronic components. A stable conductive interface with reliable conductivity and interfacial toughness is highly desired between soft substrates and rigid electronic components. Here we introduce intermolecular interactions to form strong interactions with both liquid metal and polymer matrix to create a stable heterogeneous interface with high conductivity (>40000 S m^{-1}), extreme stretchability (~1000%), toughness (~20 MJ m-3) and good adhesion (>2 MPa) with electronic components after heating (~120 °C). Leveraging the thermal transition and re-solidified ability of the composite, maximum strain tolerance of >600% for the chip-integrated conductive trace is achieved without encapsulation, which facilitates the testing and replacement of electronic components. Furthermore, a complex chip-integrated circuit and a wireless NFC device are fabricated to demonstrate our materials showing the huge potential applications in stretchable and wearable electronics.

Poster Presentation - Abstract 80

Astrocytic L-lactate signaling in the anterior cingulate cortex is essential for schema memory and neuronal mitochondrial biogenesis

AKTER Mastura

Astrocyte-derived L-lactate was shown to confer beneficial effects on synaptic plasticity and cognitive functions. However, how astrocytic G_i signaling in the anterior cingulate cortex (ACC) modulates L-lactate levels and schema memory is not clear. Here, using chemogenetic approach and well-established behavioral paradigm, we demonstrate that astrocytic G_i pathway activation in ACC causes significant impairment in flavor-place paired associates (PA) learning, schema formation, and PA memory retrieval in rats. It also impairs new PA learning even if a prior associative schema exists. These impairments were mediated by decreased L-lactate in ACC due to astrocytic G_i activation. Concurrent exogenous L-lactate administration bilaterally into the ACC rescues these impairments. Furthermore, we show that the impaired schema memory formation was associated with a decreased neuronal mitochondrial biogenesis caused by decreased L-lactate level in ACC upon G_i activation. Our study also reveals that L-lactate mediated mitochondrial biogenesis is dependent on monocarboxylate transporter 2 and NMDA receptor activity – discovering a previously unrecognized signaling role of L-lactate. These findings expand our understanding of the role of astrocytes and L-lactate in brain functions.

Poster Presentation - Abstract 81

Instant fabrication of angiogenic patch for effective vascular regeneration

BUI Thi Van Anh

Objectives: To investigate a novel protein patch that can deliver effectively a cocktail of angiogenic factors to treat peripheral artery disease.

Methods: Firstly, the concentrations of chosen angiogenic factors, including vascular endothelial growth factor (VEGF), fibroblast growth factor 2 (FGF2), epidermal growth factor (EGF), insulin-like growth factor-1 (IGF) were optimized by migration and tube formation assay on endothelial cells. Then, a combination of 4 factors at optimal concentrations was determined the angiogenic effects. The cytoprotective effects of the combination on endothelial cells were also examined. Parallely, droplet technique was employed to produce a patch from sodium alginate and ε-poly-l-lysine. The patch was evaluated release kinetics of both small protein (VEGF) and large protein (Bovine serum albumin). The released growth factors from the patch were tested their function by migration assay. Adaptation of the patch *in vivo* by encapsulating and releasing DiI in mouse legs was measured. Therapeutic effects of the patch are testing on hindlimb mouse models.

Result: The combination of angiogenic factors with 50ng/mL VEGF, 20ng/mL FGF2, 10ng/mL EGF, and 10ng/mL IGF showed the strongest angiogenic effects *in vitro* compared to single factor and other combinations. It recruited the most migrated endothelial cells, maintained the most tube-like structures, and highly promoted cell proliferation. The combination of angiogenic factors significantly protected endothelial cells from ichemic injury and inflammation injury. Simultaneously, the patch demonstrated stable release kinetics. The combination of angiogenic factors encapsulated and secreted by the patch maintained good function through migration effect. Patch encapsulating DiI slowly released DiI to the muscle of the mice. Angiogenic factor patch fully protected the ischemic hindlimbs from limb loss compared to the control group.

Conclusion: Our patch strategy successfully deliver proteins to the target tissue. The angiogenic patch indicated a novel approach for peripheral artery disease treatment.

