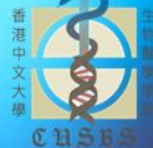


School of Biomedical Sciences



# Postgraduate Research Day 2018



Lo Kwee-Seong Integrated Biomedical Sciences Building, Area 39  
The Chinese University of Hong Kong  
8<sup>th</sup> to 9<sup>th</sup> November 2018



# SBS Postgraduate Research Day 2018



Faculty of Medicine  
The Chinese University of Hong Kong

## **Welcome Message from the Director of School of Biomedical Sciences**

I am most delighted to welcome you all to the *SBS Postgraduate Research Day 2018*, the annual flagship event of the School of Biomedical Sciences organized solely by and for our students. Stepping into its ninth year, the *Postgraduate Research Day 2018* continues to serve as an excellent platform for our students to share their achievements with their peers and supervisors.

This year, this event will be further expanded to attract more participants to join our Postgraduate Research Day 2018. We are honored to invite the graduate students and their supervisors from the Peking University and the Chinese Academy of Sciences Kunming Institute of Zoology to join the event. I would like to extend my warmest welcome to the delegations for joining this annual flagship event. This is certainly a very valuable opportunity to broaden our postgraduate students' exposure and to enhance the academic exchange among our students and their counterparts at other institutions.

It is the vision of our School to nurture future scientists who are abreast of biomedical advances and are able to do cutting-edge research. We are most proud of our young, creative, and dynamic students. Their thirst for limitless knowledge and intellectual advancement is the engine that constantly propels our staff to reach new heights. The success of the Postgraduate Research Day is a re-assurance of our commitment and effort.

Facilitating our students to achieve research and academic excellence by providing the environment and training is not our only goal. We strive to provide a holistic education to our students such that they can achieve excellence in whatever career they decide to pursue after they leave the School. I certainly believe the annual Postgraduate Research Day offers the best opportunity for our students to sharpen their other attributes such as leadership, devotion, organization and social skills.

I would like to take this opportunity to thank all individuals involved in planning, organizing, and coordinating this event, particularly members of the Organizing Committee of *SBS Postgraduate Research Day 2018* for their thoughtful planning and dedication in making this event a success. I would also like to extend my heartfelt gratitude to the Graduate Education Office for its continued support to our graduate education and students.

On behalf of all staff of the School of Biomedical Sciences, I wish you all a very successful *Postgraduate Research Day 2018*.



Wai-Yee Chan, Ph.D.  
Professor of Biomedical Sciences &  
Director, School of Biomedical Sciences  
October 2018

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**Advisers:**

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Ms. PANG Tak Keung Bruce

**Special Acknowledgements:**

Ms CHAN Hiu Ying Isabel        Ms. LAU Yin Yi Carmen  
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Ms. KWAN Mei Nui Becky        Ms. WONG Ka Yee Kylie

## Acknowledgements

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

### **School of Biomedical Sciences:**

Prof. CHAN Man Lok Andrew

Prof. CHAN Sun On

Prof. CHAN Wood Yee Woody

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### **Kunming Institute of Zoology:**

Prof. JIAO Baowei

Prog. ZHENG Ping

**The organizing committee would like to thank the following postgraduate students for providing the Program Book cover design and illustrations.**

Mr. CHENG Chak Kwong Andy

Mr. CHEUNG Ka Wing Otto

Ms. WANG Lingyi Claire

Ms. XIE Yuxin

## Rundown of Postgraduate Research Day 2018

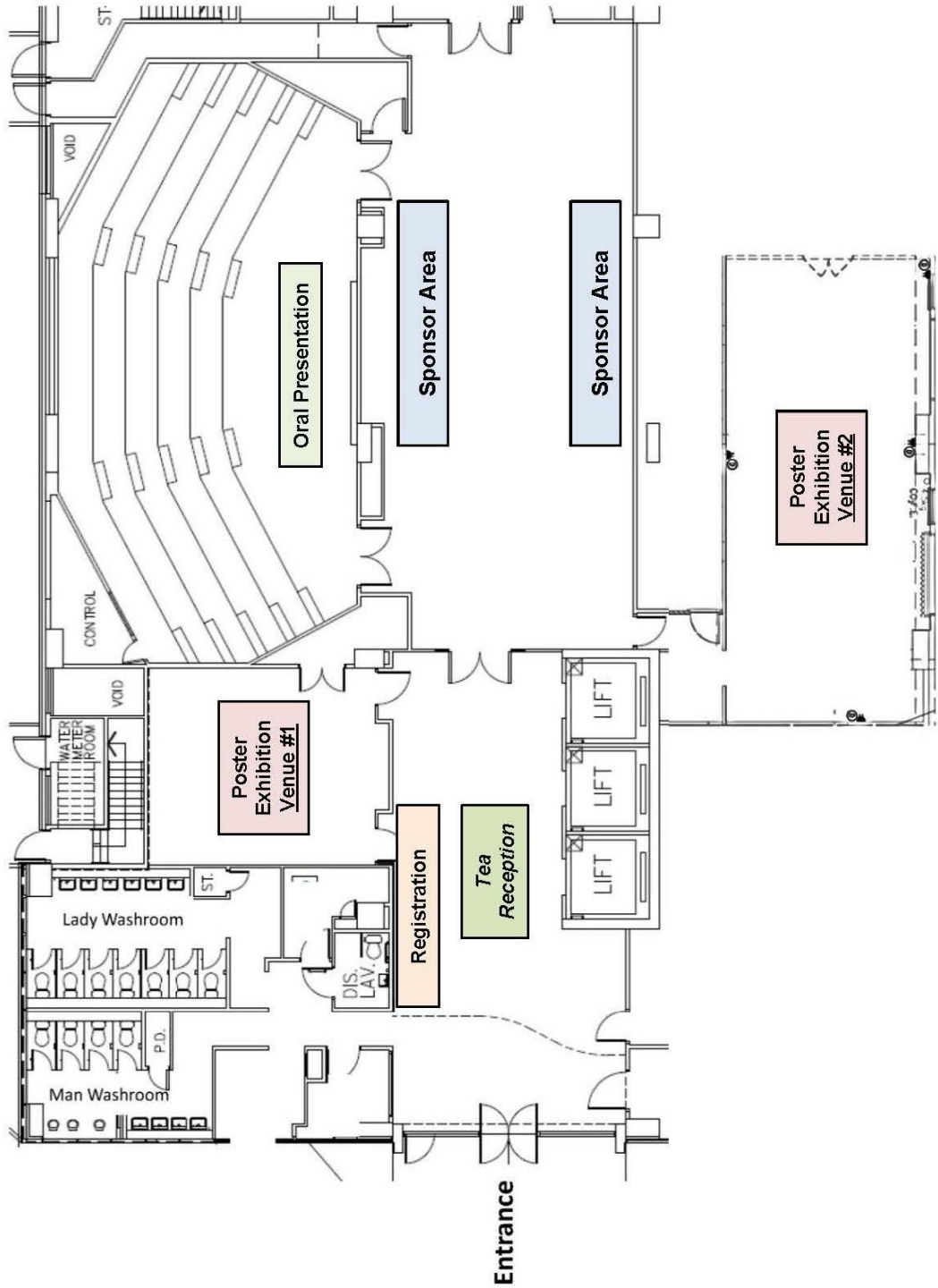
| November 8 <sup>th</sup> (Thursday – Day 1)    |   |  |                                     |                               |  |
|--|---|--|-------------------------------------|-------------------------------|--|
| <b>09:15 - 09:30</b>                           | <b>Registration (Ground Hall, SBS, Area 39)</b>   |  |                                     |                               |  |
| <b>09:35 – 10:50</b>                           | <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="background-color: #d9ead3; width: 50%;"><b>Posters NVMB 01-10 (Venue #1)</b></td> <td style="background-color: #f4cccc; width: 50%;"><b>Posters DRB 01-12 (Venue #2)</b></td> </tr> <tr> <td colspan="2" style="text-align: center;"><i>Tea Break (10 minutes)</i></td> </tr> </table> | <b>Posters NVMB 01-10 (Venue #1)</b>           | <b>Posters DRB 01-12 (Venue #2)</b> | <i>Tea Break (10 minutes)</i> |  |
| <b>Posters NVMB 01-10 (Venue #1)</b>           | <b>Posters DRB 01-12 (Venue #2)</b>   |  |                                     |                               |  |
| <i>Tea Break (10 minutes)</i>                  |   |  |                                     |                               |  |
| <b>11:00 – 12:00</b>                           | <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="background-color: #d9ead3; width: 50%;"><b>Posters NVMB 11-20 (Venue #1)</b></td> <td style="background-color: #f4cccc; width: 50%;"><b>Posters DRB 13-23 (Venue #2)</b></td> </tr> <tr> <td colspan="2" style="text-align: center;"><b>Lunch</b></td> </tr> </table>                  | <b>Posters NVMB 11-20 (Venue #1)</b>           | <b>Posters DRB 13-23 (Venue #2)</b> | <b>Lunch</b>                  |  |
| <b>Posters NVMB 11-20 (Venue #1)</b>           | <b>Posters DRB 13-23 (Venue #2)</b>   |  |                                     |                               |  |
| <b>Lunch</b>                                   |   |  |                                     |                               |  |
| <b>14:00 – 15:20</b>                           | <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="background-color: #d9ead3; width: 50%;"><b>Posters CBET 01-09 (Venue #1)</b></td> <td style="background-color: #f4cccc; width: 50%;"><b>Posters CBET 10-18(Venue #2)</b></td> </tr> <tr> <td colspan="2" style="text-align: center;"><i>Tea Break (10 minutes)</i></td> </tr> </table> | <b>Posters CBET 01-09 (Venue #1)</b>           | <b>Posters CBET 10-18(Venue #2)</b> | <i>Tea Break (10 minutes)</i> |  |
| <b>Posters CBET 01-09 (Venue #1)</b>           | <b>Posters CBET 10-18(Venue #2)</b>   |  |                                     |                               |  |
| <i>Tea Break (10 minutes)</i>                  |   |  |                                     |                               |  |
| <b>15:30 – 16:30</b>                           | <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="background-color: #d9ead3; width: 50%;"><b>Posters CBET 19-22 &amp; KIZ (Venue #1)</b></td> <td style="background-color: #f4cccc; width: 50%;"><b>Posters CBET 23-26 &amp; PKU</b></td> </tr> </table>   | <b>Posters CBET 19-22 &amp; KIZ (Venue #1)</b> | <b>Posters CBET 23-26 &amp; PKU</b> |                               |  |
| <b>Posters CBET 19-22 &amp; KIZ (Venue #1)</b> | <b>Posters CBET 23-26 &amp; PKU</b>   |  |                                     |                               |  |
| <b>16:30 – 17:30</b>                           | <i>Core lab visiting in School of Biomedical Sciences</i>   |  |                                     |                               |  |
| <b>18:00</b>                                   | <i>Dinner</i>   |  |                                     |                               |  |
| November 9 <sup>th</sup> (Friday – Day 2)      |   |  |                                     |                               |  |
| <b>09:00 – 09:30</b>                           | Opening Ceremony (Room G02)   |  |                                     |                               |  |
| <b>09:00 – 09:05</b>                           | Introduction (special thanks to VIPs and Guests)  |  |                                     |                               |  |
| <b>09:05 – 09:15</b>                           | Speech by School Director (Professor CHAN Wai Yee)  |  |                                     |                               |  |
| <b>09:15 – 09:20</b>                           | Brief introduction: rules of oral presentation (Room G02)   |  |                                     |                               |  |
| <b>09:20 - 09:25</b>                           | Group photos  |  |                                     |                               |  |
| <b>09:30 – 10:15</b>                           | <b>Oral Presentation No. 1-3</b>  |  |                                     |                               |  |
|  | <i>Tea Break (15 minutes)</i>   |  |                                     |                               |  |
| <b>10:15 – 11:00</b>                           | <b>Oral Presentation No. 4-6</b>  |  |                                     |                               |  |
| <b>12:00 -13:00</b>                            | <b>Lunch</b>  |  |                                     |                               |  |
| <b>13:00 - 14:00</b>                           | <i>Inauguration Seminar for iTERM</i>   |  |                                     |                               |  |
| <b>14:00 – 15:00</b>                           | <b>Oral Presentation No. 7-10</b>   |  |                                     |                               |  |
|  | <i>Tea Break (15 minutes)</i>   |  |                                     |                               |  |
| <b>15:15 – 16:15</b>                           | <b>Oral Presentation No. 10-14</b>  |  |                                     |                               |  |
| <b>16:50 – 17:15</b>                           | <i>Prize Presentation Ceremony and group photos (Room G02)</i>  |  |                                     |                               |  |
| <b>18:00</b>                                   | <i>Dinner</i>   |  |                                     |                               |  |

Venue #1: Room G01, LKSIBSB

Venue #2: G02B, LKSIBSB

**Map of the Meeting Venue**

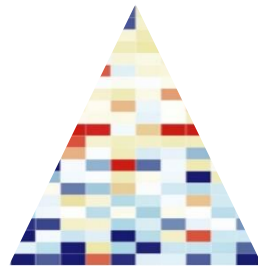
G/F, Lo Kwee-Seong Integrated Biomedical Sciences Building, Area 39



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| Peking University .....                                 | - 80 - |
| Kunming Institute of Zoology .....                      | - 86 - |

# CANCER BIOLOGY AND EXPERIMENTAL THERAPEUTICS THEME



## Cancer Biology and Experimental Therapeutics

| Title  | Author         | Abstract Number |
|--|----------------|-----------------|
| Study of CCCTC-binding Factor (CTCF) Genetic Aberrations and Dysregulation in Head and Neck Squamous Cell Carcinoma (HNSCC)                          | Chan Sze Man   | CBET-1          |
| Investigation of therapeutic effect of Fuzheng Huayu capsule, salvianolic acid B and baicalein on retrorsine-induced liver fibrosis                  | Chen Xinmeng   | CBET-2          |
| Super enhancer-associated master transcription factor SOX4 suppresses anti-tumor immunity in diet-induced hepatocellular carcinoma                   | Cheung Ka Wing | CBET-3          |
| Enhancing the efficacy of hepatocellular carcinoma immunotherapy by specific inhibition of histone deacetylase 8                                     | Feng Yu        | CBET-4          |
| Taxonomical and functional profiling of Atopic Dermatitis-associated skin microbiome through whole-genome shotgun sequencing                         | Hou Jinpao     | CBET-5          |
| Parkinson's Diseases: A Focus on the Gastrointestinal Tract  | Huang Bosheng  | CBET-6          |
| Enhancer Regulation of Myeloid-Derived Suppressor Cells in Hepatocellular Carcinoma  | Law Wing Yan   | CBET-7          |
| Targeting tumor stroma with quercetin to enhance chemotherapy in head and neck cancer  | Li Hui         | CBET-8          |
| Analysis of Pten-null mouse astrocytes revealed overlapping transcribed genes with human glioblastoma transcriptome                                  | Liu Tian       | CBET-9          |
| Delineating the immunosuppressive fibrotic environment underlying the intrinsic resistance of hepatocellular carcinoma to immune checkpoint blockade | Liu Xiaoyu     | CBET-10         |
| <i>RAC1</i> aberrations in Head and Neck Squamous Cell Carcinoma (HNSCC)   | Ngan Hoi Lam   | CBET-11         |
| Acute lung injury induced by pyrrolizidine alkaloids   | Song Zijing    | CBET-12         |
| Metabolic-immunoregulatory role of CCRK-mTOR signaling in NAFLD-associated hepatocellular carcinoma  | Tang Wenshu    | CBET-13         |

|  |                |         |
|--|----------------|---------|
| First Draft Genome of the Storage Mite <i>Tyrophagus putrescentiae</i>   | Wan Tsz Yau    | CBET-14 |
| <i>ALK</i> Genomic and Transcriptomic aberrations in head and neck squamous cell carcinoma   | Wang Lan       | CBET-15 |
| Functional analysis of FHL1- a tumor suppressor gene in human liver cancer   | Wang Lingyi    | CBET-16 |
| Hybrid De Novo Assembly of <i>Branchiostoma belcheri</i> Beihai Amphioxus Genome   | Wang Mingqiang | CBET-17 |
| Identification and functional characterization of circRNAs with critical roles in pancreatic ductal adenocarcinoma   | Wong Chi Hin   | CBET-18 |
| Differences of pyrrolizidine alkaloids-induced cytotoxicity between hepatic sinusoidal endothelial cells and hepatocytes   | Wong Ka Yan    | CBET-19 |
| Differential Adaptive Responses to Metabolic Stress in Lung Adenocarcinoma Cell Lines  | Wong Kin Lok   | CBET-20 |
| Genome-wide DNA methylation analysis of human hepatocellular carcinoma   | Wu Qiong       | CBET-21 |
| The genome assembly of <i>Blomia tropicalis</i>  | Xiong Qing     | CBET-22 |
| Single-cell transcriptomic analysis of immune-checkpoint resistance in hepatocellular carcinoma  | Xiong Zhewen   | CBET-23 |
| Epigenomic profiling of primary hepatocellular carcinoma reveals super-enhancer-associated chromatin regulator network   | Xu Liangliang  | CBET-24 |
| Hepatic cell cycle-related kinase (CCRK) shapes a metastatic-prone liver microenvironment via crosstalk between myeloid-derived suppressor cell (MDSC) and natural killer T cell (NKT) | Zeng Xuezhen   | CBET-25 |
| Drug repositioning for schizophrenia and depression/anxiety disorders: A machine learning approach leveraging expression data  | Zhao Kai       | CBET-26 |

***CBET-1*****Study of CCCTC-binding Factor (CTCF) Genetic Aberrations and Dysregulation in Head and Neck Squamous Cell Carcinoma (HNSCC)**

CHAN Sze Man, Hoi-Lam Ngan, Yuchen Liu, Wenying Piao, Vivian Wai Yan Lui

**Supervisor:** Vivian Wai Yan Lui

CCCTC-binding Factor (CTCF) is a ubiquitously expressed 11-zinc finger (ZF) DNA-binding protein that plays highly versatile genome regulatory functions including organization of topological domains in the nucleus, chromatin insulation, transcriptional activation or repression, and genomic imprinting. *CTCF* has been reported to have dual roles in carcinogenesis, tumor-suppressive and pro-tumorigenic, likely in a cancer type-specific manner. Recently, Bornstein et al. has reported that *CTCF* truncating mutations (potentially loss-of-function) are associated with progression of Head and Neck Squamous Cell Carcinoma (HNSCC), suggesting that *CTCF* wild-type may act as a tumor suppressor in this cancer type. Survival analysis of head and neck cancer patients in The Cancer Genome Atlas (TCGA) revealed that *CTCF* mutations are associated with poor overall survival ( $P = 0.0206$ , 15 months vs 56.9 months) and disease-free survival ( $P = 0.0078$ , 12.975 months vs 71.22 months), and *CTCF* downregulation is found to be associated with reduced disease-free survival ( $P = 0.0018$ , 13.01 months vs 42.77 months) in HNSCC patients. Intriguingly, we found that *CTCF* is specifically upregulated in HNSCC tumors (vs. paired normal epithelium) based on the RNA-seq data of 43 pairs of normal-tumor tissues ( $P < 0.0001$ ). This is consistent with our observed upregulation of *CTCF* protein expression in tumor primary cultures and cancer cell lines relative to normal head and neck squamous cells. To investigate the potential role(s) of *CTCF* in HNSCC, we overexpressed or downregulated *CTCF* in HNSCC cell lines and examined the consequences on cancer cell proliferation. We found that specific knockdown of *CTCF* resulted in inhibition of HNSCC cell proliferation by around 10% (N=6), while exogenous overexpression of *CTCF* promoted HNSCC cell proliferation by about 40% (N=6). Our results implicate a likely pro-tumorigenic role of *CTCF* in HNSCC.

## Investigation of therapeutic effect of Fuzheng Huayu capsule, salvianolic acid B and baicalein on retrorsine-induced liver fibrosis

Xinmeng CHEN<sup>1</sup>, Jiang MA<sup>1</sup>, Qihe XU<sup>2</sup>, Ge LIN<sup>1</sup>

(<sup>1</sup>School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR; <sup>2</sup>Department of Renal Medicine, King's College London, London, UK)

Pyrrolizidine alkaloids (PAs) are one of the most common phytotoxins causing hepatotoxicity, and PA-induced liver injury (PA-ILI) contributes significantly to herbal hepatotoxicity. PAs can induce acute hepatotoxicity, known as hepatic sinusoidal obstruction syndrome (HSOS) with typical manifestations of hepatomegaly, jaundice and ascites. Chronic exposure to small amounts of PAs may imperceptibly induce chronic liver diseases such as liver fibrosis and cirrhosis that may not show overt symptoms in the early stage during disease progression. In addition, PA-induced chronic illness, especially liver fibrosis, are common in the survivors who suffered from acute PA-induced HSOS. Currently, clinical treatment of PA-ILI is usually supportive and symptomatic for the acute HSOS. While therapies targeting chronic diseases including liver fibrosis are largely unexplored. Therefore, it is significant and timely to study PA-induced liver fibrosis and also investigate potential therapies.

In order to induce liver fibrosis, retrorsine (RTS), a representative toxic PA, was given orally to the rats twice with 40 and 20 mg/kg on day 1 and 7. For anti-fibrotic treatment, Fuzheng Huayu (FZHY) capsule, a commonly used Traditional Chinese Medicine (TCM) proprietary product for liver fibrosis, salvianolic acid B (SalB) and baicalein (BAI), the TCM-derived bioactive compounds were studied. FZHY (202.5 mg/kg/day), SalB (20 mg/kg/day or twice per day) or BAI (40 mg/kg/day or twice per day) was administered orally to the rats starting at day 15 for 2 consecutive weeks. All treated rats were sacrificed at 4 weeks after the first dose of RTS. Serum transaminase (ALT) and bilirubin levels, and diagnostic evaluation of hepatocellular injury were found in the normal ranges in all treated groups. FZHY, SalB and BAI attenuated RTS-induced liver architecture disorder and fibrosis progression as evidenced by the results of H&E staining and Sirius red staining. Comparing with RTS-treated rats, hydroxyproline (Hyp) content, a specific marker of fibrosis, was decreased significantly in FZHY, SalB and BAI treated groups. In addition, Hyp content in twice per day of SalB treated group was significantly less than that in once per day treatment. In BAI treated groups, Hyp content did not show significant differences in two regimens.

In conclusion, our results demonstrated that FZHY, SalB and BAI all have therapeutic effect on RTS-induced liver fibrosis. Among three treatment drugs/agents, FZHY capsule had better therapeutic effect than the others. SalB (twice per day) and BAI had similar therapeutic effect. All

these three demonstrated promising antifibrotic activities on RTS-induced liver fibrosis and deserve further investigations for their therapeutic values for PA-induced liver fibrosis and the underlying mechanisms.

[Supported by GRF grant from Research Grants Council of HKSAR (GRF Project No.: CUHK 14111816)] and Direct Grant from CUHK (Project No. 4054302)]

**Super enhancer-associated master transcription factor SOX4 suppresses anti-tumor immunity in diet-induced hepatocellular carcinoma**

CHEUNG Ka Wing, WU Feng, TANG Wenshu, YANG Weiqin, ZHOU Jingying, TAN Patrick, YIP Yuk Lap Kevin, TO Ka Fai, CHENG Sze Lok Alfred

**Supervisor:** Alfred Cheng Sze Lok

Hepatocellular carcinoma (HCC) has become a prominent global health threat due to its occurrence and lethality. Increasing prevalence of non-alcoholic fatty liver disease (NAFLD) have been found culpable for HCC initiation, via disruption of the liver microenvironment. Super enhancers (SEs) and master transcription factors (TFs) translate microenvironmental changes into chromatin remodelling and activation, which subsequently disrupt the transcriptome profile and initiate oncogenesis. This project aims at profiling the SE status and unveiling the master regulators involved in diet-induced HCC progression. Nanoscale chromatin immunoprecipitation sequencing (nano ChIP-seq) against histone mark H3K27ac in 10 pairs of primary human NAFLD-HCC tumors and adjacent non-tumor tissues revealed potential tissue-specific SEs (averaged 541 and 512 per HCC tumor and non-tumor tissues, respectively). Global mRNA profile was detected by RNA sequencing (RNA-seq) to support the enhancer-target gene transcription axes in the enriched metabolic and immune response pathways. A non-alcoholic steatohepatitis-induced HCC (NASH-HCC) mouse model was established by subcutaneous injection of a diabetogenic agent streptozotocin (STZ) (200 mg) two days after birth followed by a 19-week high-fat high-carbohydrate (HFHC) diet from the age of 4 weeks. Another carcinogen diethylnitrosamine-induced HCC (DEN-HCC) mouse model was established by intraperitoneal injection of DEN (25 mg/kg) followed by a 22-week HFHC diet from the age of 6 weeks. Tumor-enabling SEs were profiled in primary human NAFLD-HCC tissues and a network of master TFs were identified using integrated bioinformatic analysis. Intriguingly, tumor-enabling SEs target critical genes involved in PDGFB signalling and NAFLD pathogenesis, which are significantly correlated with high master TF SOX4 expression. Notable overexpression of Sox4 and Pdgfb was also observed in liver tumors in two HCC mouse models, significantly correlating with the CD11b<sup>+</sup> CD11c<sup>+</sup> IL10<sup>+</sup> IDO<sup>+</sup> tolerogenic dendritic cell population. Integrated analysis of ChIP-seq and RNA-seq in primary human tissues revealed a super enhancer-associated master regulatory network in NAFLD-HCC development. Our findings suggest that epigenetic regulation of the SOX4 and PDGFB may contribute to an immunosuppressive liver tumor environment, providing insights for novel therapeutic strategy against this rapidly-increasing dreadful disease.

**Enhancing the efficacy of hepatocellular carcinoma immunotherapy by specific inhibition of histone deacetylase 8**

FENG Yu<sup>1</sup>, Yang Weiqin<sup>1</sup>, Zhou Jingying<sup>1</sup>, Otto Cheung<sup>1</sup>, Chen Zhiwei<sup>2</sup>, Alfred Cheng<sup>1</sup>

**Supervisor:** Alfred Cheng

Hepatocellular carcinoma, as the worldwide fifth leading cancer, has exerted great threats to human health. Immune checkpoint therapies that targeting co-inhibitory molecules like programmed death 1 (PD-1)/PD-ligand 1 (L1) axis exhibited notable anti-tumor effect in various cancer types via enhancing cytotoxicity T lymphocytes (CTL). Unfortunately, responding rate of PD-1/PD-L1-based immunotherapy remains lower than 20% due to lack of CTL infiltration in tumor mass in HCC. We have previously unfolded the oncogenic role of HDAC8 in hepatocellular carcinogenesis, while the detailed immunoregulatory function of HDAC8 has not been studied yet.

Here, we report that HDAC8 inhibition drove remarkable enhancement on tumor CTL infiltration in a chemokine-dependent manner in an orthotopic mouse HCC model. Interestingly, the inhibition of HDAC8 also significantly reduced regulatory T (Treg) cells proportion and ameliorated the suppressive tumor microenvironment. Above all, HDAC8 inhibition hereby substantially improved the efficacy of PD-1/PD-L1 axis blockade to eradicate large hepatoma and extended the surviving period for more than one year with no recurrence observed. Furthermore, we observed that combinatorial treatment further developed potent T cell memory in circulatory system, which protected cured mice from re-challenge of the same HCC cell line. In vitro study demonstrated that HDAC8 inhibitor promotes high mobility group box 1 (HMGB1) release, which may trigger danger-associated molecule pattern (DAMP) signal and immunogenic cell death. This may explain why HDAC8 inhibitor provides long-lasting protection against HCC since recent publication unveiled the linkage between immunogenic cell death and memory formation. In summary, our study illustrated the crosstalk between epigenetics and tumor immunology and shed light on rationally combining epigenetic and immunotherapy to reach a better efficacy. Besides, further mechanistically investigation on T cell memory generation will be considered.

## Taxonomical and functional profiling of Atopic Dermatitis-associated skin microbiome through whole-genome shotgun sequencing

Jin pao Hou<sup>1</sup>, Wing Ki Yau<sup>2</sup>, Mai Shi<sup>1</sup>, Xi Zeng<sup>1</sup>, Ting Fan Leung<sup>2</sup>, Kwok Wing Tsui<sup>1,3,4</sup>

Author information

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4. Centre for Microbial Genomics and Proteomics, The Chinese University of Hong Kong, Hong Kong.

**Supervisor:** Prof. TSUI Kwok Wing Stephen

**Background:** Atopic dermatitis (AD), also known as eczema, is a common chronic skin disease characterized by *Staphylococcus aureus* colonization. However, other members of the skin microbiota may be equally important. AD skin microbiome has not been fully characterized.

**Methods:** Skin swabs at the right antecubital fossa from 50 AD patients and 50 controls were subjected to WGS sequencing. The raw reads were preprocessed by quality filtering and host reads removal using KneadData. Taxonomical and functional profiling of the metagenome are performed using MetaPhlan2 and HUMAnN2, respectively.

**Results:** We obtained 239 skin metagenomes (42 controls, 89 ezun and 108 ezaf) comprising 2.46 billion microbial reads. A significantly higher proportion of human reads was found in AD skin metagenomes. AD skin has shown a lower microbial diversity. Not only *S.aureus* was increased (6.18% VS. 70.44% in mean proportion), but also *S. hominis* (12% VS. 0.07%) and *Propionibacterium acnes* (20.08% VS. 0.97%) were reduced in AD patients compared to controls ( $p$ -value < 0.01). In addition, 12 out of the top 20 most abundant MetaCyc pathways are significantly higher in AD metagenome, among which was L-histidine degradation. Interestingly, the enhancement of this pathway in AD may imply filaggrin (FLG) dysfunction, a histidine-rich protein frequently reported to be associated with AD susceptibility.

**Conclusion:** AD skin has a lower overall microbial diversity with *S. aureus* overrepresentation and *S. hominis* and *P. acnes* underrepresentation. Histidine degradation pathway are enhanced in AD skin and can contribute to FLG abnormality. Analysis of strain level variation is needed for a better understanding of association between skin microbiome and AD pathogenesis.

**Parkinson's Diseases: a Focus on the Gastrointestinal Tract**

Ianto B. Huang<sup>1</sup>, Zengbing Lu<sup>1</sup>, Gang Lu<sup>2</sup>, John A. Rudd<sup>1,3,4</sup>

1School of Biomedical Sciences, Faculty of Medicine, 2Division of Neurosurgery, Department of Surgery, Prince of Wales Hospital, 3Brain and Mind Institute, and 4Laboratory Animal Services Centre, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, P.R. China

**Supervisor:** Prof. John A. Rudd

Parkinson's disease (PD) is a chronic and progressive movement disorder. The aetiology of the disease may involve exposure to environmental toxins, such as rotenone, one of the oldest but still globally used herbicides. PD may involve a malfunction and death of dopaminergic neurons in the substantia nigra, with associated increases in alpha-synuclein. Clinical studies have shown that the gastrointestinal tract also shows dopaminergic neuronal loss and increases in alpha-synuclein. Clinically, constipation may develop at least twenty years ahead of the central symptoms of the disease. In preclinical studies, chronic rotenone treatment induces delayed gastric emptying and causes enteric neuronal dysfunction. Gastric slow waves are generated by interstitial cells of Cajal to enable peristalsis. However, there are no reports showing that rotenone causes a disruption of slow wave activity or causes gastric dysrhythmia, or whether treating gastric dysrhythmia could delay the onset of PD.

We established a low dose (1 mg/kg, daily, for 10 weeks) rotenone-induced PD rat model with an implanted radiotelemetry device record gastric myoelectrical activity, blood pressure, and temperature. Rotenone-induced constipation and a disruption of slow waves with gastric dysrhythmia, ahead of expected CNS pathology; blood pressure was relatively unaffected but there were periods of hypothermia. In vitro, rotenone (10-100 uM) caused enteric neuronal apoptosis, and ghrelin (100 nM-10 uM) was neuroprotective. Future studies will assess if ghrelin mimetics can protect the gastrointestinal tract from rotenone-induced toxicity, whilst also delaying or preventing the central motor manifestations of PD.

## **Targeting tumor stroma with quercetin to enhance chemotherapy in head and neck cancer**

LI Hui, PIAO Wen Ying, LUI Wai Yan Vivian

**Supervisor:** LUI Wai Yan Vivian

Head and neck squamous cell carcinoma (HNSCC) is an aggressive cancer with a high recurrence rate. Recurrences occur in ~25% cases of early -stage patients (Stage I-II) and ~50-60% cases of advanced stage (Stage II-IV) patients. Cumulative evidences suggest that tumor stroma, especially cancer-associated fibroblast (CAF) play a protective role and development of drug -resistance in a tumor, thus resulting in incomplete cure of tumor by drug treatment, leading to recurrences.

Quercetin is a flavonoid purified from natural plants, reported to facilitate drug delivery to tumor side by suppressing CAFs and reducing collagen accumulation. In this study, we hypothesize that targeting CAFs with quercetin can enhance anti-tumor effects of cisplatin in HNSCC.

Tumor cells co-cultured with CAFs are more likely to mimic tumor microenvironment. Thus, we established a co-culture model with tumor cells and CAFs derived from HNSCC patients to investigate its drug sensitivity. Our data show that combination of quercetin and cisplatin elicited a significantly greater growth inhibition (70% of cell inhibition) than cisplatin (37%) or quercetin (15%) alone.

We then established stroma-rich xenograft models using primary cell lines from HNSCC patients to investigate the effect of quercetin in vivo. Quercetin reduce the expression of  $\alpha$ -SMA compared with control. This indicates that there are less CAFs in quercetin treated group than that in control.

In conclusion, quercetin can reduce CAFs within tumor bulks which has the potential to enhance antitumor effect when combined with cisplatin.

## **Enhancer Regulation of Myeloid-Derived Suppressor Cells in Hepatocellular Carcinoma**

Rhoda Wing Yan Law, Man Liu, Jingying Zhou, Alfred Sze Lok Cheng

**Supervisor:** Prof. Alfred Cheng

Hepatocellular carcinoma (HCC) is the most common liver cancer. The suppressive tumor microenvironment (TME) plays an instrumental role in HCC development and progression. Myeloid-derived suppressor cells (MDSCs) are immature myeloid cells which are major component of TME that undermine immune surveillance and facilitate tumor progression and immune escape. As a key driver of liver fibrosis, activated hepatic stellate cell (HSC) has been shown to induce MDSC generation through cell-cell contact and/or secreted soluble factors. Given the crucial role of enhancer in cell lineage specification, we aim to elucidate the enhancer regulation of MDSC in the immunosuppressive liver TME. Using human peripheral blood mononuclear cells (PBMCs) from healthy donors, we have successfully recapitulated the immunomodulatory effect of HSC on MDSC generation. Using single cell-RNA sequencing from HCC patient samples and RNA sequencing from in vitro-derived MDSCs, signature genes which have differential expression in MDSC were identified. Comprehensive epigenetic drug screening and expression analysis suggested that histone H3 lysine 27 acetylation (H3K27ac) contributes to HSC-induced MDSC generation. We further demonstrated that BET inhibitor JQ1, which disrupts bromodomain protein 4 (BRD4) association with H3K27ac-enriched active enhancers, controls MDSC signature gene expressions and abrogates MDSC generation and suppressive function. Epiregulin is one of the signature genes which may be controlled by enhancer, as verified by qRT-PCR and qChIP-PCR. These results reveal that enhancers are crucial in MDSC identity formation and function, which may provide novel therapeutic targets to restore the anti-tumor immune response in HCC patients.

**Analysis of Pten-null mouse astrocytes revealed overlapping transcribed genes with human glioblastoma transcriptome**

LIU Tian, OR Mei Yu, Penelope, WONG Chi Wai, CHEUNG Kwok Kuen, Stanley, WANG Yubing, CHAN Man Lok, Andrew

**Supervisor:** Andrew M. Chan

Glioblastoma is an aggressive form of brain cancer with universally poor prognosis. Even with optimal therapy, the cancer recurs frequently. Phosphatase and tensin homolog (PTEN) is a tumor suppressor gene whose mutations are often found in multiple human cancers. PTEN encodes a lipid phosphatase and serves as a negative regulator of the phosphoinositide 3-kinase (PI3K) pathway. Cumulative evidence suggests that PTEN mutations play significant roles in tumorigenesis of glioblastoma as 60% of glioblastoma patients acquired mutations in this gene. Our laboratory has generated a knockout (KO) mouse line of PTEN. We isolated the cortex of newborn mice for astrocytes primary culture and then performed RNA-seq analyses for both wild type (WT) and KO astrocytes. The RNA-seq results revealed significant transcriptome alterations between WT and KO astrocytes with 1826 genes being upregulated and 1175 genes downregulated in PTEN KO group. Those 3001 differentially expressed genes (DEGs) were integrated with online database related to human glioblastoma and a total of 60 overlapping DEGs were selected for further qRT-PCR validation. 4 genes (RCC1, NEK2, CHK2 and G0S2) were validated to be significantly upregulated in PTEN KO astrocytes. Regulator of chromatin condensation 1 (RCC1) has been reported to be a major guanine-nucleotide exchange factor for Ran GTPase and participates in various biological processes such as spindle assembly during mitosis. PTEN has also been found to regulate spindle assembly at the centrosomes. Our major hypothesis is that PTEN deficiency or PI3K pathway hyperactivation may lead to the upregulation of RCC1, and this in turn promotes tumorigenesis of glioblastoma by dysregulating cell mitosis. To further explore the RCC1 roles, we will establish both RCC1 knockdown and RCC1 overexpressing glioblastoma cell lines to find out how it affects cell mitosis thus promoting glioblastoma. In summary, these studies will help in exploring roles of PTEN and its regulated pathways in tumorigenesis of glioblastoma.