Poster Presentation - Abstract 82

Autophagy is impaired in neutrophils from Atorvastatin-induced Intracerebral haemorrhage zebrafish larvae

CHAN Yu Suen
Supervisor: Prof CHENG Shuk Han

Intracerebral haemorrhage (ICH) is a severe form of stroke that results from bleeding within the brain tissue, often leading to brain damage and death. Zebrafish have emerged as a promising model organism to study ICH pathophysiology and potential treatments. In this study, we investigated the role of neutrophils in ICH zebrafish larvae and tested the hypothesis that changes in neutrophil activity involve autophagy impairment.

To induce ICH, zebrafish larvae were exposed to atorvastatin for 24 hours, we found that the survival rate of ICH zebrafish larvae in the first week was around 90%, which is significantly higher than the survival rate observed in humans (approximately 65-75%). Additionally, more than 90% of zebrafish larvae were able to recover from hematoma without the need for medication. These findings suggest that the zebrafish may have a greater capacity for recovery from ICH compared to humans.

To investigate the neutrophil on ICH zebrafish larvae, neutrophil activity was tracked using transgenic zebrafish Tg(*Lyz: DsRed)* and *Tg(Lyz: GFP)* from 1 day post-ICH to 7 days post-ICH. Our results showed that neutrophils were recruited to the injured brain only from 1-day post-ICH to 2-day post-ICH, with an increased production of neutrophils at 1-day post-ICH. However, after 3 days post-ICH, neutrophil activity resumed to average levels, even though the injury site and hematoma persisted.

We further investigated the autophagy activity in neutrophils using LC3B puncta tracking by immunofluorescence in *Tg(Lyz: DsRed; LC3B: GFP*) zebrafish larvae. Our findings revealed that neutrophils from ICH zebrafish larvae had low expression of LC3B after 3 days post-ICH, indicating autophagy impairment. To verify the importance of autophagy impairment in neutrophils for ICH zebrafish larvae recovery, we administered an autophagy inducer from 2-day post-ICH to 3-day post-ICH. Our results showed that the neutrophil number increased, and the hematoma recovery was prolonged, supporting the notion that autophagy impairment in neutrophils may be necessary for zebrafish larvae recovery from ICH.

In short, our study provides insights into the mechanisms underlying neutrophil activity and autophagy impairment in ICH zebrafish larvae and highlights the potential of zebrafish as a valuable model organism for studying ICH pathophysiology and testing potential treatments.

Poster Presentation - Abstract 83

The Role of Defective TFEB-Autophagic Flux Axis in Endothelial Dysfunction in Diabetic Mice

Lei HE[1], Lei ZHAO[2], Cheng-Lin ZHANG[3], Qinghua CHEN[1], Yu HUANG[1]

[1]Department of Biomedical Sciences, City University of Hong Kong, Hong Kong, China.
[2]School of Biomedical Sciences, Chinese University of Hong Kong, Hong Kong, China.
[3]Department of Pathophysiology, School of Basic Medical Sciences, Shenzhen University Health Science Center, Shenzhen 518060, China.

Introduction: Endothelial nitric oxide synthase (eNOS) monomerization and uncoupling are crucial players in the development of vascular dysfunction in diabetes although the underlying mechanisms are not fully understood. Transcription factor EB (TFEB), a master regulator of autophagy and lysosomal biogenesis, and inflammation.
Aims: The present study aims to investigate whether TFEB-mediated autophagic flux in diabetic endothelial dysfunction through regulating eNOS monomerization/dimerization and eNOS activity.
Methods: Diabetic *db/db* mice were randomly assigned to induce calorie restriction and the aortas of *db/db* mice were used to conduct *ex vivo* organ culture, functional study on wire myograph and mitochondrial ROS (mtROS) production. Low-temperature Western blotting and Western blotting were used to determine the levels of protein of target genes. Adenovirus was used to overexpress TFEB both *in vivo* and *in vitro*.
Results: Autophagic flux is impaired in the endothelium of diabetic *db/db* mice and in human endothelial cells exposed to advanced glycation end products or oxidized low-density lipoprotein. Pharmacological inhibition of autophagic flux by chloroquine or bafilomycin A1 were sufficient to induce eNOS monomerization and attenuate NO bioavailability through increasing mitochondrial reactive oxygen species (mtROS). By contrast, restoration of autophagic flux by overexpressing TFEB reduced endothelial oxidative stress, enhanced eNOS dimerization and improved endothelium-dependent relaxations (EDR) in diabetic mouse aortas. Meanwhile, inhibition of mammalian target of rapamycin kinase by rapamycin increased TFEB nuclear localization, decreased mtROS accumulation, facilitated eNOS dimerization, and improved endothelial function in *db/db* mice. Furthermore, calorie restriction also increased aortic TFEB expression, improved autophagic flux, and restored EDR in diabetic mice.
Conclusion: In summary, this study uncovered that mtROS-induced eNOS monomerization is closely associated with the impaired TFEB-autophagic flux axis leading to endothelial dysfunction in diabetic mice.
Keywords: Autophagy, TFEB, eNOS, endothelial dysfunction, diabetes

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Poster Presentation - Abstract 84

Identification of a novel cell death in a severe cryoinjury heart of neonatal mouse model

HERNANDEZ CORTES Sinai

Cardiac disease is one of the most common causes of death worldwide. As a result of myocardial infarction (MI), there is damage to blood vessels, and severe loss of cardiomyocytes that lack proliferation after injury. Neonatal mice have great capacity for heart regeneration that is limited if the damage is greater than 20% in the left ventricle (LV) even within 7 days of being born. Among other MI models, cryoinjury shares similar features to human infarcted cardiac tissue, inducing the decrease of cell cycle activity in cardiomyocytes and the formation of fibrotic scar. Here, using cryoinjury in neonatal mouse model enables to mimics the features of myocardial infarction similar to human infarcted heart tissue. Through mild and severe cryoinjury in the newborn mice, a mapping of differential expressed genes (DEG's) analysis was performed. Using transcriptome sequencing, a mapping of observed upregulated genes. The DEG's from the severe cryoinjury lead the identification of key markers of genes that participate in ferroptosis pathways, a novel intracellular cell death iron-dependent distinct of other types of cell death such as apoptosis, necrosis, and autophagy, in the severe injured cryoinjury. Since a severe cryoinjury does not induce heart regeneration in the newborn, this model enables testing interventions seeking the molecular mechanisms to stimulate regeneration not only for adult but also pediatric heart diseases.

Poster Presentation - Abstract 85

Develop The Strategy of Inducing Hair Regeneration By Increasing CD133 Population in Dermal Papilla Cells

HO Thi Quynh Mai

Hair is the organ which plays various important functions in mammals such as protection, thermal regulation, sexual as well as social interaction, etc. Therefore, alopecia syndrome that causes hair fall out would bring the critical limitation to patients. In current study, we aim at inducing hair regeneration by increasing CD133 population in dermal papilla cells. Firstly, we try to prove CD133 as essential functional marker for hair inductivity by investigating CD133 expression in dermal papilla cells. Secondly, since CD133 plays the important role in hair inducing ability, we try to enhance hair regrowth through the increase of CD133 population in dermal papilla cells. In this part, the first minor objective we need to conduct is examining effect of potential agents such as CHIR, Rapamycin and Valproic acid on CD133 expression in dermal papilla cells. As appropriate agents at optimal concentrations are identified, we would apply these conditions in vivo to examine hair regeneration capability. Subsequently, we could develop the potential strategy in hair loss therapy

Poster Presentation - Abstract 86

Developing light-inducible lipid nanoparticle (LiLNP) mRNA vectors for ocular gene editing therapy