*CBET-10***Delineating the immunosuppressive fibrotic environment underlying the intrinsic resistance of hepatocellular carcinoma to immune checkpoint blockade**

Xiaoyu Liu, Man Liu, Jingying Zhou, Weiqin Yang, Yu Feng, Alfred Sze-Lok Cheng

**Supervisor:** Alfred Sze-Lok CHENG

Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related death. Immune checkpoint blockade (ICB), in particular anti-programmed cell death-1 (PD-1) or its ligand (PD-L1), exhibits promising benefits but yet low response rate for HCC patients. Since most HCC occurs in the setting of fibrosis or cirrhosis, we aim to uncover the key factors that drive the ICB non-responsiveness in fibrosis-associated HCC. Two orthotopic fibrosis-HCC mouse models were established by gavage of carbon tetrachloride (CCl<sub>4</sub>) followed by intrahepatic injection of either Hepa1-6 or RIL-175 mouse hepatoma cell line. We found that liver fibrosis promotes HCC development dramatically. Similar to the clinical scenario, around 50% of fibrotic HCCs showed no response to anti-PD-L1 treatment. In the non-responders, both monocytic myeloid-derived suppressive cells (M-MDSCs) and polymorphonuclear (P)-MDSCs were enriched in the tumor-surrounding liver and positively correlated with tumor growth. However, only M-MDSC proportion was consistently correlated with reduced T cell proportion and its cytotoxic functions in the non-responders. Furthermore, P-MDSC depletion by anti-Ly6G in fibrotic HCC did not delay tumor growth, implying that targeting M-MDSCs may be a potential therapeutic approach to improve HCC immunotherapy.

**CBET-11****RAC1 aberrations in Head and Neck Squamous Cell Carcinoma (HNSCC)**Hoi-Lam NGAN, Vivian Wai Yan Lui**Supervisor:** Vivian Wai Yan Lui

*RAC1* is a known oncogene. Genomic aberrations of *RAC1* is highly relevant to melanoma due to its high prevalence and the presence of a hotspot *RAC1* mutation (*RAC1* p.P29S). Yet, little is known about *RAC1* in other human cancers, including head and neck squamous cell carcinoma (HNSCC). In the Human Protein Atlas, pan-cancer immunohistochemistry (IHC) staining analysis showed that *RAC1* protein expression is detected in 9/20 cancer types with HNSCC being one of the expressing one. Pan-cancer mutation analysis of 32 cancer types from the Cancer Genome Atlas (TCGA) reveals that *RAC1* mutations only affect 12/32 cancer types and HNSCC is the second most frequently affected cancer type, after melanoma, having 2.64% of *RAC1* somatic mutations reported. Thus, *RAC1* aberrations may have a role in HNSCC tumorigenesis. Clinical correlation analyses of the TCGA HNSCC provisional cohort showed that patients with somatic *RAC1* aberrations (gene amplification/copy number gain/hotspot mutations) are strongly associated with both poor overall survival (OS)( $P=4.555e-5$ ; Median survival of 30.91 months vs 68.43 months) and poor disease-free survival (DFS) ( $P=5.49e-5$ ; Median survival of 27.89 month vs n.a.) in HNSCC, when compared with those without *RAC1* aberrations. *RAC1* aberrations are also associated with enriched *TP53* mutation ( $P=2.07e-6$ ), and low rate of HPV infection ( $P=7.397e-6$ ; 7.7% vs. 87.2%). Further, *RAC1*-altered patients appear to be more advanced (T staging T3/4 vs T1/2;  $P=0.0312$ ) as well as having more gross/microscopic extension ( $P=0.0111$ ; 59/119 vs 54/201) than the *RAC1*-unaltered subgroup. These evidences strongly suggest that HNSCC with *RAC1* aberrations are more aggressive. Lastly, mutational profile of *RAC1* mutated HNSCC tumors demonstrated enriched mutations of three tumor suppressor genes: *FAT1*, *FAT4* and *CSMD3* ( $P=0.0001$ ,  $P=0.0038$  and  $P=0.0467$  respectively). This may reveal a different mechanism for *RAC1* mutated HNSCC tumorigenesis and warrant future investigation.

## Acute lung injury induced by pyrrolizidine alkaloids

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Pyrrolizidine alkaloids (PAs) are widely distributed in thousands of species of plants. Among them, PAs of retronecine-type and otonecine-type have been reported to be toxic to liver. Monocrotaline (MCT), one of retronecine-type PAs has been demonstrated to be pulmonary toxic and used to establish animal models of pulmonary arterial hypertension for more than 40 years. However, except for MCT, whether the other toxic PAs may also induce lung injury is poorly understood. The present study aimed to investigate if pulmonary toxicity is common for all of the toxic PAs.

Riddelliine (RID) and clivorine (CLI), the representative hepatotoxic PAs of retronecine type and otonecine-type respectively were orally administrated to rats at 0.2 mmol/kg, and also compared with the same dosage of MCT treatment for 48 hours. Pyrrole-protein adducts, a biomarker of PA-induced toxicity, were detected in lungs of all PA-treated rats, indicating pulmonary toxicity induced by all PAs tested. The results of hematoxylin and eosin (H&E) staining showed all PAs induced changes of endothelial layer and smooth muscle layer of pulmonary arteries within 48 hours after dosing. The expression of alpha-smooth muscle actin (alpha-SMA), a specific marker of smooth muscle cells (SMCs), significantly increased in lungs of all PA-treated rats using western blot and immunohistochemical (IHC) staining, demonstrating abnormal SMCs proliferation in the early stage of PA-induced lung injury. Additionally, IHC double staining of caveolin-1(cav-1), a marker of endothelial cells (ECs), and alpha-SMA showed breakdown of endothelial monolayer in lung of all PA-treated rats with more severity in RID-treated and MCT-treated rats than CLI-treated rats.

In conclusion, the results demonstrated that both otonecine-type and retronecine-type PAs induced pulmonary toxicity. Damage of ECs and disordered proliferation of SMCs involved in the mechanism of PA-induced pulmonary toxicity in the initial stage.

[Supported by GRF grant from Research Grants Council of HKSAR (GRF Project No.: CUHK 14160817) and Direct Grant from CUHK (4054376)]

*CBET-13***Metabolic-immunoregulatory role of CCRK-mTOR signaling in NAFLD-associated hepatocellular carcinoma**Wenshu TANG, Jingying ZHOU, Weiqin YANG, Yu FENG, Myth T.S. MOK, Alfred S.L. CHENG**Supervisor:** Alfred S.L. CHENG, Jingying ZHOU

Obesity increases the risks of steatosis, non-alcoholic steatohepatitis (NASH) and HCC development. Non-alcoholic fatty liver disease (NAFLD) associated-hepatocellular carcinoma (HCC) is a male-predominant cancer mainly caused by metabolic disorder. Our previous findings has demonstrated that cell cycle-related kinase (CCRK), which is a direct downstream of androgen receptor (AR), functions as a signaling hub connecting multiple oncogenic circuits to drive metabolic and immunosuppressive reprogramming in the liver. CCRK upregulates mTORC1 pathway, resulting in deregulated lipid/glucose metabolism, immunosuppression and tumorigenesis (Nat Commun 2018). In this study, we further elucidate that CCRK activates mTORC1 through SLC3A2 and SLC7A5 dependent amino acid transportation. We performed immune cell profiling in a high-fat high-carbohydrate diet (HFHC)-induced obesity and NASH murine model and uncovered activation of CCRK-mTORC1 signaling cascade and specific reduction in cytolytic natural killer T (NKT) cells, which subsequently enhanced tumor growth of orthotopically-implanted HCC tumor cells. Notably, treatment of the mTORC1/C2 dual kinase inhibitor vistusertib (AZD2014), currently undergone phase I/II clinical trials, not only abrogated mTORC1/SREBP2 signaling and plasma cholesterol levels, but also derepressed the cytolytic NKT cells in plasma and liver leading to reduced tumorigenicity. Furthermore, mTORC1/SREBP2 positively associated with plasma cholesterol level as well as liver NKT in the streptozotocin (STZ)-HFHC induced spontaneous NAFLD-HCC mouse model, which will be utilized to further study the therapeutic effect of vistusertib in vivo. Collectively, this study will elucidate the metabolic-immunosuppressive role of CCRK-mTOR signaling in NAFLD-associated HCC and further provide insights into development of therapeutic strategies against the CCRK-mTOR reinforced HCC.

*CBET-14***First Draft Genome of the Storage Mite *Tyrophagus putrescentiae***Angel Tsz Yau WAN, Stephen Kwok Wing TSUI**Supervisor:** TSUI Kwok Wing Stephen

*Tyrophagus* (T.) *putrescentiae*, known as storage mite and mold mite, is an important mite species that infests a wide variety of stored foods, especially those with high protein and fat contents. *T. putrescentiae* is associated with allergic disorders including asthma and allergic rhinitis like other domestic mite. The identification of allergen sequences and structure in this species is important for clinical diagnosis and allergen-specific immunotherapy. In this study, we aimed to construct a high-quality reference genome of *T. putrescentiae* to find known canonical allergens and predict novel allergens. We employed a hybrid approach to assemble the *T. putrescentiae* genome. Next generation sequencing was performed by sequencing of 330,182,370 clean reads from the paired-end library generated from Illumina HiSeq 4000. A total of 13.2 Gb raw data containing 1,908,442 filtered sub reads with average read length of 6.42 Kb was generated on PacBio SEQUEL. We assembled a draft genome of 97.3 Mb in size, containing 903 contigs and 176 scaffolds. The scaffold N50 and contig N50 were 2,914 kb and 302 kb respectively. The genome completeness determined was 91.6% using BUSCOs (v3) analysis with 1,066 core genes from *arthropoda\_odb9* dataset. This study paves the way for studying the genome of *T. putrescentiae* and investigating the difference between storage mites and house dust mites by comparative genomics.

**CBET-15****Genomic aberrations of *ALK* in head and neck squamous cell carcinoma**

WANG Lan, LIU Yuchen, PIAO Wenying, POON Hui Yan Peony, YEUNG Chun Kit Thomas,  
LUI Wai Yan Vivian

**Supervisor:** LUI Wai Yan Vivian

Anaplastic lymphoma kinase (*ALK*) is a receptor tyrosine kinase coded by the gene *ALK*, which belongs to a subfamily of the insulin receptor superfamily. *ALK* signalling is involved in cell proliferation, differentiation, and development, etc. There are several major downstream signaling pathways activated by *ALK*, including the MAPK and PI3K pathways. Thus far, *ALK* aberrations play important roles in the oncogenesis of non-small cell lung cancer and glioblastoma. Head and neck cancer squamous cell carcinoma (HNSCC) is an aggressive cancer with diverse genomic heterogeneity. Genomic aberrations of *ALK* are found in a subset of head and neck cancer. Yet, the biological contribution of these HNC-associated *ALK* aberrations is largely unknown.

Here, we analysis the genomic and transcriptomic aberrations of *ALK* in HNC using the US TCGA HNSCC database (TCGA, Provisional; N=528; [www.cbioportal.org](http://www.cbioportal.org)). Among the 43 pairs of normal-tumor samples, we found that *ALK* mRNA expression is specifically upregulated in the tumors (~2-fold, P=0.001). The remaining 457 tumors show an average of 1.5-fold upregulation (P=0.039) of *ALK* transcript when compared to the normal head and neck epithelium. Whole-exome sequencing data reveal the presence of 3.5% of somatic *ALK* mutations, 0.4% *ALK* amplification, 17.4% *ALK* copy number gain in HNSCC (N=510). In our small Asian HNSCC cohort (n=53), we also find that 18.9% patients harbour *ALK* germline variants (n=10) with unknown clinical significance. *ALK* mutations are not associated with HPV status (P=0.717) and *TP53* mutations (P=0.604) in HNSCC patients. However, *ALK*-mutated patients are found to be largely advanced cases (93.33%, Stage III and Stage IV), but do not associated with overall survival (P=0.921), likely due to the limited cases with *ALK* mutations. Protein expression profiling reveals that *ALK*-altered primary tumors have increased PIK3CA and CHEK2 protein overexpression (P<0.001, 0.0001, respectively), as well as under-expression of PRAS40(pT246) and VEGFR2 (P<0.001, 0.001, respectively). In conclusion, *ALK* aberrations may contribute to HNSCC aggressiveness. Future investigations are warranted to study the biological effects of *ALK* aberrations on HNSCC oncogenesis.

**Acknowledgements**

VWYL received funding supports from the Research Grant Council, Hong Kong (General Research Fund: #1711484, #17121616; # 14168571; Theme-based Research: T12-401/13-R), Health and Medical Research Fund (HMRF #15160691), the University-Industry Collaboration Program (UIM/329; Innovation and Technology Fund, Hong Kong), and the Hong Kong Cancer Fund, Hong Kong.

YCL received funding (Postdoctoral Hub of UIM/329) from the Innovation and Technology Fund, Hong Kong government.

WP received funding (Postdoctoral Fellowship) from the Faculty of Medicine, the Chinese University of Hong Kong, Hong Kong.

*CBET-16***Functional analysis of FHL1- a tumor suppressor gene in human liver cancer**Lingyi Wang**Supervisor:** TSUI Kwok Wing Stephen

Liver cancer is one of the most common types of cancer among human malignancies. Four and a half LIM domains 1 (FHL1), as a tumor suppressor gene, is frequently downregulated in multiple types of human cancer. However, the role and specific mechanisms of FHL1 as a tumor suppressor in liver cancer are poorly understood. The present study aimed to investigate the role and associated mechanisms of FHL1 in human liver cancer. The level of FHL1 mRNA in hepatocellular carcinoma (HCC) tissue specimens and cell lines derived from the human liver was determined using reverse transcription polymerase chain reaction. Cell viability, cell migration and cell cycle assays were performed while knockdown FHL1 in PLC5 cell line. The inhibitory effects of FHL1 on liver cancer cell growth were associated with S-phase arrest. In addition, promoted cell migration was observed in liver cells upon the downregulation of FHL1. Our data suggest that reduced expression of FHL1 may play an important role in the development and progression of liver cancer and that FHL1 may be a useful target for liver cancer gene therapy.

## Hybrid De Novo Assembly of *Branchiostoma belcheri* Beihai Amphioxus Genome

Ming-Qiang Wang, Kevin Yi Yang, Junyuan Chen, Bingyu Mao, Stephen Kwok-Wing Tsui

**Supervisor:** TSUI Kwok Wing Stephen

*Branchiostoma belcheri*, also known as Chinese amphioxus, is the closest living invertebrate relative of the vertebrates. It is widely used as a model system for studying evolutionary developmental biology and the origin of vertebrates. Beihai amphioxus is a subspecies of *B. belcheri* that inhabits in Guangxi Beihai, China. The previously reported amphioxus genome generated by short reads sequencing technologies are highly fragmentary, which hinders the downstream analysis and further applications. Here we report the sequencing and assembly of the 640Mb Beihai amphioxus genome using a hybrid approach that combined Pacific Bioscience Single Molecule Real-Time (SMRT) long reads and Illumina short reads sequencing technology. Specifically, a total of 80 Gb genomic data, including 20X PacBio long reads from Sequel sequencing platform, 66X paired-end reads and 56X mate-pair reads from Illumina HiSeq 2000 sequencing platforms, were generated to achieve a high-quality Beihai amphioxus genome. We performed hybrid de novo assembly, scaffolding, gap filling and polishing bioinformatics pipeline to obtain the assembly result which contains 92,511 contigs and 28,014 scaffolds with the contig N50 of 12 kb and scaffold N50 of 73 kb, respectively. The assembly genome contains 907 (92.7%, out of 978) BUSCO core genes collected from metazoa\_odb9 database, with 796 (81.4%) of them being complete. The assembly result contains 15M repetitive sequences, which contains 67% simply repeats, 11% SINEs and 7% low-complexity sequence, accounting for 2.28% of the whole genome. Evidence-driven gene prediction method based on RNA-Seq data has identified 63,612 transcripts and 44,087 protein coding sequences in this Beihai amphioxus genome. We anticipate the Beihai amphioxus genome would improve our knowledge on the genetic diversity of this species, meanwhile providing a valuable genetic resource for the scientific community to further understand the vertebrate evolution.

*CBET-18***Identification and functional characterization of circRNAs with critical roles in pancreatic ductal adenocarcinoma**Chi Hin Wong, Yangchao Chen**Supervisor:** Yangchao Chen

Circular RNA (circRNA) is a novel class of non-coding RNA that regulates gene expression. However, the role of circRNAs in pancreatic ductal adenocarcinoma (PDAC) is largely unknown. Here, we performed RNA sequencing of non-tumor human pancreatic ductal epithelial HPDE and PDAC cell lines PANC-1 and SW1990, and identified 169 differentially expressed circRNAs in PDAC. We selected the up-regulated circRNAs and characterized their unique circular structures. We found that circR, derived from exon 4 and 5 of precursor mRNA R, was significantly up-regulated in both PDAC cells and tissues. Importantly, knockdown of circR inhibited PDAC cell viability, clonogenic ability, migration and invasion, and induced G0/G1 cell cycle arrest and apoptosis. Via bioinformatics analysis and luciferase reporter assay, we found that circR absorbed multiples microRNAs (miRNAs) including miR-15a, miR-497-5p and miR-891b. Also, knockdown of circR inhibited the expression of miRNAs targets: BMI-1, FGF2, FGFR1, HOTTIP and CBL-B. Collectively, circR functioned as “sponges” of miR-15a-5p, miR-497-5p and miR-891b-5p, and in turn increased the expression of BMI-1, FGF2, FGFR1, HOTTIP and CBL-B to promote cell proliferation and invasion in PDAC.

*CBET-19***Differences of pyrrolizidine alkaloids-induced cytotoxicity between hepatic sinusoidal endothelial cells and hepatocytes**KY Wong, YS He, B Feng, G Lin

Pyrrolizidine alkaloids (PAs) are a group of phytotoxins that contaminate foods and natural products in our daily life. PAs exert their toxicity through the formation of dehydro-PAs mediated by cytochrome P450 (CYPs) enzymes and especially CYP3A4 isozyme in human liver. The exposure to toxic PAs can cause acute liver injury represented by the lethal hepatic sinusoidal obstruction syndrome (HSOS). The initiation and progression of HSOS involve damages in hepatic sinusoidal endothelial cells (HSECs) and hepatocytes (HCs), and HSECs are particularly more susceptible to PA intoxication than HCs. The present study aims to investigate the differences of PA-induced cytotoxicity between HSECs and HCs.

## Differential Adaptive Responses to Metabolic Stress in Lung Adenocarcinoma Cell Lines

Wong Kin Lok, Chan Man Lok Andrew, Or Mei Yu

**Supervisor:** Professor Chan Man Lok Andrew

A considerable amount of selective pressure is exerted on tumour cells from nutrient deprivation in the tumour microenvironments whether in primary, invading or metastatic sites. Of lung cancers, the most common histological type is non-small-cell lung cancer (NSCLC) where the brain is a common organ for metastasis.