HUANG Yifan

Lipid nanoparticles (LNPs) underpin the great success of Covid-19 as mRNA vaccines and is on the rise as a reliable tool for mRNA therapeutics. Nevertheless, the efficacy of LNPs for delivering mRNA was reported to be too low to reach a therapeutic effect, largely due to the extremely poor mRNA endosomal escape efficiency (~1%), which is the major bottleneck of LNP-based delivery. Herein, we will develop light-inducible LNP (LiLNP) to enhance the mRNA endosomal release via light-induced ROS-mediated membrane opening and test its feasibility as an ocular gene therapy vector. In the preliminary study, I tested several LNPs encapsulating Cre mRNA in the eyes of the Cre reporter mouse lines by two injection routes (subretinal and intravitreal injections). These LNPs contain different ionizable lipids, including one containing MC3, which was used in the first FDA-approved LNP drug Onpattro. Most of the LNPs tested enabled effective Cre expression in different subsets of retinal cells depending on the injection route. Intravitreal injection resulted in strong fluorescence expression in Müller glia, retinal ganglion cells (RGCs) and the cells in ciliary bodies, while retinal pigment epithelium (RPE) and photoreceptor cells infection could be detected in the eye receiving a subretinal injection. Next, in collaboration with Dr. Gigi Lo's lab, we will develop LiLNP by conjugating photosensitizer (e.g. PcZn) into the lipids which could be activated by far-red light to enhance mRNA intracellular release. After determining the safety and effectiveness of photosensitizer-conjugated LiLNP and light treatment, we will further examine the *in vivo* genome editing efficiency of CRISPR mRNA-LiLNP, attesting its potential application for ocular gene editing therapy. We hope that the successful development of LiLNP mRNA vector could empower gene editing therapy for inherited blindness with improved safety and efficacy profiles.

Poster Presentation - Abstract 87

Ejection of mitochondria and TNF-α activation mediate hypertonicity-induced adipocytes dedifferentiation

LIU Guopan

Purpose: Recent studies demonstrated that elevated osmolarity could induce adipocyte dedifferentiation, representing an appealing procedure to generate multipotent stem cells. Here we aim to elucidate the molecular mechanisms that underlie osmotic inducion of adipocyte reprogramming. We focused on the autocrine activities of the dedifferentiating adipocytes and investigated the TNF-α-Wnt signaling axis, with the ultimate goal to enhance the efficiency of adipocyte-derived stem cells.

Method: 3T3-L1 cell and stromal vascular fraction (SVF) were induced by 1 μM dexamethasone, 10 μg/ml insulin, 500 μM IBMX and 10 μM rosiglitazone to construct the adipocytes model. To induce reprograming, the adipocytes were cultured under the hypertonic pressure in 2% PEG 300 medium. . The dedifferentiation of adipocytes were monitored by aspect ratio measurement, Oil Red staining and qPCR to examine the morphology, lipid droplet sizes, and adipose-specific gene expression, respectively. Furthermore, the stem-cell capacity of dedifferentiated adipocytes was validated by alkaline phosphatase (ALP) expression for osteogenic differentiation. To elucidate the mechanism of the osmotic stress-induced dedifferentiation, the extracellular vesicles (EVs) were collected the reprograming cells, and mass spectrometry was used to define the proteome. To investigate the stress reponse, ELISA and qPCR were applied to assess the TNF-α-signaling pathway. To counteract the effects of the TNF-a signaling, TNF–α neutralizing antibody (20ng/ml) was applied. . Cell apoptosis was during reprograming was evaluated by the annexin v flow cytometry 24h later (N=3). For Wnt signaling investigation, the activation of β-catenin was validated by active β-catenin Western blot and immunostaining. In addition, we applied 10 μM Wnt agonist 1 that activates β-catenin to induce the multipotent adipocyte dedifferentiation in the absence of osmotic stress.

Result: In response to the hypertonic treatment the adipocytes became elongated and lost the lipid droplets. The dedifferentiation was accompanied by gaining of osteogenic capacity. Proteomic and biochemical analysis revealed that the EVs contained mitochondria-derive vesicles positive (MDV) of UQCRC2, NDUFA9, and VDAC. The hypertonic EVs also could trigger the release of TNF-α, leading to activation of Wnt-β-catenin signaling and adipocyte dedifferentiation. In parallel, we observed that direct activation of Wnt-β-catenin signaling using Wnt agonist 1 could efficiently induce adipocyte dedifferentiation while circumventing the apoptotic effect of the hypertonic treatment.