Utilising different lung adenocarcinoma cell lines, we show differential usage of oxidative phosphorylation and glycolysis among these cell lines using the Seahorse metabolomics analyser. A1115 is a rare brain metastatic lung adenocarcinoma cell line that exhibits a high dependence in glycolysis and was found to be very sensitive to glucose deprivation while less so from oxidative phosphorylation inhibition treatment. This suggests a highly reliance on Warburg effect for survival. Through prolonged selection in low glucose, glucose independent A1115 sublines (LG) were isolated in low glucose medium (0.15 g/L) where these sublines demonstrated a higher resistance to glucose deprivation than control.

In order to examine the possible mechanisms for the switch to glucose independence in the sublines, a parallel investigation using Transcriptomic analysis (RNAseq) and Metabolomics analysis (LC/MS on metabolites) comparing parental A1115 in high glucose medium (4.5 g/L) and LG in low glucose medium (0.15 g/L) was conducted. Integrative enrichment analysis of both transcriptomics and metabolomics suggested the alanine, aspartate and glutamate metabolism pathway to have major differences between levels of transcripts of enzymes as well as associated metabolites, where within this pathway, qPCR and Western blotting analysis confirmed elevated transcript and protein levels of Carbamoyl phosphate synthetase I (CPS1) – an enzyme responsible in the rate-limiting step in the biosynthesis of urea. Thus it would be interesting to further investigate the mechanism behind how CPS1 can induce survival from prolonged glucose deprivation.

*CBET-21***Genome-wide DNA methylation analysis of human hepatocellular carcinoma**Qiong WU, Kevin Y. YIP and Alfred S. CHENG**Supervisor:** Alfred Cheng

As one of the most common liver cancers, hepatocellular carcinoma (HCC) is considered as the 5th most common cancer in the world and is responsible for 5% of all malignant tumors in humans. In this study, we want to delineate the DNA methylome in human HCCs and identify the key differentially methylated genes crucial for hepatocarcinogenesis. Furthermore, we also want to distinguish the methylation profiles between chronic hepatitis B (HBV)- and Nonalcoholic fatty liver disease (NAFLD)-associated HCCs. In our whole-genome bisulfite sequencing (WGBS) analysis of 10 human HBV-associated tumors, 10 human NAFLD-associated tumors and 1 public normal liver tissue, methylation state at all CpG sites in the genome of HCC tumors are comprehensively studied.

**The genome assembly of *Blomia tropicalis***

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**Supervisor:** Prof. Stephen Tsui

House dust mite allergens are the main cause of human allergic diseases, especially respiratory diseases like asthma and allergic rhinitis. Among all the house dust mites, *Blomia tropicalis* is prevalent in tropical and sub-tropical areas, including South China and Southeast Asia. *Blomia tropicalis* belongs to the superfamily of Glycyphagidae, initially described as a storage mite and now considered as a house dust mite.

A high-quality genome assembly would facilitate our scientific research to *Blomia tropicalis*, and even clinical diagnosis and therapy to associated allergic diseases. Here we combined two sequencing technologies (Ion Torrent S5 and Pacbio Sequel) for genomic DNA sequencing and applied Illumina HiSeq 2500 for transcriptome sequencing, and assembled a high-quality *Blomia tropicalis* genome. The assembled *Blomia tropicalis* genome has a size of 63,587,129 bp, 152 scaffolds (scaffolds N50= 1,512,722 bp) and 295 contigs (contigs N50= 517,564 bp). The BUSCO assessment (91.4% complete) reveals the high completeness of this assembled *Blomia tropicalis* genome. So far, 7424 protein-coding genes have been annotated, besides 35 rRNA genes and 157 tRNA genes.

Further, we plan to reveal novel allergens of *Blomia tropicalis* and confirm them by clinical and immunology tests. Also, we wish to illustrate biological mechanisms that how *Blomia tropicalis* results in human allergic diseases based on this high-quality genome assembly.

## Single-cell transcriptomic analysis of immune-checkpoint resistance in hepatocellular carcinoma

Zhewen Xiong, Jingying Zhou, Yu Feng, Xuezheng Zeng, Alfred Sze-Lok Cheng

**Supervisor:** Prof. Alfred Sze-Lok Cheng

Binding of programmed death-ligand 1 (PD-L1) to its receptor programmed death 1 (PD-1) on activated T cells inhibits anti-tumor immunity by counteracting T cell-activating signals. Although use of immune checkpoint inhibitors (ICI) targeting the PD-1/PD-L1 has yielded impressive results in cancer patients, resistance to these therapies has increasingly been observed, especially in hepatocellular carcinoma (HCC). To elucidate the mechanisms of adaptive resistance, we generated a novel anti-PD-L1 treatment resistant mouse hepatoma cell Hepa1-6 (PD-L1R) by 6-generation of *in vivo* selection. To first identify the transcriptional re-programming and intratumor genomic heterogeneity of resistant tumors, we performed single-cell RNA-sequencing (scRNA-seq) profiles of more than 8,000 single cells isolated from *in vivo* anti-PD-L1 treated tumors generated from parental- or PD-L1R-Hepa1-6 cells. Using K-means clustering and t-distributed stochastic neighbor embedding (t-SNE), we identified 8 distinct clusters with significantly different expression of HCC-associated genes and immune-related genes via biaxial scatter plots. Heatmap of the 100 most differentially expressed genes showed vast heterogeneity of transcriptional patterns in responsiveness tumors and resistance tumors. To investigate the tumor-cell extrinsic factors for adaptive resistance, we analyzed immune profiling of the responsiveness tumors and resistance tumors by flow cytometry. The data showed that adaptive resistance was associated with immunosuppression represented by lower anti-tumor CD8<sup>+</sup> T cells but higher exhausted T cells, intermediate Th17 cells, myeloid-derived suppressor cells (MDSCs) as well as T regulatory cells (Tregs) in tumor microenvironment. These findings suggested that adaptive resistance may be controlled by both tumor intrinsic transcriptional re-programming and genomic heterogeneity as well as its associated immunosuppression. Further investigation on the mechanisms and functions of crosstalk between tumor and tumor microenvironment to identify novel druggable targets for combination therapy will be considered.

*CBET-24***Epigenomic profiling of primary hepatocellular carcinoma reveals super-enhancer-associated chromatin regulator network**

Liangliang XU, Feng WU, Otto K.W. CHEUNG, Lemuel L.M. SZETO, Myth T.S. MOK, Alfred S.L. Cheng

**Supervisor:** Alfred S.L. Cheng

Hepatocellular carcinoma (HCC) is the second most common cause of mortality from cancer worldwide. Parallel with the growing epidemics of obesity and diabetes, non-alcoholic fatty liver disease (NAFLD) has become the predominant cause leading to HCC in a dysregulated metabolic background. Recent HCC genomics studies present only a paucity of recurrent gene mutations, which lead to a sparkling interest in the epigenetic regulation of hepatic carcinogenesis. Chromatin modifications convert the metabolic insults from NAFLD to transcriptional program that contributes to HCC development. Super-enhancers (SEs) are subclass of regulatory elements with unusually strong enrichment for the binding of transcriptional coactivators to fulfill cell identity. Currently, it has been proposed that dysregulation of SEs underlies the development of diseases including cancers, but the role of SEs in NAFLD-associated HCC is still unknown.

In order to investigate the alterations of histone modifications in NAFLD-associated HCC development, nanoscale chromatin immunoprecipitation sequencing of multiple histone marks were performed. By integrating 60 epigenomic profiles (including H3K4me1, H3K4me3, H3K27ac, H3K27me3 and H3K18ac) from primary NAFLD-associated HCCs and matched non-tumor tissues, we identified recurrent SEs enriched in chromatin regulators that contribute to fatty liver, lipid metabolism and metabolic syndrome. Interestingly, we observed significant enrichment of recurrent active SEs in chromatin regulators, including SIRT7, SUZ12, CBX8, DNMT3B, BRPF3, CBX4, DNMT3A. Co-immunoprecipitation and chromatin profiling indicated that the most significant chromatin regulator SIRT7 may coordinate with the Polycomb repressive complex to maintain tumor suppressor gene silencing. Notably, through CRISPR/Cas9-induced knockout liver cell lines, we showed that deletion of two distinct SE regions significantly reduced SIRT7 expression and attenuated the tumorigenic potential of HCC cells. In summary, our results show that the dysregulation of SEs contributes to chromatin regulator mis-expression in human hepatic carcinogenesis, thus representing novel therapeutic targets for NAFLD-associated HCCs.

*CBET-25*

**Hepatic cell cycle-related kinase (CCRK) shapes a metastatic-prone liver microenvironment via crosstalk between myeloid-derived suppressor cell (MDSC) and natural killer T cell (NKT)**

Xuezheng Zeng, Jingying Zhou, Zhewen Xiong, Hanyong Sun, Weiqin Yang, Wenshu Tang, Yu Feng, Alfred Sze-Lok Cheng

**Supervisor:** Prof. Alfred CHENG

Metastasis is a prominent cause of cancer-related death governed by both cancer cell-intrinsic mechanisms and extrinsic microenvironment. Clinical observations demonstrated liver as a common metastatic site for various cancers, which may be due to its immune tolerant environment. Myeloid-derived suppressor cell (MDSC) is a heterogeneous cell population of immature myeloid cells that contribute to the formation of a favorable metastatic environment partially via suppression of immune effector cells. However, the underlying mechanisms in liver tropism of tumor metastasis remain poorly understood. We have previously discovered that cell cycle-related kinase (CCRK) can promote local liver cancer hepatocellular carcinoma (HCC) development via MDSCs. Thus, we hypothesize that the accumulation of hepatic MDSCs induced by CCRK may contribute to the formation of a favorable metastatic liver microenvironment. By construction of a liver-specific CCRK inducible transgenic (TG) mouse model by a Cre/loxP system, induction of CCRK expression by tamoxifen injection could increase MDSCs, predominantly CD11b+Gr-1+Ly6G+Ly6Clow polymorphonuclear (PMN)-MDSCs liver accumulation specifically in male mice with upregulated Cxcl1 and Gcsf expression. Notably, intrahepatic injection of a mouse hepatoma cell line Hep1-6 in male TG mice developed larger tumors compared to control positively associated with increased PMN-MDSCs levels in liver, which was abolished by PMN-MDSC depletion. Notably, intrasplenic injection of a mouse melanoma cell line B16F10 exhibited enhanced liver metastasis in male TG mice compared to control mice, as shown by *in vivo* imaging. Moreover, enlarged liver size and tumor weight, as well as increased level of liver-infiltrating PMN-MDSCs were observed in TG mice, while further depletion of PMN-MDSCs suppressed metastasis in liver. Mechanistically, anti-tumor NKT cells rather than NK cells, CD8+T cells were negatively correlated with tumor weight and MDSC proportion, indicating that the cross-talk between MDSC and NKT might lead to liver metastasis. Our findings suggest that hepatic CCRK expression create a tumor growth- and metastasis-supportive liver microenvironment via enhancing immunosuppression. Additional colorectal cancer metastasis models will be evaluated in our established TG mice. Moreover, the roles and underlying mechanisms of CCRK-PMN-MDSC interactions in the establishment of liver metastasis await further investigation.

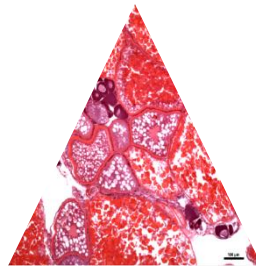
*CBET-26***Drug repositioning for schizophrenia and depression/anxiety disorders: A machine learning approach leveraging expression data**Kai Zhao, Hon-Cheong So**Supervisor:** Hon-Cheong So

Development of new medications is a lengthy and costly process, and drug repositioning might help to shorten the development cycle. We present a machine learning (ML) workflow to drug repositioning by predicting indication for a particular disease based on drug expression profiles, with a focus on applications in psychiatry. Drugs that are not originally indicated for the disease but with high predicted probabilities serve as candidates for repurposing. This approach is widely applicable to any chemicals or drugs with expression profiles measured, even if drug targets are unknown. It is also highly flexible as virtually any supervised learning algorithms can be used.

We employed the ML approach to identify repositioning opportunities for schizophrenia as well as depression and anxiety disorders. We applied, including deep neural networks (DNN), support vector machines (SVM), elastic net regression, random forest and gradient boosted trees. The predictive performance of the five approaches in cross-validation did not differ substantially, with SVM slightly outperforming the others. However, other methods also reveal literature-supported repositioning candidates of different mechanisms of actions. As a further validation, we showed that the repositioning hits are enriched for psychiatric medications considered in clinical trials. We also examined the correlation between predicted probabilities of treatment potential and the number of related research articles, and found significant correlations for all methods, especially DNN. Finally, we propose that ML may provide a new avenue to exploring drug mechanisms via examining the variable importance of gene features.

Keywords: anxiety disorder, drug repositioning, machine learning, major depressive disorder, schizophrenia.

# DEVELOPMENTAL AND REGENERATIVE BIOLOGY THEME



## Developmental and Regenerative Biology

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*DRB-1***Hyaluronic acid drives mesenchymal stem cell-derived extracellular matrix assembly by directly being involved in fibronectin fibrillogenesis.**

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**Supervisor:** Anna Blocki

Mesenchymal stem cells (MSCs) secrete a unique and very powerful panel of factors (extracellular matrix (ECM), including cytokines) that mediate pro-reparative and pro-regenerative processes. They are therefore employed in a wide range of cell-based therapies, also for the treatment of various ischemic diseases. Nonetheless, MSC-based therapy is facing many challenges, as the hostile inflammatory and ischemic environment in the affected tissue area impairs MSC integration, survival and hence therapy efficacy.

To overcome this limitation, we decided to directly engineer MSC-derived ECM in vitro, customized in its bioactivity to address the inflammatory and ischemic tissue environment. This approach allows to generate a bio-instructive matrix under controlled conditions to be directly applied in sufficient amounts into the affected areas.

We decided to harness the well-known pro-regenerative and anti-inflammatory bioactivity of high molecular weight hyaluronic acid (HA) by allowing MSCs to assemble their ECM in the presence of exogenously added HA. This prominent glycosaminoglycan of the ECM appears early in tissue regeneration. We therefore hypothesized that HA might also be involved in initial ECM assembly and therefore enhance the accumulation of MSC-derived ECM.

Human bone marrow MSCs deposited an ECM rich in fibronectin and collagen I over a time course of 2 to 6 days. The exogenously added HA significantly increased the amount of deposited fibrillar fibronectin in a dose-dependent manner. Confocal imaging confirmed a strong co-localization of fibronectin, HA and integrin  $\alpha 5$ , thus the presence of HA in fibrillar adhesions. Inhibition of HA synthesis resulted in a decrease of fibronectin fibril formation in the ECM, which could be partially rescued by exogenously added HA.

As initial fibronectin assembly is essential for the deposition of further ECM components such as collagen I, it is not surprising that we observed a HA dose-dependent increase in assembled collagen I in the MSC-derived in vitro ECMs.

The current results implicate HA to be a necessary component of fibrillar adhesions and involved in the cell-guided fibronectin fibrillogenesis process. As major constituents of provisional ECM in wound healing and early development, a HA and fibronectin enriched matrix derived from human MSCs exhibits great potential to be used as biomaterial for the treatment of ischemic diseases.

*DRB-2***BRE is essential to maintain proper cell cycle regulation and pluripotency in mESCs in responses to DNA damage**Chung Cheuk Yiu Tenny, Lo Hau Yi Paulisally, Lee Ka Ho Kenneth**Supervisor:** Professor Lee Ka Ho Kenneth

Pluripotent mouse embryonic stem cells (mESCs) can undergo self-renewal and long-term proliferation, together with the ability to differentiate into all cell types in the body. DNA damage can be generated naturally during proliferation or caused by external factors such as gamma irradiation. Inability to properly repair the DNA lesion will lead to genomic instability and mutations. Consequently, the build-up of mutation in mESCs may lead to metastasis and developmental defect. Hence, it is essential that the genetic integrity is maintained to prevent accumulation of mutation in mESCs.

mESCs have unique cell cycle regulation response which is essential to maintain their genetic integrity and pluripotency. Shortened G1 phase contributes to the rapid proliferation rate of mESCs. Absence of G1 checkpoint following DNA damage is essential to remove damaged cells by apoptosis and maintain pluripotency of the mESCs. While functional G2/M cell cycle checkpoint is important for mESCs to repair DNA damage and prevent acquisition of mutation during mitosis. In our study we found that BRE is essential for mESCs to escape from G1 checkpoint while activate G2/M cell cycle checkpoint following DNA damage. Meanwhile, p53 was found to be prolonged expressed in BRE<sup>-/-</sup> mESCs and inhibit Nanog expression. Consequently, lead to loss of pluripotency markers in BRE<sup>-/-</sup> mESCs days after gamma irradiation. In conclusion, abnormal prolonged of G1 phase together with the prolonged p53 expression in BRE<sup>-/-</sup> mESCs after DNA damage consequently lead to the loss of pluripotency in BRE<sup>-/-</sup> mESCs. Our results demonstrate the role of BRE in maintaining proper cell cycle regulation and pluripotency in mESCs in responses to DNA damage. It accounts for the low birth rate of BRE<sup>-/-</sup> mice as we observed.

*DRB-3*

**The role of Jmjd1a in neural stem cells and brain aging**

Gao Lin

**Supervisor:** Jiang Xiaohua

Jmjd1a regulates function of NSCs and homeostasis of the brain, which are highly related to brain aging and neurological diseases.

*DRB-4***The role and mechanism of zinc in regulating oocyte maturation in zebrafish**Duo Huang, Jianzhen Li, Christopher H.K. Cheng**Supervisor:** Prof. Christopher H.K. Cheng

Oocyte maturation represents primary resumption from prophase I to metaphase II. Oocyte maturation is tightly regulated by luteinizing hormone. Zinc is an essential element for normal function in reproduction, serving as both a structural component and a signaling molecule. Action of zinc on oocyte maturation seems to show species specificity. Insight into the mechanistic role of zinc in oocyte maturation is required to understand the complex regulation network of meiosis at molecular level and from an evolution aspect. Our preliminary data demonstrated that (1) both total and free zinc contents are increased during folliculogenesis; (2) zinc can promote oocyte maturation in a dose-, time- and stage-dependent manner; (3) the expression of zinc transporters is altered during folliculogenesis and oocyte maturation. For future studies, zinc dynamics during oocyte maturation will be assessed. Knockout zinc transporter fish will be constructed to identify the upstream regulators of zinc during oocyte maturation. Inhibitors of cAMP-signaling pathway will be employed to identify the downstream signaling pathway of zinc-mediated oocyte maturation.

**DRB-5****An effect of PROGRAMIN 2 to reverse the senescence of human umbilical cord perivascular stem cells (HUCPVSCs) and human fibroblasts**Grete Laane, Dr. Paulisally Lo, Prof. Kenneth Lee**Supervisor:** Prof. Kenneth Lee

Mesenchymal stem cells (MSCs) are one of the most attractive stem cell source for tissue engineering and cell therapy. For example, human umbilical cord perivascular stem cells (HUCPVSCs) possess a potential to self-renew and differentiate into various types of cells such as neuronal stem cell, osteoclasts and all the types of cells found in the blood. These cells are also easy to harvest and free from ethical complications, which is an advantage compared to the stem cells derived from the bone marrow or adipose tissue. Despite all the good qualities, HUCPVSCs age very fast, which limits their use in laboratory and tissue engineering in general. Therefore, it is crucial to enhance the proliferation capacity of these cells. The aim of this project is to test an ability of the small molecule PROGRAMIN 2 to reverse the senescence of HUCPVSCs. Those senescent cells were treated with PROGRAMIN 2 to compare the presence of various proliferation markers such as phosphor histone H3 (PH3). Results showed a higher expression of PH3 when senescent cells were treated with PROGRAMIN 2 for 1, 3, 5 and 7 days. Also the number of the cells had increased after the treatment with the small molecule. This indicates that PROGRAMIN 2 has potential to activate the proliferation in senescent cells. The same experiments were also performed on the human fibroblasts, which showed similar results proving the effect of the PROGRAMIN 2 also on the differentiated cells. Future investigations will involve the beta-galactosidase staining as a senescence marker and q-PCR analysis to test the expression of the genes related to the cellular proliferation and senescence such as Ki67, p21, p16 and BRE.

**DRB-6****Improving oocyte quality through mitochondrial transfer from mesenchymal stem cells.**

Wing-Tung Lee, Yan Qian, Jinyue Liao, Hoi-Ching Suen, Chun-Shui Luk, Ting-Hei Chan, Kin-Wing Ng, Tin-Lap Lee

**Supervisor:** Prof. Lee Tin Lap

It has established that advanced maternal age (AMA) is a key risk factor for infertility, poor oocytes quality, miscarriage and genetic disorders. In Hong Kong, it is expected that more than half of mothers in Hong Kong will be giving birth at the age of 35 or above soon. Despite in vitro fertilization (IVF) could increase the chance of pregnancy for AMA women, the failure rate remains high (25% to 50%). It was suggested that oogenesis and preimplantation development could be affected by mitochondrial content. Therefore, age-dependent changes of mitochondrion could contribute to the oocyte and embryo quality. Recently we have successfully discovered that mesenchymal stem cells (MSCs) could transfer mitochondria to mouse oocytes via tunneling nanotubes (TNT). The transfer led to significant improvement on mitochondrial function by 13.3% and maturation rate by 80.4% in mitochondrial defective oocytes model. We also tested the transfer effect on aging animal model which markedly rescued aged oocytes mitochondrial function by 56.6% and maturation rate by more than 200%. In conclusion, mitochondrial transfer from MSCs via TNT is a novel and natural therapy to improve aged oocyte quality. This drives us to develop an easy-to-use microfluidics platform to allow consistent and standardized autologous mitochondria transfer in IVM procedure in the future.

*DRB-7***RhoA/ROCK pathway regulates the migration of enteric neural crest cells in mice**Leung Cheuk Ling, Taida Huang, Yip Wing Ching, Chan Wood Yee**Supervisor:** Prof. Chan Wood Yee

The enteric nervous system is composed of cells arising from vagal and sacral neural crest cells. During embryonic development, vagal neural crest cells migrate from the proximal foregut to the distal hindgut whereas sacral neural crest cells migrate in an opposite direction from the distal end of the hindgut to the proximal hindgut. However, little is known about the molecular mechanism regulating the migration of these two groups of neural crest cells. In the present study, to identify signalling molecules that are involved in the enteric neural crest cell (ENCC) migration, we first performed transcriptomic analyses through RNA sequencing, and also immunofluorescence staining on ENCCs. Our results showed that molecules along the RhoA/ROCK pathway including RhoA, ROCK, MLCK and cofilin that are important for cytoskeletal modifications and cell motility in a number of migratory cell types were also found to be highly expressed in ENCCs. Using a Förster resonance energy transfer (FRET)-based biosensor, active RhoA was shown to be expressed at the leading edge of migrating ENCCs in vitro, implicating that RhoA may drive neural crest cells moving towards a particular direction. Moreover, glial cell-derived neurotrophic factor (GDNF) was also shown to be chemoattractive to migrating vagal ENCCs but not sacral ENCCs. In conclusion, our results indicated strongly that the RhoA/ROCK pathway is important in regulating the migration of enteric neural crest cells. Next, we plan to image enteric neural crest cells with the FRET-based biosensor in an organotypic gut culture ex vivo to further confirm the role of the RhoA signalling pathway ex vivo. ROCK inhibitor Y-27632 will also be used to evaluate the effects of the inhibition of the pathway on the expression of cytoskeletal proteins and the migratory behaviours of enteric neural crest cells.

*DRB-8*

## **Human fetal bone marrow-derived mesenchymal stem cells promotes the proliferation of pancreatic progenitor cells via insulin-like growth factor signalling**

Li Xing Yu, Wu Shang Ying, Leung Po Sing

**Supervisor:** Leung Po Sing

Pancreatic progenitor cells (PPCs) are the primary source for all pancreatic cells, including beta-cells and thus the proliferation and survival of PPCs offers an alternative to stem cell therapy for diabetic medicine. Meanwhile, mesenchymal stem cells (MSCs) with multipotentiality are procured from various sources and they possess the ability to enhance the proliferation of different cell types of interest. Despite this, the regulatory role of MSCs in the expansion and survival of PPCs remains poorly understood. One of the known morphogenic factors secreted from MSCs is insulin-like growth factor 1 (IGF1), which has been reported to modulate the growth of various progenitor cells. Against this background, we aimed to explore the mechanism-of-action whereby MSCs induce PPCs proliferation by means of our established co-culture system of human PPCs with human fetal bone marrow-derived MSCs.

Our results with BrdU assay and Ki67 immunofluorescent staining showed that MSCs conditioned medium (CM) was able to promote PPC proliferation, and that IGF1 gene expression levels were elevated in MSCs after co-culture. In addition, we observed an increase in the gene expression of IGF1 receptors in PPCs. Moreover, administration of IGF1 protein was found to accelerate the proliferation of PPCs in a dose dependent manner, as demonstrated by BrdU assay. Furthermore, we sought to further identify the potential role of IGF1 in our co-culture system. We thus employed PPP, an inhibitor of IGF-1 receptor, coupled with BrdU and Ki67 assays. Results revealed that PPP diminished the proliferative effects induced by the co-culture system. On the other hand, we observed a reduction of cell death in MSCs-CM group in relation to the starvation group, as evidenced by cell death Elisa kit. Consistently, Annexin V assay also detected apoptosis in serum-free conditions, which was confirmed by arrested cell growth in G0/G1 phase using flow cytometry; these effects were blocked by the MSCs-CM. Besides, protein expression of Bcl-2 was upregulated whereas BAX was downregulated by the MSCs-CM compared with the starvation conditions. Interestingly, we observed upregulated expression of phospho-Akt level under MSCs-CM condition compared with serum free group, which was confirmed with the RNA-seq data, suggestive of an involvement of PI3K-Akt signaling during PPC proliferation.

In conclusion, our data indicate that MSCs stimulate the proliferation of human PPCs, probably via the mediation of IGF1 and its related signaling pathway, and that our protocol may provide a mechanism-driven basis for the proliferation and differentiation of PPCs into clinically transplantable islets.

**DRB-9****Identification of essential factors in *Xenopus* heart regeneration**Lin Yijyun, Zhao Hui**Supervisor:** Zhao Hui

Cardiovascular diseases were ranked globally in Top 10 causes of deaths. Among 56.9 million deaths, 15.2 million deaths were caused by ischaemic heart disease and stroke in last 15 years. Cardiovascular diseases commonly caused myocardial damage. However, the mammal heart has limited ability on regeneration after myocardial damage. *Xenopus* is an amphibian species that phylogenetically closer to mammals but still remains its well regeneration ability in heart. To determine the reason as to how the *Xenopus* regenerate its heart, I setup a heart regeneration model by applying heart apical resection in *Xenopus*. I used the isobaric tags for relative and absolute quantitation (iTRAQ) mass spectrometry technique to find out the underlying mechanism of *Xenopus* heart regeneration. The iTRAQ-labeling technique could not only illustrate the protein activation or inactivation levels but could also quantify the protein expression through the isobaric labels. Furthermore, the GO analysis showed that the *Xenopus* heart could be repaired after 60 days from surgery. The top 15 pathways from day 1 to day 3 after surgery samples involved pathways such as neutrophil degranulation and apoptosis. The day 4 to day 16 pathway analysis indicated that the immune responses were reduced and translation related molecular activity was more abundant. In summary, the mass spectrometry results provided an insight into the molecular proteins level changes after *Xenopus* heart surgery. I will further focus on whether cardiomyocyte proliferation or diploidy/polyploidy phenotype contribute to the heart regeneration ability in *Xenopus* based on the mass spectrometry results.

*DRB-10***Mitochondrial biogenesis is a bottleneck of early embryonic development**Long Qi, Zhao Hui**Supervisor:** Zhao Hui

Quality control of embryonic development is an important event in reproduction process. There are three bottlenecks happened in oogenesis, fertilization and early embryonic development, which plays a key role in eliminating the embryos with genetic abnormality, development abnormality and environment abnormality. However, the role of mitochondria in this process remains obscure. To address this problem, we use morpholino targeting mitochondria biogenesis related genes and monitor the development abnormality of *Xenopus laevis* embryos. Our results show that mitochondrial biogenesis plays an essential role in controlling the development bottleneck during the gastrulation. Dysregulation of mitochondrial biogenesis will delay the gastrulation process, resulting in partial development failure and smaller body size. Our study suggests that the quantity of mitochondria replication, rather than ATP production rate of mitochondria, is a key factor for this bottleneck.

*DRB-11***Inducible CRISPR/Cas9 system for genome editing**MA Xun, HE Xiangjun, ZHANG Chenzi, TAN Chunlai, ZHANG Xueyan, FENG Bo**Supervisor:** FENG Bo

Genome editing by manipulating functional DNA sequences in host genome is a fundamental strategy for biomedical research. The Type II prokaryotic clustered regularly interspaced short palindromic repeats (CRISPR) system has open a new era for this field. The development of conditional genome editing methods give hope for understanding biological process in a particular tissue or at a specific time point. Here we report the development of an inducible method for CRISPR/Cas9 genome editing with 4-hydroxytamoxifen (4-OHT) by fusing the Cas9 enzyme with the hormone-binding domain of the estrogen receptor (ERT2). Our system provides an efficient, simplified method for conditional genome modification for both human and animal cells.

*DRB-12***Bufalin, a Traditional Chinese Medicine, for chemoprevention of colorectal cancer**Tung Him NG, Xiao SUN, Kathy W.Y. SHAM, William K.K. WU, Christopher H.K. CHENG**Supervisor:** Prof. Christopher H.K. CHENG

Despite the advancement in treatments against colorectal cancer (CRC), the clinical outcomes of patients with CRC, particularly patients with distant metastasis, remain dismal. Chemoprevention could be another strategy to reduce CRC incidences and related mortalities. Therefore, it is imperative to identify a promising chemopreventive agent to prevent this malignancy. Bufalin is a cardiac glycoside extracted from a traditional Chinese medicine Chansu. It has been adopted in clinical settings for cancer treatment with no significant sign of toxicity. Also, the use of cardiac glycosides was reported to be associated with reduced risks and recurrence of CRC. These evidences render Bufalin a potential candidate for CRC prevention. In this studies, the role of Bufalin in CRC chemoprevention is to be addressed. Colitis-associated CRC was induced by Azoxymethane (AOM)/Dextran Sulphate Sodium (DSS) administration in BALB/c mice. Mice were provided with Bufalin (0.5 mg/kg body weight) or vehicle every two day throughout the studies. After administration of Bufalin for 16 weeks, the mice exhibited a significant reduction in the number and size of colonic tumors as compared to mice treated with vehicle. Shortening of colon and enlargement of spleen after AOM/DSS treatment were also significantly alleviated after Bufalin treatment. Moreover, pro-inflammatory mediators including tumor necrosis factor-alpha, interleukin-6 and cyclooxygenase-2 were significantly downregulated in Bufalin treated mice. Furthermore, Bufalin promoted colon cancer cell apoptosis and suppressed proliferation. Taken together, our results indicated that Bufalin is protective against colitis-associated CRC induced by AOM/DSS treatment. Bufalin could potentially be adopted as a chemopreventive agent to prevent CRC.

*DRB-13***Endogenous enteric neurons in the hindgut are required for the normal colonization of transplanted enteric neural crest cells in the mouse**Tiantian PAN, Wood Yee CHAN**Supervisor:** Wood Yee CHAN

Hirschsprung's disease (HSCR) is a common congenital gut motility disorder characterized by the reduction or absence of enteric neurons in the distal colon. The Dominant megacolon (Dom) mouse, which carries a spontaneous mutation in Sox10, exhibits HSCR-like phenotypes, and has been used as an animal model of HSCR. In Dom embryos, the migration and differentiation of neural crest cells (NCCs) are abnormal, leading to hypoganglionosis (reduction of enteric neurons) and aganglionosis (absence of enteric neurons) in distal parts of the hindgut. However, it is still unknown whether these phenotypes exhibited by the Dom mouse are a result of abnormalities in NCCs only (cell-autonomous effects), alterations of the gut microenvironment (niche effects), or both (combinatorial effects). Our previous study has already shown that Dom hindgut segment explanted at E13.5 developed normally in an organotypic culture for up to 10 days. In the present study, Dom hindgut explants at E13.5 were used as recipient gut segments for transplantation of donor enteric neural crest cells to find out differences in the migration and differentiation of the donor cells. To obtain donor enteric neural crest cells (ENCCs), midgut segments were dissected from E12.5 transgenic mouse embryos in which all cells were ubiquitously labelled with GFP and cultured on a fibronectin-coated surface. GFP-labelled ENCCs emigrated from the midgut segments were collected one day later, sorted by FACs with p75 antibodies, and transplanted to the recipient hindgut explants, which were then cultured *ex vivo* for 5 days. We found that transplanted ENCCs survived, migrated, and differentiated into TuJ1-immunoreactive enteric neurons in normoganglionic and hypoganglionic segments of the hindgut explant. When transplanted to aganglionic segments of the hindgut, ENCCs were still able to differentiate but they were not able to form normal neuronal network as those found in normoganglionic and hypoganglionic hindgut segments. Our results indicated that endogenous enteric neurons were required for the proper engraftment and colonization of transplanted ENCCs within the hindgut explant.

*DRB-14***Generation of skeletal tissue on chips for personalized therapeutics**Bruce Tak Keung Pang<sup>1</sup>, Hon Son Ooi<sup>1</sup>, Megan Yi Ping Ho<sup>2</sup>, Chao Wan<sup>1</sup>

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**Supervisor:** Chao Wan

The life expectancy of human being is increasing with the advancement of medicine and biotechnology. Aging related diseases are challenging public health issues. Osteoporosis, diabetes associated bone loss and cancer bone metastasis are among those severe diseases affecting the skeleton in the elderly. Statistically, at least one third of women and one fifth of men over 50 year-old suffer from osteoporosis around the world and it is defined by the international osteoporosis foundation as a global serious health concern. The current therapies for those skeletal diseases remain unsatisfactory. There are significant clinical demands for discovery of novel therapeutic approaches for those devastating skeletal diseases. With the recent development of bioengineering and biomedicine, more attention is paid on novel research platforms such as 3D skeletal tissue models that could be used for personalized drug screen or regenerative therapeutics. Here, we aim to develop a novel approach in establishing the osteocyte chip system to speed up the process in understanding the mechanisms of bone metastasis, discovering new drugs and improving the efficacy of the drugs by boosting up the screening progress. We have created the osteon-mimicking osteocyte chips by newly developed methods, and successfully created the osteon mimicking pattern in silicon wafer and PDMS mold by soft lithography. Next, we developed a novel approach that could transfer the high resolution concentric osteon-simulating pattern into biocompatible hydrogel in a clear and sharp manner. We established a specialized cell sedimentation method in patterning and localizing the MC3T3E1 cells to form accurate osteon concentric pattern in the hydrogel chips. The live/dead cell staining indicated that the high cell viability was preserved by using this patterning method. The proliferation of MC3T3E1 cells embedded in the osteocyte chips was well-maintained as revealed by the alamar blue assay. Furthermore, the expression of osteogenic marker genes including Runx2, Osterix and Col1 $\alpha$ 1 was shown to be upregulated following culture. Thus, we have established a novel method that can fabricate the osteocyte chips with high cell viability, cell proliferation and satisfied osteogenic differentiation potential. We expect that the skeletal tissue on chips will serve as a 3D physiologically-relevant model to facilitate the drug screen or diseasing modeling for the skeletal tissue.

*DRB-15***Transplantation of Retinal Ganglion Cells derived from Male Germline Stem Cell into Glaucoma Mice**Hoi Ching SUEN, Yan QIAN, Jinyue LIAO, Chun Shui LUK, Wing Tung LEE, Tin-Lap LEE**Supervisor:** Prof Lee Tin-Lap

Glaucoma is characterized by retinal ganglion cell (RGC) degeneration and is the leading cause of blindness worldwide. However, current treatments such as eye drops or surgery are limited and do not target the loss of RGC. Regenerative therapy using embryonic stem cells (ESCs) holds a promising option but ethical concern hinders clinical applications on human subjects. We hypothesized that RGCs could be generated from mouse spermatogonial stem cells (SSCs) as regenerative treatment for glaucoma. We first collected SSCs from mouse testes and they were subsequently transdifferentiated to ESC-like cells (SSC-ESCs) through a non-viral and non-invasive approach. SSC-ESCs exhibited stem cell properties similar to ESCs in terms of morphology and pluripotency gene expression profile. These cells then underwent retinal differentiation to differentiate towards retinal lineages and developed optic vesicles in 7-10 days in in vitro culture, while some cells generated neurites in 13-16 days. These neuron-like cells were positive for RGC-specific marker *Brn3b* and other neural markers, thus confirming their RGC identity. SSC-ESC-derived RGCs exhibited response to light in microelectrode array (MEA). To enable efficient isolation of RGCs by fluorescence-activated cell sorting (FACS), we generated *Brn3b*-GFP reporter SSC-ESCs. GFP<sup>+</sup> RGCs were isolated by FACS and able to survive in subsequent in vitro culture. We also transplanted SSC-ESC-derived RGCs to drug-induced glaucoma animal model and observed their survival in vivo in host retina. SSC-ESC-derived RGCs can thus potentially be a novel alternative to replace the damaged RGCs in glaucomatous retina.