Conclusion: High osmolarity prompts the adipocytes to release MDV, which in turn enhances the secretion of TNF-α as a pro-inflammatory cytokine during the stress response. Importantly, TNF-α is essential for the activation of the Wnt/β-catenin signaling that drives adipocyte dedifferentiation. A caveat of the hypertonic treatment is apoptosis, which could be circumvented by direct activation of the Wnt/β-catenin signaling using Wnt agonist 1.

Keywords: Multipotent stem cells, adipocyte dedifferentiation, hypertonic treatment, mitochondria-derived vesicles, TNF-α, Wnt/β-catenin signaling.

Poster Presentation - Abstract 88

Microglial circadian clocks regulate neural cell recruitments

LU Qingqing

Circadian clocks are endogenous oscillators built on a transcriptional-translational negative feedback loop which represent in nearly all cell types, including microglia. Microglia are phagocytes in central nervous system and are critical in immune responses and homeostasis maintain. However, how circadian clocks are involved in regulation of microglia functions are still not clear. Here, to confirm the existence and address the regulator of circadian clock in microglia, we tested the expression level of core circadian gene *Bmal1* with microglia activation markers *Iba1* and *itgam*, and the result shows they all have a rhythmic expression pattern. The circadian rhythm of these target genes will be modified after activation by lipopolysaccharide (LPS) treatment, suggest the resting and activated microglia have different circadian rhythms. Next, we examined how altered circadian clocks affect microglial functions. To answer this question, we built the stereotaxic injection system to inject GFP tagged microglia into the corpus callosum in the mouse brain. The injected resting microglia recruit oligodendrocyte progenitor cells (OPCs) to the injection site, while activated ones lose this ability. Interestingly, microglia with Bmal1 deficiency do not recruit OPCs, suggesting circadian clocks may regulate the cell recruitments from microglia. As cytokines are signals secreted from microglia, we test their expression by cytokine screening assay. In our results, several cytokines, such as CCL2, CCL3, CCL5, CCL12 and CXCL12, are significantly modified immediately after LPS injection, suggest that they may be the candidates regulated by circadian clock. Our study shows that the circadian clock of microglia regulates microglia function after immune activation, for example stopping recruiting OPCs.

Poster Presentation - Abstract 89

Postnatal eye size in mice is controlled by SREBP2-mediated transcriptional repression of *Lrp2* and *Bmp2*

MAI Shuyi
Supervisor: Dr. Wenjun XIONG

Eye size is a key parameter of visual function, but the precise mechanisms of eye size control remain poorly understood. Here, we discovered that the lipogenic transcription factor sterol regulatory element-binding protein 2 (SREBP2) has an unanticipated function in the retinal pigment epithelium (RPE) to promote eye size in postnatal mice. SREBP2 transcriptionally represses low density lipoprotein receptor-related protein 2 (*Lrp2*), which has been shown to restrict eye overgrowth. Bone morphogenetic protein 2 (BMP2) is the downstream effector of *Srebp2* and *Lrp2*, and *Bmp2* is suppressed by SREBP2 transcriptionally but activated by *Lrp2*. During postnatal development, SREBP2 protein expression in the RPE decreases whereas that of *Lrp2* and *Bmp2* increases as the eye growth rate reduces. *Bmp2* is the key determinant of eye size such that its level in mouse RPE inversely correlates with eye size. Notably, RPE-specific *Bmp2* overexpression by adeno-associated virus effectively prevents the phenotypes caused by *Lrp2* knock out. Together, our study shows that rapid postnatal eye size increase is governed by an RPE-derived signaling pathway, which consists of both positive and negative regulators of eye growth.

Poster Presentation - Abstract 90

An investigation of a new therapeutic target on atherosclerosis

Yujie PU, Li WANG, Yu HUANG

Department of Biomedical Sciences, City University of Hong Kong

Background and Objectives

TANK binding kinase 1 (TBK1) activation stimulates inflammatory response upon pathogens infection. Endothelial cells (ECs) lining the arteries are critical players in maintaining vascular homeostasis, and loss of a quiescent endothelial phenotype in response to proinflammatory risk factors contributes to the early formation of atherosclerotic lesions. However, whether endothelial TBK1 contributes to inflamed endothelial dysfunction and atherogenesis is largely unknown. This study aims to identify the precise role of endothelial TBK1 in atherogenesis.