*DRB-16***In vitro 3D liver model utilizing iPSC induced hepatocytes for drug testing**HY Tsang, HY Lo, KK Lee**Supervisor:** Professor Lee Ka Ho Kenneth

Liver is responsible for the detoxification in our bodies, and it is exposed to different threats in everyday life. Despite of the strong regenerating property of the organ, when the exposure of toxic substances is prolonged and beyond its limits, the damage could be irreversible, as the extracellular matrix proteins which are supposed to heal wound over accumulates, forming extensive scar tissues in the liver. Indeed, chronic liver diseases including liver cirrhosis have been one of the highest leading causes of death in Hong Kong and in other developed countries for these decades. At this moment, no cures can yet be found for liver cirrhosis. One major challenge in the drug discovery process is the lack of representativeness in the in vitro cell model which fails most of the pre-clinical trials, as liver cells growing on a petri dish do not resemble the biological responses of their in vivo counterparts. To better mimic the human microenvironment, we adopted the 3D bio-printing technique, where cells were printed in bioink made of alginate and gelatin. In our study, we found that liver cells show higher proliferation and functionality in 3D bioink scaffold, as the cell-cell interactions and cell-ECM interactions are regained. On the other hand, functional hepatocytes were successfully generated from human iPSC, using cheaper small molecules method instead of the traditional expensive growth factor protocol. We hope to provide novel insights into the personalized drug testing model with the 3D bio-printed iPSC induced hepatocytes.

*DRB-17***Psoralen exerts distinct mode of actions in regulating osteoblast and osteoclast differentiation**WANG YANYAN**Supervisor: WAN CHAO**

Osteoporosis is the most common metabolic bone disease characterized by a decrease in bone mass and deterioration in bone microarchitecture, leading to bone fragility and high risk of fractures. In recent years, more attentions are paid to plant-based therapies including traditional Chinese medicines (TCM) to treat osteoporosis. Psoralen (PS), a coumarin derivative compound, is the main active ingredient extracted from *Psoralea corylifolia* L. fruit which is one of the widely used herbs in Chinese formulas to treat osteoporosis. However, the underlying cellular and molecular mechanisms of PS on osteoblast and osteoclast function remain poorly understood. In this study, we showed that PS enhanced MC3T3-E1 pre-osteoblast cell differentiation and calcified nodule formation as indicated by Alkaline Phosphatase (ALP) and Alizarin Red S staining, respectively. PS also up-regulated the mRNA expression of osteogenic marker genes such as Runx2, Osterix (OSX), ALP, Osteopontin (OPN), Osteocalcin (OCN) detected by Real-time PCR. This was associated with up-regulation of HIF-1 $\alpha$ . In addition, tartrate-resistant acid phosphate (TRAP) staining showed that PS inhibited bone marrow mononuclear cells (BMMNCs) differentiation into osteoclasts. Real-time PCR showed that PS suppressed the mRNA level of TRAP, Calcitonin receptor (CTR), Nuclear factor of activated T-cells c1 (NFATC1), Cathepsin K (CtsK), Carbonic anhydraseII(CAII) and c-Src. Protein level of NFATC1, the key transcription factor of osteoclast differentiation, was shown to be downregulated by PS. Our preliminary data suggest that PS might promote bone formation associated with upregulation of HIF-1 $\alpha$  in osteoblasts while inhibit osteoclastogenesis through downregulation of NFATC1 during osteoclast differentiation that deserves further investigation.

*DRB-18***Role of igf3 on gonad development and sex reversal in zebrafish**Yuxin Xie, Lianhe Chu, Yun Liu, Xiao Sun, Jianzhen Li, Christopher H.K. Cheng**Supervisor:** Christopher H.K. Cheng

The insulin-like growth factor (IGF) system plays a fundamental role in several physiological processes in vertebrates. Distinct from the conventional IGF1 and IGF2 which are expressed in various tissues of a number of species, a gonad-specific subtype igf3 has been identified in teleost by our group previously. Due to this characteristic, role of igf3 on fish reproduction has been intensively studied in different fish types. However, all the existing studies were based on in vitro experiments. In order to investigate its function more comprehensively and thoroughly, in vivo igf3 mutant model is urgently needed.

Using transcription activator-like effector nucleases (TALENs)-mediated gene knockout, we successfully disrupted Igf3 in zebrafish. Interestingly, all the adult mutants are males. Since the mechanism of zebrafish sex differentiation has long been unsolved, it is promising that igf3 might be an important gene in this process. We first showed that the igf3 expression level in fish individuals was increased from 5 days post fertilization (dpf) to 60 dpf, and spontaneously split into a lower group and a higher group starting from around 24 to 26 dpf, also known as the sex decision stage of zebrafish. Moreover, the expression of igf3 at the sex decision stage seemed to be correlated with the sex-specific markers, *cyp19a1a* for females and *amh* for males, suggesting that the differential expression of igf3 might be associated with gender. And for spatial expression, we detected Igf3 in the germ cells of developing gonads by immunostaining.

Then we investigated the functional role of igf3 by tracking gonad development in wild-type and igf3 knockout fish through histology. Intriguingly, at 45 dpf, majority of igf3 knockout males appeared delayed spermatogenesis, while the gonads of all igf3 mutant females were ovotestes, which is a sign of sex reversal. Later at 60 dpf, spermatogenesis of mutant males came back to normal, whereas ovarian development of igf3 knockout females was further terminated as marked by degenerative ovaries. We also demonstrated that igf3 mutants display an abnormal decrease of germ cell proliferation at 30 dpf, which could probably compromise oocyte survival and cause female-to-male sex reversal. We will then construct igf3 mutant rescue line in the near future.

*DRB-19***Cytosolic Interactome of KDM3A and Its Role in Bone Regeneration**XUE Shaolong**Supervisor:** Jiang Xiaohua

Kdm3a is a crucial histone lysine demethylase which was previously found to regulate numerous biological processes. However, the current studies only addressed its demethylase domain (C-terminal jmjC domain), which acted as a nuclear transcriptional co-activator and histone modulator. Our previous studies have found a trace existence of KDM3A in the cytosol of multiple cell lines and tissues, especially the bone-derived MSC cells. This particular adult stem cells are largely responsible for the critical bone regeneration balance, which is disrupted in Kdm3a KO mice and resulted in a severe osteoporosis phenotype in their femur and tibia. Thus, we verified the genuine existence of Kdm3a in multiple human primary cells and well-established cell lines. Meanwhile, we are trying to map its interactome with the Virotrap to decypher KDM3A's role in regulating bone MSCs' function which may attribute to the bone regeneration.

*DRB-20***The effects of alcohol drinking on the expression and function of FGF21 in pancreatic islets of type 2 diabetes**Baochen Yang, Yi Wang and Po Sing Leung**Supervisor:** Leung Po Sing

Fibroblast growth factor 21 (FGF21) is a potent metabolic regulator for lipid and glucose homeostasis; it is primarily expressed in the liver but is also found in adipose tissue and the pancreas. In view of this fact, FGF21 has pharmacotherapeutic value for obesity-related type 2 diabetes mellitus (T2DM) and metabolic syndromes. It is known that chronic heavy ethanol consumption increases the risks of T2DM, via increased insulin resistance and  $\beta$ -cell apoptosis. In this context, recent studies have shown that FGF21 has an indirect action on the alcohol metabolism, affecting gastric emptying rate and initial alcohol metabolism. Meanwhile, convergent data have also shown that circulating FGF21 levels are markedly increased by acute and sub-chronic intake of alcohol in humans and mice. In light of these findings, we thus hypothesize that the pattern of alcohol drinking modulates the expression and function of FGF21 in islets and obesity-induced T2DM .

In order to test the hypothesis, we sought to use MIN 6 cell line ( $\beta$ -cell line) and high-fat-diet (HFD) induced T2DM mice as well as isolated human islets. Our results showed that the insulin gene expression was downregulated under binge drinking condition. In addition, the mRNA expression levels of FGF21,  $\beta$ -klotho, and FGF receptors (FGFR1, FGFR2, FGFR3 and FGFR4), as well as FRS2 protein phosphorylation in MIN6 cells were significantly upregulated. In corroboration, consistent results were also observed in HFD induced T2DM mice under binge alcohol condition. Furthermore, similar results were found in normal human islets. In conclusion, our data indicate that exposure to high concentration of alcohol may lead to  $\beta$ -cell dysfunction, probably via impaired FGF21 signalling in islets, and that FGF21 signaling has a role in regulating alcohol metabolism and islet function .

*DRB-21***Complete disruption of *ULK1*, *FAT10* and *CtIP* genes by homology-independent multiallelic knock-in yielded distinct functional outcomes**

Chenzi Zhang<sup>1</sup>, Xiangjun He<sup>1</sup>, Yvonne K Kwok<sup>2</sup>, Feng Wang<sup>1</sup>, Hui Zhao<sup>1,8</sup>, Kin Wah Suen<sup>2</sup>, Chi Chiu Wang<sup>2,3</sup>, Jianwei Ren<sup>4</sup>, George G. Chen<sup>4,6</sup>, Paul B. S. Lai<sup>4,5,6</sup>, Yin Xia<sup>1</sup>, Andrew M Chan<sup>1,8</sup>, Wai-Yee Chan<sup>1,7,8\*</sup>, Bo Feng<sup>1,7,8\*</sup>

1 Key Laboratory for Regenerative

**Supervisor: FENG BO**

**Background:**

Cultured human cells are pivotal models to study human gene functions, but targeted gene disruption for complete loss-of-function in diploid or aneuploid cells has been a challenge. The recently developed CRISPR/Cas9-mediated homology-independent DNA knock-in approach permits targeted insertion of large DNA at high efficiency, thus providing a promising tool. Using this approach, studies have reported complete gene disruption for a few selected genes, but functional outcomes have not been examined. Hence, we took advantage of the promoterless fluorescence reporter systems established in our recent study, and investigated functional outcomes after gene disruption.

**Results:**

Exemplified by using hyperploid human cell line LO2, we demonstrated that simultaneous knock-in of dual reporters through CRISPR/Cas9-induced homology-independent DNA repair permits one-step generation of cells carrying complete gene disruption at multiple alleles. Through knocking-in large DNA fragments at a coding exon, we generated stable single-cell clones carrying complete disruption of all four copies of *ULK1* gene, lacking all three copies of intact *FAT10* gene, or devoid of intact *CtIP* gene at both alleles. Importantly, we fully confirmed the depletion of *ULK1* and *FAT10* transcripts as well as corresponding proteins; and in subsequent functional analysis of the *ULK1*<sup>-/-</sup> and *FAT10*<sup>-/-</sup> cell clones, we observed defect in mitophagy and cytokine-induced cell death, respectively; which are consistent with previous reports.

Rather interestingly, despite the complete disruption of *CtIP* at both alleles was successfully achieved, the cell clones obtained preserved in-frame aberrant *CtIP* transcripts and produced proteins. Strikingly, the *CtIP*-disrupted clones raised through another two distinct targeting strategies also carried varied but in-frame aberrant *CtIP* transcripts. Sequencing analysis suggested that diverse DNA processing and alternative RNA splicing were involved in generating the various in-frame aberrant *CtIP* transcripts, and some minor events were biasedly enriched among the *CtIP*<sup>-/-</sup> cell clones.

**Conclusion:**

Multiallelic gene disruption could be readily introduced through CRISPR/Cas9-induced homology-independent knock-in of dual fluorescence reporters followed by direct tracing and cell isolation. Robust cellular mechanisms exist to spare essential genes from loss-of-function modifications, by generating partial functional transcripts through diverse DNA and RNA processing mechanisms.

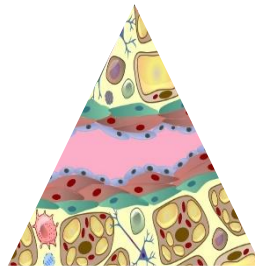
*DRB-22***Role of Follistatin-like 1 (FSTL1) in kidney fibrosis**ZHANG Yu, HUANG Huihui, WANG Yang, XIA Yin**Supervisor:** XIA Yin

Kidney fibrosis is a final common pathway of progressive chronic kidney disease, and is characterized by the increase of interstitial fibroblasts and myofibroblasts, and the excessive production and accumulation of extracellular matrix (ECM) proteins within the kidney. Wnt / $\beta$ -catenin signaling has been found to promote renal fibrosis by facilitating tubular epithelial-mesenchymal transition (EMT) and promoting myofibroblast differentiation, proliferation and function. Follistatin-like 1 (FSTL1) is a member of the follistatin family, characterized by the FS domain found in follistatin. FSTL1 has been studied in heart injury, lung development and injury, arthritis, and wound healing. The roles of FSTL1 in kidney are largely unknown. In the present study, we showed that FSTL1 expression in the kidney was dramatically increased 1, 3 and 7 days after unilateral ureteral obstruction (UUO), while it was only slightly increased by ischemia/reperfusion (I/R) and cisplatin nephrotoxicity in mice. Immunofluorescent staining showed that FSTL1 expressed in fibroblasts in the kidneys with UUO. Blockage of FSTL1 with a neutralizing anti-FSTL1 antibody aggravated epithelial injury and renal fibrosis after UUO. In addition, we found that neutralization of FSTL1 enhanced Wnt- $\beta$ -catenin signaling. Therefore, our study suggested that FSTL1 inhibits kidney fibrosis by suppressing canonical Wnt signaling.

*DRB-23***Molecular control of hypoxia regulation on type II collagen secretion by chondrocytes**ZHAO Yichen, Wan Chao**Supervisor:** Prof. Wan Chao

Type II collagen (Col II) is a major component of the extracellular matrix (ECM) produced by chondrocytes. The regulation of Col II production and secretion of chondrocytes involves in multiple factors including hypoxia, mechanical force, inflammation and aging. Hypoxia inducible factor- $\alpha$  (HIF- $\alpha$ ) is the key transcription factor that regulates genes related to chondrogenesis under hypoxic microenvironment. However, the molecular mechanisms of hypoxia/HIF- $\alpha$  regulation on Col II secretion remain unclear. In this study, we found that hypoxia decreased Col II secretion in the supernatant while increased its accumulation in the cytosol of chondrocytes compared with that under normoxic conditions. Col II protein level in the lysosome isolated from chondrocytes under hypoxia was elevated than that under normoxia. The phenotype was accompanied by alterations of a panel of marker genes or proteins of autophagosome and lysosome formation. The autophagosome marker LC3 and the lysosome marker LAMP1 and cathepsin D (CtsD) were shown to be upregulated in chondrocytes under hypoxia compared with that under normoxia. The numbers of autophagosome and lysosome were increased in chondrocytes under hypoxia than that under normoxia. In addition, we showed that the transcription factor EB (TFEB), a key regulator of lysosomal biogenesis, was transcriptional regulated by HIF-1 $\alpha$  and HIF-2 $\alpha$  through distinct transcriptional binding sites in its promoter region, indicating that HIF- $\alpha$  involves in the regulation of lysosome biogenesis. Interestingly, Col II secretion in the supernatant of chondrocytes with lysosome inhibition was decreased compared with the controls. Our results suggest that hypoxia regulates the secretion of Col II by chondrocytes through the autophagy-lysosomal pathway, and the normal lysosome function is required by Col II secretion.

NEURAL, VASCULAR, AND  
METABOLIC BIOLOGY THEME



## Neural, Vascular, and Metabolic Biology

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*NVMB-1***The GHRH-R signaling pathway in modulating cell death of retinoblastoma cells**CHEN Binbin, YU Qiuxiao, LIANG Weicheng, REN Jialin, LEUNG Pui-Ying, CHAN Sun-On**Supervisor:** Prof. CHAN SUN-ON

Retinoblastoma (RB) is a rare hazardous cancer of retina in children, and the incidence is nearly 4% among all pediatric cancers. It has been previously reported that the receptor for growth hormone-releasing hormone (GHRH-R) is specifically and highly expressed in RB. GHRH-R antagonists, MIA-602, has been shown to inhibit proliferation and induce apoptosis in RB cells. In our study we chose cell line of retinoblastoma cells (Y79), and retinal pigmented epithelial cells (ARPE-19) as control, to identify the signaling mechanism mediated by MIA-602. After examining the change of p-mTOR, p-AKT, Caspase-3, Caspase-8, Caspase-9, and cytochrome c, we found that the mTOR-AKT and the intrinsic apoptotic signaling pathways play an important role in the MIA-602 induced cell death. Besides that, we also used PrePPI and FpClass prediction software to identify protein candidates that interact potentially with GHRH-R. Using immuno-precipitation (IP), we found an interaction between GHRH-R and insulin receptor substrate 1 (IRS1), suggesting a potential role of IRS1 in the GHRH-R mediating cell survival. We are currently investigating the function of IRS1 on modulating the signaling pathways in MIA-602 mediated cell death in retinoblastoma cells.

*NVMB-2***Reduction of endoplasmic reticulum stress through AMPK activation mediates endothelial benefits of GLP-1R agonist exenatide against hyperhomocysteinemia**CHENG Chak Kwong, LAU Chi Wai, TIAN Xiao Yu, HUANG Yu**Supervisor:** Prof. HUANG Yu

Hyperhomocysteinemia (HHcy) is an independent risk factor for both cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM). Homocysteine (Hcy) can induce endoplasmic reticulum (ER) stress to injure endothelial cells, resulting in endothelial dysfunction. Glucagon-like peptide 1 (GLP-1) analogue exenatide was shown to ameliorate ER stress but its detailed mechanism remains to be investigated. Therefore, the present study focuses upon how exenatide lowers ER stress in endothelial cells against high Hcy. Isolated aortae of wild-type mice were pre-incubated with exendin-4 (Ex4, a form of exenatide), followed by the acute exposure to Hcy (300  $\mu\text{mol/L}$ ). Mice were fed with high methionine low folate (HMLF) diet to induce HHcy and these mice were chronically treated with Ex4 via subcutaneous injection (1 nmol/kg) for 4 consecutive weeks. Vascular reactivity of arteries was examined in myograph, The reactive oxygen species (ROS) level in arteries was assessed by both confocal microscopy of dihydroethidium (DHE) staining and lucigenin-enhanced chemiluminescence method. Protein and mRNA expression levels of the signaling pathway were measured by Western blotting and RT-PCR, respectively, in both human umbilical vein endothelial cells (HUVECs) and diet-induced HHcy mice. Both acute and chronic Ex4 treatments improved endothelium-dependent relaxations in response to acetylcholine in Hcy-treated arteries and in arteries from HHcy mice. Ex4 treatment decreased the levels of ER stress markers, phosphorylated eIF2 $\alpha$ , spliced XBP-1 and ATF6. In addition, exenatide can promote proper protein folding in ER to limit Hcy-induced ER stress, through the activation of AMP-activated protein kinase (AMPK). The present results shed light on the therapeutic potential of exenatide against CVD and/or T2DM in association with hyperhomocysteinemia.

*NVMB-3***The role of interactions of platelets with brain-specific neuronal glycolipids in the modulation of neuronal functions during neurological disorders**Ekaterina Kopeikina**Supervisor:** Prof. Eugene Ponomarev

In the intact CNS platelets are separated from the neurons and glial cells by the blood-brain barrier (BBB) structure. However, during pathological condition such as the trauma, infection, neurodegeneration, inflammation BBB become compromised leading to direct interaction of neurons with platelets. We have previously found that brain-specific sialated gangliosides within neuronal lipid rafts directly activated platelets. Here we plan to investigate the possible role of platelet-lipid raft interactions in the modulation of neural activity in mouse model of epilepsy. We found that the seizure durations were significantly reduced in wild-type (WT) mice with depleted platelets. At the same time, ST3-deficient, which lack of brain-specific gangliosides in neuronal lipid rafts, had lower levels of peak seizures with lower mortality rate when compared with WT controls. The activity of neurons increases when neuronal cells are co-incubated with platelets, both in primarily neural culture and in brain slice organotypic cultures. Thus, we expect to elucidate particular molecular mechanisms how integration of platelets with neuronal lipid rafts modulate neuronal functions and promote seizures.