Methods

Constitutively active TBK1 was transfected to human ECs to investigate the signaling cascade driving endothelial-to-mesenchymal transition (EndMT) after TBK1 gain of function. *ApoE-/-* mice were treated with endothelial-specific *AAV-Cas9-sgTBK1* or TBK1 inhibitor GSK8612 (1 mg/kg/day) to examine whether inhibition of TBK1 *in vivo* suppresses EndMT and delays atherogenesis.

Results

TANK-binding kinase 1 (TBK1) activity was increased in atherosclerotic endothelium. We next overexpressed the constitutive TBK1 in human ECs and performed RNA sequencing analysis. Atherosclerosis and EndMT signaling pathways were top enriched by Gene-disease association (GAD) and Hallmark pathway analysis in human endothelial cells overexpressed with constitutive TBK1, which was confirmed by real-time PCR, western blot, and immunostaining. Endothelial-specific TBK1 knockdown or oral treatment of GSK8612 (1 mg/kg/day) for one month in *ApoE-/-* suppressed the formation of atherosclerotic plaques and the expression of EndMT markers in mouse aortas and carotid arteries without alteration of metabolic parameters.

Conclusions

The present study reveals that endothelial TBK1 is a novel therapeutic target of anti-EndMT and anti-atherogenesis. TBK1 inhibitors may act as potentially effective drugs for the treatment of patients with atherosclerotic vascular disease. This study is supported by HMRF 07181286 and SRFS2021-4S04.

Poster Presentation - Abstract 91

Dissecting the mechanism and function of lamTOR1 in two-pore channel 2 (TPC2) calcium release and lysosomal activity

SI Xiaotong
Supervisor: Dr. YUE Jianbo

Lysosomal activity is crucial for normal cellular function, including autophagy, endocytosis and mTOR activation. Acidification plays an essential role in maintaining lysosomal function, because lysosomal enzymes can only be active under acidic pH. The proton pump, v-ATPase is the one to generate pH gradient across lysosomal membrane, although its regulation mechanism remains elusive. Two-pore channel 2 (TPC2) is cation-selective channel, locating on late endosomes and lysosomes. TPC2 is one of the major Ca^{2+} channels to maintain Ca^{2+} concentration gradient in lysosome. Although debated, TPC2 was reported to regulate lysosomal pH, and to be a selective Na^{+} channel as well. TPC2 deficient cells were found to inhibit fusion between autophagosome and lysosome. However, the role of TPC2 in lysosomal function is still controversial. Here, our lab previously identified lamTOR1 to be a TPC2 binding protein, a scaffold protein for mTORC1 activation. Cellular calcium measurement confirmed that loss of lamTOR1 inhibits TPC2 calcium release by using a NAADP analog, TPC2-A1-N. Also, immune preparticipation by using NAADP-conjunct beads confirmed that lamTOR1 binds to NAADP. However, whether lamTOR1 binds to NAADP directly, and whether lamTOR1 regulates lysosome function are still under discussion.

Keywords: Two-pore channel 2 (TPC2), LamTOR1, mTOR, Cholesterol

Poster Presentation - Abstract 92

Overexpression of *Dkk1* in the retinal pigment epithelium promotes mouse eye size growth during early postnatal development

Tian TANG, Shuyi MAI, Shengyu WU, Wenjun XIONG

Purpose: Precise size regulation of ocular growth is a prerequisite for normal vision, but the underlying mechanisms are largely unknown. Dickkopf 1 (DKK1) is a secreted glycoprotein, which suppresses the β-CATENIN-dependent WNT pathway while stimulates the β-CATENIN-independent WNT pathway. DKK1 was reported to regulate ocular growth during the embryonic development and axial elongation during the formation of myopia in adult animals. In this study, we sought to study whether and how the retinal pigment epithelium (RPE) regulates mouse eye size via DKK1 during the rapid eye size growth period in early postnatal development.

Methods: AAV serotype 8 was subretinally injected in neonatal mice, and the hBest1 promoter was used to drive *Dkk1* expression in the RPE cells specifically. AAV8-hBest1-*Dkk1* or the control virus AAV8-hBest1-EGFP was injected into the right eyes of wild-type mice at postnatal day (P) 0. Injected mice were harvested at P14 for eye size measurement and retinal histology examination.