*NVMB-4***KLF2 Suppresses Vascular Calcification through Inhibition of Endothelial BMP/Smad Pathway**HUANG Juan, LUO Jiangyun, HUANG Yu**Supervisor:** Huang Yu

Vascular calcification is a common vascular complication of diabetes, fibrotic renal diseases and atherosclerosis, and is associated with an increased risk of cardiovascular mortality. The bone morphogenetic proteins (BMPs) have been implicated as mediators of calcification in the vascular wall. However, the regulatory mechanism of BMP/Smad pathway in the progression of vascular calcification is largely unknown. Here, we show that KLF2, a transcription factor induced by athero-protective shear stress, negatively regulates BMP/Smad pathway. Specifically, KLF2 knockdown in human umbilical vein endothelial cells (HUVECs) increases expression levels of BMP2/4/6, total and phosphorylated Smad1 and Smad5, and decreases expression of Smad6 (an inhibitory Smad). By contrast, KLF2 overexpression downregulates expression of BMP2/4/6 and Smad1, and upregulates Smad6 expression. In addition, KLF2 overexpression also induces the expression of BMPER that functions as an endothelial BMP antagonist. Endothelial cells are constantly exposed to mechanical forces generated by blood flow. Different flow patterns induce distinct cellular responses. Disturbed flow (DF) induces vascular inflammation and promotes atherogenesis, while laminar shear stress (LSS) produces anti-inflammatory and athero-protective effects. We found that LSS decreases expression of BMP4 and increases expression of BMPER and Smad6, suggesting an inhibition of BMP/Smad signaling. Moreover, KLF2 silencing using shRNA abolishes the inhibitory effect of LSS on the expression of BMP4, BMPER and Smad6, suggesting that KLF2 is likely to mediate the suppressive effect of LSS on BMP pathway. On the other hand, DF decreases BMPER and increases BMP4 and p-Smad1/5, suggesting an activation of BMP/Smad signaling. KLF2 overexpression reverses the activation BMP/Smad signaling induced by DF. Taken together, our present study suggests that targeting the KLF2-BMP/Smad signaling cascade may hold promise as a novel drug target against vascular calcification.

*NVMB-5***KLF2 Suppresses Vascular Calcification through Inhibition of Endothelial BMP/Smad Pathway**Juan Huang, Jiang-Yun Luo, Yu Huang**Supervisor:** Yu Huang

Vascular calcification is a common vascular complication of diabetes, fibrotic renal diseases and atherosclerosis, and is associated with an increased risk of cardiovascular mortality. The bone morphogenetic proteins (BMPs) have been implicated as mediators of calcification in the vascular wall. However, the regulatory mechanism of BMP/Smad pathway in the progression of vascular calcification is largely unknown. Here, we show that KLF2, a transcription factor induced by athero-protective shear stress, negatively regulates BMP/Smad pathway. Specifically, KLF2 knockdown in human umbilical vein endothelial cells (HUVECs) increases expression levels of BMP2/4/6, total and phosphorylated Smad1 and Smad5, and decreases expression of Smad6 (an inhibitory Smad). By contrast, KLF2 overexpression downregulates expression of BMP2/4/6 and Smad1, and upregulates Smad6 expression. In addition, KLF2 overexpression also induces the expression of BMPER that functions as an endothelial BMP antagonist. Endothelial cells are constantly exposed to mechanical forces generated by blood flow. Different flow patterns induce distinct cellular responses. Disturbed flow (DF) induces vascular inflammation and promotes atherogenesis, while laminar shear stress (LSS) produces anti-inflammatory and athero-protective effects. We found that LSS decreases expression of BMP4 and increases expression of BMPER and Smad6, suggesting an inhibition of BMP/Smad signaling. Moreover, KLF2 silencing using shRNA abolishes the inhibitory effect of LSS on the expression of BMP4, BMPER and Smad6, suggesting that KLF2 is likely to mediate the suppressive effect of LSS on BMP pathway. On the other hand, DF decreases BMPER and increases BMP4 and p-Smad1/5, suggesting an activation of BMP/Smad signaling. KLF2 overexpression reverses the activation BMP/Smad signaling induced by DF. Taken together, our present study suggests that targeting the KLF2-BMP/Smad signaling cascade may hold promise as a novel drug target against vascular calcification. (Supported by RGC-CRF and RGC-GRF grants)

**NVMB-6****Myeloid Bmal1 Deletion Promotes Vascular Remodeling in Hypertension**

HUO Mingyu, ZHANG Hongsong, LAU Chi Wai, TIAN Danyang, WU Yalan, Ajay Chawla,  
HUANG Yu, TIAN Xiao Yu

**Supervisor:** HUANG Yu

**Background**

The molecular clock plays an essential role in regulating cardiovascular function and immune responses through rhythmic expression of clock-controlled transcripts and their biological functions. Our previous study showed that myeloid-specific deletion of Bmal1 promotes atherosclerosis in ApoE<sup>-/-</sup> mice by enhancing monocyte trafficking to plaques. The role of Bmal1 in vascular inflammation in hypertension is unknown.

We propose that Bmal1 contributes to vascular inflammation and immune cell infiltration in the adventitia thus accelerates hypertension; while also promotes renal inflammation and dysfunction.

**Methods**

We use the Bmal1FloxP/FloxP (Bmal1MWT) as control and Bmal1FloxP/FloxP;LysMCre/+ (Bmal1MKO) as myeloid-specific Bmal1-deficient mice housed under 12 hour light/dark cycle at 22°C. Hypertension was induced by angiotensin II infusion via osmotic pump for 28 days and blood pressure was measured using CODA tail-cuff system and implantable radio telemetry.

**Results**

We found higher systolic blood pressure (131.5±7.2 vs 117.8±9.7mmHg) in Bmal1MKO compared to Bmal1MWT mice after angiotensin II infusion. Flow cytometric analysis showed more total macrophages (CD11b+F4/80+) and more Ly6c<sup>+</sup> macrophages in the kidney from Bmal1MKO mice. Endothelium-dependent vasodilation in mesenteric arteries and endothelium-dependent contraction in carotid arteries were further impaired in hypertensive Bmal1MKO mice, indicating Bmal1 deletion promotes hypertension-induced vascular dysfunction. Masson's trichrome staining of aorta showed thicker tunica media and higher media-to-lumen ratio with more macrophages infiltration in the adventitia in Bmal1MKO mice after angiotensin II infusion. The mRNA expressions of MMP9/13 in aorta from Bmal1MKO mice were much higher, meaning that imbalance of extracellular matrix induced by upregulation of MMPs played important roles in vascular remodeling and dysfunction.

**Conclusions**

Our results indicate myeloid Bmal1 deletion exacerbates hypertension and hypertension-induced vascular inflammation. Further experiments will be carried out to examine the mechanisms between Bmal1 and MMPs in the development of vascular remodeling in hypertensive mice.

Supported by China NSFC 9173910019, Hong Kong Health Bureau HMRP-RFS 01150057

NVMB-7

**The Effect of Pre-gestational Type 1 Diabetes on Fetal Programming of Defective Brown Adipose Tissue in the Offspring**

LIN Kenneth N., LEE Leo M.Y., WANG Chi Chiu, SHUM Alisa S.W.

**Supervisor:** Prof. SHUM Alisa

Offspring of mothers with type 1 diabetes mellitus (DM) are more prone to develop obesity and type 2 DM. Recent studies show that brown adipose tissue (BAT), which serves to regulate body temperature in the newborn, is important in adults for preventing obesity via dissipating energy as heat. This study aims to investigate our hypothesis that maternal diabetes impairs fetal BAT development, such that offspring will be more inclined to develop obesity in later life.

A streptozotocin-induced type 1 diabetic mouse model was used. Pregnancies were obtained by mating non-diabetic male with diabetic or non-diabetic female mice. We found that in near-term (gestational day 18) fetuses of diabetic mice, the ratio of BAT weight to body weight was significantly reduced. Oil red O staining of fetal BAT showed a prominent reduction in lipid accumulation, which was further confirmed by triglyceride content measurement. Mitochondria in BAT are critical for its thermogenic function. Results of staining fetal brown adipocytes with MitoTracker Red and measurement of mitochondrial DNA copy number revealed a marked reduction in mitochondrial mass. Notably, prominent upregulation of mRNA and protein levels of uncoupling protein 1 (Ucp1), the specific protein for BAT thermogenic process via uncoupling fuel oxidation from ATP synthesis, normally occurring prior to birth was markedly suppressed, which implicates impaired BAT thermogenic functions. Indeed, newborns of diabetic mice had a significantly lower body temperature. Despite a lower birthweight, the offspring of diabetic mice underwent rapid catch-up growth when fostered by a non-diabetic mother. Adult offspring of diabetic mice exhibited reduced ability to upregulate BAT-specific genes including Ucp1 and maintain body temperature in the cold tolerance test. When challenged with a high-fat diet after weaning, they accumulated more subcutaneous and visceral fat. Moreover, they were more insulin resistant and glucose intolerant, and showed higher incidences of diabetes.

Retinoic acid (RA) signalling plays important roles in the development of multiple organs. We have previously found that RA levels are significantly decreased in embryos of diabetic mice. To investigate whether there is any causal link between reduced RA signalling and impaired fetal BAT development in diabetic pregnancy, first, we treated pregnant non-diabetic mice with bisdiazine, a potent inhibitor of the RA synthesizing enzyme. Perturbation of RA signalling led to a decrease in lipid accumulation, mitochondrial mass and Ucp1 levels in the BAT of fetuses of non-diabetic mice. These changes were highly similar to those observed in fetuses of diabetic mice. On the contrary, maternal supplementation of RA could significantly increase lipid accumulation and Ucp1 levels in the BAT of fetuses of diabetic mice. Together, these findings suggest that perturbation of RA signalling may be a critical mechanism contributing to impaired BAT development in fetuses of diabetic pregnancy.

To conclude, offspring that have been exposed to intrauterine type 1 DM exhibit impaired fetal BAT development and show increased risks to develop obesity and type 2 DM later in life. Further study will be conducted to determine whether correction of fetal BAT development via maternal RA supplementation can reduce such risks.

*NVMB-8***Exercise ameliorates endothelial dysfunction in type2-diabetic mice through increasing microRNA-181b level**SHANG Wenbin, WANG Li, WANG Yu, LUO Jiang-Yun, TIAN Xiaoyu, HUANG Yu**Supervisor:** HUANG Yu

Endothelial dysfunction plays an essential role in the pathogenesis of diabetic vascular diseases. Our previous studies showed that exercise improves endothelial function by 5' AMP-activated protein kinase (AMPK) pathway. MicroRNA 181b (miR-181b) was reported to improve glucose homeostasis and insulin sensitivity by enhancing endothelial function. Several studies implied that miR-181b expression is controlled by shear force. However, it is still unknown whether miR-181b is involved in the vasoprotective effect of exercise and whether miR-181b expression is under the control of AMPK pathway. Therefore, we investigate the expression of miR-181b in aortas from diabetic mice with/without exercise and examine the role of the AMPK pathway in exercise-mediated miR-181b expression.

To investigate whether exercise upregulates miR-181b expression, db/db mice were subjected to treadmill exercise for 45 min per day for two months. Thereafter, mouse aorta was dissected for real-time PCR analysis. The results show that exercise significantly increases miR-181b level in the endothelium of mouse aorta compared to that from control sedentary mouse. To examine the impact of blood flow on miR-181b expression, human umbilical vein endothelial cells (HUVECs) exposed to laminar flow in a constant speed at 12 dyn/cm<sup>2</sup> for 24 hours exhibited a dramatic increase of miR-181b expression. To study the role of AMPK in miR-181b expression, 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) used to treat HUVECs in three separate time points (2, 4, 8 h) significantly induced miR-181b expression and peaked at 8 h, which can be reversed by AMPK inhibitor compound C. Consistently, glucose transporter inhibitor 2-deoxy-D-glucose (2-DG) also increased the miR-181b level. To confirm whether miR-181b has beneficial effect on endothelial function, intravenous administration of miR-181b overexpressing adenovirus to db/db mice for two weeks improved endothelial function.

Exercise increases miR-181b level in mouse vascular endothelium likely via laminar shear stress-induced AMPK activation. MiR-181b improves endothelial function in diabetic mice and may serve as a therapeutic target for the treatment of diabetic vasculopathy.

N<sup>o</sup>9**Endothelial Smad4 mediates angiogenesis and induction of beige adipocytes in adipose tissue.**WANG Chenguang, ZHANG Hongsong, HUO Mingyu, MA Ronald C, TIAN Xiao Yu, HUANG Yu**Supervisor:** HUANG Yu

**Introduction:** Brown adipose tissue (BAT) expresses higher levels of uncoupling protein-1 (UCP1) and mitochondrial content associated with increased heat production. Induction of BAT or “beige/brite” adipose tissue from white adipose tissue (WAT) is a new strategy aiming to treat obesity and insulin resistance. Cold stress increases adrenergic stimulation of WAT and upregulates UCP1 expression. Previous studies showed that angiogenesis induced by VEGFs and PDGFs contributes to formation of beige adipose tissue. However, the regulatory role of vascular endothelium in beiging of WAT remains poorly understood.

**Methods:** To specifically knock down Smad4 in endothelial cells, we used Smad4floxP/floxP as wild type (Smad4EC-WT) and Smad4floxP/floxP; Tie2CreERT2/+ as inducible endothelial-specific Smad4 knockout mice (Smad4EC-KO), Cre recombinase expression was induced by Intraperitoneal injection of tamoxifen in adult mice (8-10 weeks old). Mice were housed at 8°C for 4 days or injected with  $\beta$ 3-adrenoceptor agonist CL316,243 (CL) at a dosage of 1 mg/kg per day via subcutaneous injection for 10 day to induce beiging. RNA sequencing, qPCR and tube formation were performed on human endothelial cells (HUVECs) infected with lentiviral vector carrying scramble or SMAD4-shRNA.

**Results:** RNA-sequencing data showed that SMAD4 knockdown downregulated expression of genes involved in angiogenesis, such as VEGFA, CXCR4; macrophage recruitment and infiltration such as CCL2 and CX3CL1; and adipogenesis such as PDGFA and PDGFB, indicating a regulatory role of Smad4 in angiocrine function of endothelial cells. We further performed quantitative real-time PCR to validate the RNA-seq results. SMAD4-knockdown HUVECs exhibited a reduced capacity of tube formation. Upon cold exposure at 8°C, Smad4EC-KO mice showed an attenuated UCP1 upregulation in subcutaneous WAT as determined by Western blotting and immunohistochemistry. Flow cytometric analysis of sWAT stromal vascular fraction showed a 73% reduction of CD31+CD144+ endothelial cells in Smad4EC-KO mice compared with Smad4EC-WT mice. In addition, adipogenic precursor cells (CD45-CD31-Sca-1+PDGFRa+) also reduced by 58% in sWAT from Smad4EC-KO mice after cold exposure. In addition, the reduced Ki67 expression in endothelial cells and adipogenic precursors suggests an impaired proliferation of these cells.

**Conclusion:** The present results suggest a positive role of Smad4 in endothelial cells in angiogenesis and adipogenesis; the latter two contribute to beiging of WAT.

**Keywords**

SMAD4, Angiogenesis, Adipose, Endothelium, Growth factors

*NVMB-10***Serum Exosomal Arginase 1 Regulates Endothelial Functions in Diabetes**

WANG Yifan, ZHANG Huina, LIU Jian, QU Dan, HUANG Yuhong, MA Ronald Ching Wan,

WANG Li, HUANG Yu

**Supervisor:** HUANG Yu**Objectives**

Exosomes are abundant in blood. The molecules carried by exosomes vary under physiological and pathological conditions, reflecting the disease profile of their parental cells upon exosome secretion. Exosomal molecules can be delivered to recipient cells via circulation and participate in subsequent cellular processes, representing a new way of cell-cell communication. Vascular endothelial cells are constantly exposed to the circulating exosomes in blood. Therefore, we aim to investigate how endothelial cells respond to serum exosomes and its implication in diabetic vasculopathy.

**Methods**

To test vascular function, mouse aortas were dissected and isometric force was measured using wire myograph. Second-order resistance mesenteric arteries were cannulated onto pressure myograph to measure flow-mediated dilatation. To measure NO bioavailability, human umbilical vein endothelial cells (HUVECs) were stained with NO-sensitive fluorescent dye and NO production was detected by confocal microscopy. Gene expression were measured by q-PCR and Western blot.

**Results**

The present study shows vascular endothelial cells were able to take up PKH67-labeled serum exosomes isolated from diabetic (db/db) mouse, as indicated by increased incorporation of fluorescence into aortic endothelial cells. db/db serum exosomes reduced NO production in endothelial cells, and severely impaired NO-dependent vascular relaxation in non-diabetic (db/m+) mouse conduit and resistant arteries. Proteomic analysis showed significant increase of Arginase 1 expression in serum exosomes of db/db mouse compared to db/m+ mouse. The impaired vascular function can be rescued by co-treatment with heparin (exosome uptake inhibitor) or eNOS substrate L-arginine. Silence of Arg1 in vivo restores endothelial functions in db/db mouse. Overexpression of Arg1 in vivo leads to endothelial dysfunction in db/m+ mouse.

**Conclusion**

The present results demonstrate that db/db serum exosomes deliver functional Arg1 to endothelial cells, reducing NO bioavailability and impair endothelium-dependent relaxation in mouse arteries. We have identified a previously undefined role of serum exosomes in the development of diabetic vasculopathy.

*NVMB-11***Inhibition of FGF2 ameliorates diabetic nephropathy through Hippo pathway**WANG Yu, WANG Li, SHANG Wenbin**Supervisor:** HUANG Yu

Fibrosis is one of the most important features in the pathogenesis of chronic kidney disease (CKD) in diabetic patient. Recent studies showed that basic fibroblast growth factor (FGF2) involves in renal fibrosis. The Hippo pathway is an evolutionarily conserved kinase cascade that exerts crucial effects on regulating cell proliferation, organ size and tissue regeneration. Recently, Yap/Taz, the key components in the Hippo pathway have been reported to stimulate fibroblast activation and kidney fibrosis. However, the underlying mechanism is largely unknown. In the present study, we found FGF2 is overexpressed in kidney epithelial cell after exposing to high glucose (30mM) for 24h in kidney epithelial cell line, which is accompanied by increased expression of Yap/Taz target gene CTGF, CYR61, suggesting an activating of Yap/Taz. Furthermore, Yap/Taz overexpression upregulates FGF2 expression in renal proximal tubule epithelial cell HK2. In addition, simvastatin, which suppresses Yap/Taz through activating Hippo pathway, can also suppress the expression of FGF2. In conclusion, Yap/Taz mediate high glucose-induced diabetic nephropathy through increasing FGF2 expression.