Results: RPE-specific overexpression of *Dkk1* induced enlarged eye size, with nearly 20% increases in equatorial diameter (ED) and axial length (AL). In addition, *Dkk1* overexpression caused elongated retina radius and uniform thinning of the whole retina without affecting the normal retinal stratification. The ZO-1 staining result showed that the RPE cells infected with AAV8-hBest1-*Dkk1* showed a significant increase in the average cell size (area).

Conclusions: Targeted overexpression of *Dkk1* in the RPE promotes eye enlargement in mice during the early postnatal period, accompanied by overall retinal thinning and increased RPE size. Further studies will focus on the cellular and molecular mechanisms accounting for the *Dkk1*-induced eye enlargement phenotype.

Poster Presentation - Abstract 93

Hydrogel-based Bioprinting of "Liver Capsule"

WANG Wanying

Tissue engineering and regenerative medicine (TERM) is a promising solution for organ shortage. Hydrogel-based bioprinting is able to mimic the extracellular matrix (ECM) and natural-tissue-like intricate structures. This study attempted to develop a bio-printable and biodegradable ECM-derived hydrogel system, and to construct functional "liver capsules" as a demonstration for liver failure treatment. Hepatocytes and human umbilical vein endothelial cells (HUVEC) were precisely co-printed in an interlaced manner to create vascularized, heterogeneous cell-laden tissue constructs compatible with hepatic physiological environments and can endothelialize *in situ*. Morphology, mechanical properties, and biocompatibility evaluations of the printed hydrogel were performed to ensure cell viability and proliferation. Cell distribution indicating angiogenesis could be observed, demonstrating the potential utilization of the proposed system to be implanted into lesions of the damaged liver.

Key Words: bioprinting; hydrogels; endothelialization; tissue engineering

Poster Presentation - Abstract 94

Mechanism study of Syk function in terminal erythropoiesis

WANG Weixi

Erythropoiesis is the differentiation process that red blood cells (RBCs) generated from hematopoietic stem cells. Terminal erythropoiesis refers to the differentiation from erythroid-committed precursors to erythrocytes, which consists of chromatin condensation, enucleation and reticulocyte maturation. RBCs exit from the cell cycle properly is crucial for normal erythropoiesis and organogenesis.

Spleen tyrosine kinase (Syk) is recognized as a key mediator of immunoreceptor signaling inflammatory cells, involving with various kinds of diseases pathogenesis. However, Functional roles and molecular mechanism of Syk in erythropoiesis are still unclear. In former study, we found that Syk deletion leads to anemia phenotype in adult and embryonic stage and also disturbs definitive and adult erythropoiesis. Moreover, Syk impairs retinoblastoma 1 (RB1) stability and deficiency disrupts cell cycle exit. Mechanistically, Syk maintains RB1 stability by restricting ubiquitin-proteasome pathway to regulate the cell cycle exit in both mouse and human cells. Upon Epo activation, Syk recruits Src homology 2 domain-containing inositol-5-phosphatase 1 (SHIP1) to inhibit the E3 ubiquitin ligase Casitas B-lineage lymphoma (c-CBL) and thus restricting the activity of ubiquitin. In Syk deficiency condition, the activated c-CBL accelerates RB1 degradation by enhancing the ubiquitin activity, which disrupts cell cycle progression and causes enucleation failure and anemia finally.

Collectively, our study explores the underlying mechanism related with Ubiquitin-Proteasome degradation and signal transduction. The function of Syk in terminal erythropoiesis provides new sights of red blood cell enucleation and pathogenesis of anemia.

Poster Presentation - Abstract 95

Investigation of toxicity and toxins of benthic and epiphytic toxic algae (BETA)

ZHOU Shiwen

The frequency of the occurrence of benthic dinoflagellates, many of which are known to be toxic, have been affected by global climate change and human activities, and pose a great threat to human health and marine ecosystems. Hong Kong is the located in the subtropical region of the South China Sea. So far, at least six genera of BETA including *Amphidinium, Prorocentrum, Ostreopsis, Coolia, Fukuyoa, Gambierdiscus* have been found in Hong Kong Waters. However, the risks of BETA to marine environments of Hong Kong are less known. The aim of our study is i) to characterize morphological and phylogenetic features for each collected algae, ii) to evaluate their toxicity, and iii) to establish a BETA culture collection to facilitate environmental and ecological investigation. In this study, a total of 127 BETA strains including eight species were isolated and cultured based their morphology and phylogeny: *Amphidinium carterae*, *Coolia malayensis*, *Coolia palmyrensis*, *Prorocentrum rhathymum*, *Prorocentrum emarginatum*, *Prorocentrum fukuyoi*, *Prorocentrum lima*, *Fukuyoa ruetzleri*. Among these species, *Prorocentrum emarginatum* was firstly reported in Hong Kong Waters. To evaluate their risks to Hong Kong ecology, 17 strains of *Amphidinium carterae* was preliminarily evaluated for their hemolytic toxicity and all of them show potent toxicity. In addition, LC-MS/MS analysis was applied to explore their toxin profiles to facilitate a better understanding of their risks. The suspect screening of 17 strains of *Amphidinium carterae* resulted in the identification of 10 know toxins such as amphidinols from *Amphidinium* based on their mass and fragmentation patter with references.

Poster Presentation - Abstract 96

Isolation of Tissue Adipose Stem Cells based on Single-Cell Metabolism in Droplet-Based Microfluidics

ZHOU Xiaoyu

Adipose Stem cells have the great potential in tissue engineering and regenerative medicine, but remain challenges for the blockage of isolation of high quality stem cells from donor tissue and delivery to target tissue. Here, we report a novel label-free method to isolate mouse adipose stem cells (mASCs) and human adipose stem cells (hASCs) by encapsulation adipose stem cells into alginate microgel based on the differences between single cell metabolic of mASCs and hASCs with other cells. We further showed that isolated mouse adipose stem cells render higher stemness than those cells enriched with traditional procedure, also high level of proliferation and differentiation potential both culture within alginate microgel and plastic. And T2D mice receiving encapsulated ASCs shows better insulin sensitivity, inflammation regulation and other health condition compared to the mice injected with suspended ASCs. Our method provides one novel procedure of sorting and enrichment of ASCs, of which might be used as one candidate way of mesenchymal stem cell therapy.

Poster Presentation - Abstract 97

Regional comparison on ciguatoxicity, hemolytic activity, and toxin profile of the dinoflagellate *Gambierdiscus* from Kiribati and Malaysia

ZHU Jingyi

The dinoflagellates *Gambierdiscus* and *Fukuyoa* can produce Ciguatoxins (CTXs) and Maitotoxins (MTXs) that lead to ciguatera poisoning (CP). The CP hotspots, however, do not directly relate to the occurrence of the ciguatoxic *Gambierdiscus* and *Fukuyoa*. Species-wide investigations often showed no association between CTX level and the molecular identity of the dinoflagellates. In the Pacific region, Kiribati is known as a CP hotspot, while Malaysia has only three CP outbreaks reported thus far. Although ciguatoxic strains of *Gambierdiscus* were isolated from both Kiribati and Malaysia, no solid evidence on the contribution of ciguatoxic strains to the incidence of CP outbreak was recorded. The present study aims to investigate the regional differences in CP risks through region-specific toxicological assessment of *Gambierdiscus* and *Fukuyoa*. A total of 19 strains of *Gambierdiscus* and a strain of *Fukuyoa* were analyzed by cytotoxicity assay of the neuro-2a cell line, hemolytic assay of fish erythrocytes, and high-resolution mass spectrometry. *Gambierdiscus* from both Kiribati and Malaysia showed detectable ciguatoxicity; however, the Kiribati strains were more hemolytic. Putative 44-methylgambierone was identified as part of the contributors to the hemolytic activity, and other unknown hydrophilic toxins produced can be potentially linked to higher CP incidence in Kiribati.

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CITY UNIVERSITY OF HONG KONG
KOWLOON TONG, HONG KONG

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