*NVMB-12***Regulation of Orai1 in Angiotensin II-Induced Cardiac Hypertrophy**XIE Mingxu, ZHENG Changbo, YAO Xiaoqiang**Supervisor:** YAO Xiaoqiang

Heart failure, a worldwide severe disease, usually initiates with pathological cardiac hypertrophy which is a response of heart to increased workload. The cardiac hypertrophy is characterized as increased cardiomyocytes size and weakened heart contractility, develops during the process of disorders such as hypertension, myocardial infarction and coronary artery diseases. Calcium, as a ubiquitous second messenger, plays vital roles in mediating a wide range of cardiovascular diseases. Few reports determined store operated Ca<sup>2+</sup> entry (SOCE) is associated with cardiac hypertrophy yet. Here, we hypothesized Orai1-mediated SOCE is responsible for the Angiotensin II-induced cardiac hypertrophy. Our data showed after the subcutaneous implantation of Ang II osmotic pump in C57BL6 mice, the heart size significantly increased. However, this effect could be abolished by knocking down Orai1 expression levels using AAV-Orai1-shRNA. A real-time PCR results showed the hypertrophic marker genes atrial natriuretic factor (ANF), brain natriuretic peptide (BNP) and myosin heavy chain beta ( $\beta$ -MHC) upregulated in Ang II treatment group, while they remained unchanged or slightly downregulated after the treatment of AAV-Orai1-shRNA. Western blots also confirmed that the protein level changes of other markers ANF and cardiac troponin T (cTnT) are in line with the qPCR results, indicating Orai1 could rescue the Ang II perfusion induced cardiac hypertrophy *in vivo*. Moreover, Masson's Trichrome staining convinced type I collagen levels in heart increased after Angiotensin II perfusion while it is attenuated after downregulating Orai1 *in vivo*, showing the progress of cardiac fibrosis during Orai1-mediated cardiac hypertrophy. Taken together, these findings suggest that Orai1 is a novel regulator involved in Angiotensin II induced cardiac hypertrophy *in vivo*.

*NVMB-13***Tubular Cell Specific Mst1/Mst2 Deficiency Leads to Chronic Kidney Disease in Mice**

XU Chunhua, WANG Yang, WANG Li, LI Wenling, MAK King Lun Kingston, HUANG Yu, XIA Yin

**Supervisor:** HUANG Yu

The Hippo pathway is a regulator of organ size. The core components of the Hippo pathway consist of Mammalian Ste20-like kinases 1/2 (MST1/2) and their scaffold protein SAV1, large tumor suppressor 1/2 (LATS1/2) and their scaffold proteins MOB1A/1B, and two downstream effectors YAP/TAZ. Several members of the Hippo pathway including SAV1, LATS1/2, YAP and TAZ have been found to be involved in embryonic kidney development or kidney disease. However, the role of MST1/2 in kidney remains unknown.

Here, we showed for the first time that MST1 was highly expressed in all nephron segments and collecting ducts in mouse kidneys. We therefore generated tubular cell specific Mst1/Mst2 double knockout (dKO) mice by intercrossing floxed Mst1/Mst2 mice with Ksp-Cre transgenic mice. dKO mice showed increased kidney weights starting at 4 weeks of age. Body weights were comparable between WT and dKO mice up to 8 weeks of age. However, dKO mice exhibited a significant body weight loss at 6 months and later stages. Kidney structural abnormality and tubular injuries were seen as early as 4 weeks and aggravated with age, as indicated by immune cell infiltration, tubular cell death, thickening of glomerular basement membrane and tubular basement membrane, cast formation, and increased levels of urinary NGAL, a marker for kidney injury. At 6 months of age and later, protein levels of the fibrotic markers  $\alpha$ -SMA, fibronectin 1 and collagen I $\alpha$ 1 were significantly increased in dKO kidneys, and Masson's trichrome staining showed much more collagen deposition in dKO kidneys, indicating dKO mice developed renal fibrosis. Consequently, renal function was impaired at 6 months and older ages, as shown by increased serum creatinine and blood urea nitrogen (BUN) levels in dKO mice. Moreover, dKO kidneys exhibited increased expression of inflammatory factors and infiltrations of macrophages into the interstitium.

YAP activity was significantly enhanced in dKO kidneys at 4 weeks of age, coupled with increased Ki67-positive tubular cell numbers. By generating Mst1/Mst2/Yap triple knockout (tKO) mice, we found that deletion of Yap restored the kidney weights in dKO mice to WT levels, and also fully rescued the expression of all the inflammatory factors measured except TNF- $\alpha$  at 4 weeks of age. Notably, an increase in TNF- $\alpha$  was found in dKO kidneys at 2 weeks of age, when YAP was not activated yet. These results suggest that the increased kidney weights and most inflammatory responses observed in tubular Mst1/Mst2 deficient mice are dependent on YAP while TNF- $\alpha$  expression is induced via both YAP-dependent and -independent mechanisms.

Collectively, we found tubular Mst1/Mst2 deficiency leads to tubular cell hyperproliferation, inflammation, tubular injury, renal fibrosis, and renal dysfunction, indicating that tubular MST1/2 play important roles in restraining renal overgrowth and inflammation and maintaining normal tubular structure and function.

*NVMB-14***Resveratrol stimulates Na<sup>+</sup>-Ca<sup>2+</sup> exchanger on the plasma membrane to reduce cytosolic Ca<sup>2+</sup> in rat aortic smooth muscle cells**ZHANG Yunting , YAN F**Supervisor:** YAO Xiaoqiang

Background: Resveratrol has well-documented vascular relaxant and anti-hypertensive effect. Here we studied the action of resveratrol in modulating cytosolic [Ca<sup>2+</sup>] level and ATP-induced Ca<sup>2+</sup> release from sarcoplasmic reticulum (SR) in rat aortic smooth muscle cells (ASMCs) and explored the underlying mechanisms. Method and result: Cytosolic [Ca<sup>2+</sup>] and SR [Ca<sup>2+</sup>] in ASMCs were determined by Fluo-4/AM or Mag-Fluo-4/AM, respectively. Resveratrol (20, 50 and 100 μM) caused a rapid and substantial reduction in cytosolic [Ca<sup>2+</sup>] in ASMCs bathed either in the normal Hank's Balanced Salt Solution (HBSS) or in a Ca<sup>2+</sup>-free HBSS. Resveratrol pretreatment reduced ATP-induced SR Ca<sup>2+</sup> release and also lowered SR Ca<sup>2+</sup> content. In cells bathed in a Na<sup>+</sup>-free physiological saline, which favors reverse mode of Na<sup>+</sup>-Ca<sup>2+</sup> exchanger (NCX), resveratrol induced rises in cytosolic [Ca<sup>2+</sup>] and SR [Ca<sup>2+</sup>]. The effect of resveratrol on cytosolic [Ca<sup>2+</sup>] and SR [Ca<sup>2+</sup>] were inhibited by a selective NCX inhibitor, SEA0400. Conclusion: Resveratrol stimulates NCX to reduce cytosolic [Ca<sup>2+</sup>] and SR [Ca<sup>2+</sup>] in ASMCs in normal physiological saline.

*NVMB-15***MCOLN2 plays an oncogenic role in prostate cancer progression**YU Hongyan, YAO xiaoqiang**Supervisor:** YAO xiaoqiang

Prostate cancer (PCa) originated from the glandular epithelial cells is one of the most common malignancies and the second leading cause of cancer-related death in males worldwide. However, the molecular mechanism of PCa remains to be clearly defined. MCOLN2 belongs to the transient receptor potential (TRP) protein superfamily, which consists of gated, tetrameric cation channels with diverse physiological functions, particularly in sensory signaling. This protein shares a conserved structure of six transmembrane helices with differing cytoplasmic oriented N- and C-terminal domains. Previous studies suggest that MCOLN2 may play a role in the immune system by activate chemokines CCL2. However, the role of MCOLN2 during PCa development has not been studied yet. By bioinformatic analysis, we found that *mcoln2* is overexpressed in most of the PCa tissues compared with the adjacent normal tissues. The patients with higher MCOLN2 expression tend to have poor overall survival rates. And we also found that MCOLN2 is upregulated in most of PCa cell lines compared with benign prostate epithelial cell line BPH at both the mRNA and protein level by western blot and qRT-PCR analysis. Cellular and molecular biology experiments showed that knockdown endogenous MCOLN2 expression in PC3 and Du145 cells could inhibit cell growth and foci formation by CCK-8 assay and crystal violet staining. Furthermore, silencing of MCOLN2 in PC3 and Du145 cells could inhibit cells migration and invasion via transwell assay. In summary, our results indicate that MCOLN2 may play an oncogenic role in PCa progress.

*NVMB-16***A combined chemotherapy for drug resistance in ovarian cancer**YU Libo, YAO Xiaoqiang**Supervisor:** YAO Xiaoqiang

The adaptive drug resistance has been the main obstacle for chemotherapy in ovarian cancer patients. Many studies have proved that the increased drug efflux by the ATP-binding cassette (ABC) transporters was the main cause of drug resistance acquisition. We have detected that several transporter genes, especially P-gp, in PTX-resistant A2780 cell line were dramatically higher than the WT A2780. These results indicate that finding a promising way to block ABC transporters and reverse the drug resistance in tumor cells is the key point for successful ovarian cancer chemotherapy.

Zebularine was reported to be the most effective methyltransferase inhibitor with less cytotoxicity, which can repress the transcription of ABC family. First, with the treatment of zebularine, the expression of P-gp was significantly reduced in the PTX-resistant A2780 cells. Accordingly, the drug sensitivity was increased. Then BAPTA-AM, a cell permeable Ca<sup>2+</sup> chelator, was used to block the function of existed transporter pumps. Results showed that BAPTA-AM could not only inhibit the function of transporter genes with less drug expelled into the medium, but also inhibit the expression of P-gp. Furthermore, with the combination of zebularine and BAPTA-AM, the drug resistant A2780 cells showed much higher sensitivity to PTX. Therefore, this combined therapy may be applicable in the clinical treatment of PTX resistant patients.

*NVMB-17***Insulin-like Growth Factor-1 Alleviates Ocular Inflammation in a Rat Model of Acute Uveitis via regulation of NF- $\kappa$ B p65**

YU Qiuxiao, MA Ding, LIANG Weicheng, REN Jialin, CHEN Binbin, LEUNG Pui-Ying, CHAN

Sun-On

**Supervisor:** CHAN Sun On

Insulin-like growth factor-1 (IGF-1) has been shown to participate in inflammatory processes. The aim of this study is to investigate the role of IGF-1 in endotoxin-induced uveitis (EIU). EIU in rats was established by footpad injection of lipopolysaccharide (LPS, 1 mg/kg). Different doses of recombinant human IGF-1 (rhIGF-1, 0.1, 0.5, and 1  $\mu$ g/eye) and/or the specific inhibitor of IGF-1 receptor, picropodophyllin (PPP, 10  $\mu$ g/eye), were administered by intravitreal injection 2 hours before LPS administration. Ocular tissues were harvested 24 hours post LPS injection. A significant decrease of IGF-1 was observed in iris and ciliary body at both mRNA and protein levels. Exogenous IGF-1 attenuated ocular inflammation by reducing cell infiltration, protein exudation and pro-inflammatory mediator production. Blocking the IGF-1 signaling pathway with PPP reversed these protective effects. Moreover, LPS induced nuclear translocation and phosphorylation of NF- $\kappa$ B p65 in iris and ciliary body, which were suppressed significantly by rhIGF-1 treatment. In vitro, lentivirus-mediated shRNAs targeting p65 were constructed to infect human non-pigmented ciliary epithelial cells (HNPCEpiC), and chromatin immunoprecipitation (CHIP) assay was conducted to examine the interaction between p65 and IGF-1 DNA fragments. The knockdown of p65 resulted in significant increase of IGF-1 mRNA expression, and p65 was proved to pull down the DNA fragments from IGF-1 gene promoter. Our findings indicate that treatment with rhIGF-1 at the dosage of 1  $\mu$ g/eye exerted a stable beneficial effect in LPS-induced ocular inflammation, and that this effect is mediated by suppression of p65 activity.

*NVMB-18***The Functional Study of Mammalian TRAPPIII Complex in Centrosome  
Assembly and Ciliogenesis**ZHANG Caiyun**Supervisor:** Prof. YU Sidney

The transport protein particle (TRAPP) is a multi-subunit tethering protein complex that regulates endoplasmic reticulum (ER)-to-Golgi transport, intra-Golgi transport, autophagy and ciliary membrane biogenesis. So far, three forms of TRAPP complexes have been identified, TRAPP I, II, and III. TRAPPIII, which contains specific subunits TRAPPC12 and TRAPPC8, has functions in modulating ER-derive COPII vesicles, autophagy and ciliary biogenesis.

Primary cilia are microtubule-based, hair-like organelles projected from the surface of mammalian cells for motility, sensing and signaling functions. Defects in ciliary structure or function cause physiological disorders collectively called ciliopathies. We and others have discovered TRAPPIII may play a role in ciliogenesis. TRAPPC8, a subunit specific to TRAPPIII complex, is localized to the centrosomes and the basal body of cilia, in addition to its reported localization at the (ER)-to-Golgi vesicles. It binds to a number of proteins known to regulate biogenesis of the centrosome and the basal body of primary cilia. Mutations to the genes encoding these proteins cause ciliopathies. Moreover, TRAPPC8 is important for ciliogenesis and ciliary length as siRNA depletion of TRAPPC8 reduced formation of cilia, and the length of the cilia in retinal pigment epithelial cells. Effort to investigate the underlying mechanism of how TRAPPIII contributes to ciliogenesis is underway.

*NVMB-19***Treatment of Alzheimer's disease by a disease-modifying small molecule with multiple cellular actions**ZHANG Xiaoman, YANG Shengxi, MU Mingdao, RONG Kanglin, KE Ya, YUNG Wing-ho**Supervisor:** YUNG Wing Ho

Alzheimer's disease (AD) is the most common neurodegenerative disease leading to loss of memory and other cognitive functions. GSK-J4 is a newly discovered epigenetic regulator found to cross blood-brain barrier and possess anti-brain tumor function. Recently, our laboratory has discovered several beneficial effects of GSK-J4 for AD and suggested GSK-J4 to be a novel multi-modal drug for disease-modifying treatment in neurodegeneration. Our objective here is to investigate the therapeutic effects of GSK-J4 in AD and unravel the mechanisms.

9-month old 3xTg AD female mice were intraperitoneally injected with GSK-J4 (10mg/kg/per day) for 30 days. Different behavior tests were performed to assess cognitive and memory abilities. Long-term potentiation (LTP) in hippocampus in vitro was conducted to monitor synaptic plasticity. Immunohistochemistry was used to reveal changes in levels of molecules, mostly for investigation of synaptic properties related proteins including PSD-95 and brain-derived neurotrophic factor (BDNF).

The results of behavioral assays demonstrated the beneficial effects of GSK-J4 treatment in enhancing learning ability and memory, indicated by the performance of mice in Morris water maze, Y-maze and cued fear conditioning tests. Consistent with behavioral improvement, the magnitude of LTP of hippocampal CA3-CA1 pathway increased by about 30% after GSK-J4 treatment, which was accompanied by upregulated expressions of PSD-95 and BDNF in the hippocampus detected by immunohistochemistry. Taken together, our results confirmed the potential of GSK-J4 in treating AD via enhancement of synaptic functions.

*NVMB-20***The Functional Role of TRPC5 in Platelets**ZHUO Duan, LAU Eva, LO Chunyin, YAO\* Xiaoqiang**Supervisor:** YAO Xiaoqiang

The main function of platelets is to maintain normal hemostasis. Decreased platelet number and/or function results in uncontrolled hemorrhage, whereas abnormal hyper-aggregability and/or thrombus formation could cause ischemic cardiovascular and cerebrovascular disorders. Considering that vascular damage is rather common in daily life, normal platelet function need to be tightly controlled. TRPC5, known as transient receptor potential channel 5, is a Ca<sup>2+</sup>-permeable nonselective cation channel. Previously, we identified a novel function of TRPC5 in promoting blood clot formation. However, the underline mechanism is unknown yet. We plan to utilize various platelet physiological agonists (thrombin, collagen and ADP) to investigate possible role of TRPC5 channels in platelet activation and aggregation. Antiplatelet therapies are of vital importance for treating cardiovascular diseases. Through this study, we hope to find out novel functional proteins/signaling pathways important for platelet function, helping future development of safer drugs.

# PEKING UNIVERSITY

**Peking University**

| <b>Title</b>   | <b>Author</b> | <b>Abstract Number</b> |
|--|---------------|------------------------|
| Real-time visualization of the dynamics of cotranscribed genes within the transcription factory in living cells            | He Renxi      | PKU-1                  |
| Genome-Wide Evolutionary Analysis of Natural History and Adaptation in the World's Tigers                                  | Liu Yuechen   | PKU-2                  |
| Mitochondrial DNA is transported and partitioned via mitochondrial dynamic tubulation at the ER-mitochondria contact sites | Qin Jinshan   | PKU-3                  |
| Cap-specific, terminal N6-methylation by a mammalian m6Am methyltransferase  | Zhang Meiling | PKU-4                  |

*PKU-1***Real-time visualization of the dynamics of cotranscribed genes  
within the transcription factory in living cells**Renxi He, Shipeng Shao, Yujie Sun**Supervisor:** Yujie Sun

It has been widely assumed that active RNA polymerases track along the DNA templates to produce transcripts since single RNA polymerase molecule is much smaller than DNA template. However, accumulating evidence emerged in the last decades has provided support for an alternative model that active RNA polymerases tend to concentrate in discrete “factories” in nuclei during transcription. The relative large size (~100 nm diameter) and stable position of these factories may suggest that RNA polymerases are immobile while the DNA strand is mobile during transcription. However, the supposed process has never been directly observed in living cells due to the lack of efficient labeling and imaging tools. Thus, multi-color visualization of two or more genes during transcription could throw light on the controversy that whether polymerase or DNA template is mobile. To visualize the dynamics of DNA strands in the “factories” during transcription, two human genes SAMD4A (221 kb) and TNFAIP2 (11 kb) were chosen as a model, as they can be switched on rapidly and synchronously by TNF- $\alpha$ . It will take more than 1 hour for a polymerase molecule to finish the transcription of SAMD4A while this process occurs within several minutes for TNFAIP2. It has been reported that both SAMD4A and TNFAIP2 promoters are recruited into these “factories” after a 10 minutes stimulation. We have used reverse-transcription PCR and single molecule inexpensive FISH to confirm that these nascent transcripts co-localize at relevant times, verifying the responsiveness of immortalized HUVEC to TNF- $\alpha$ . Although many creative methods have been introduced for gene labeling in living cells, most of which are only feasible for gene loci containing repeats sequence. It’s still challenging for SAMD4A and TNFAIP2 dual labeling since they don’t contain enough repeat sequence nearby. Live cell imaging of DNA was previously carried out by using a florescent repressor-operator system to enrich fluorescent proteins at specific chromatin sites. We use CRISPR/Cas9 system to knock two optimized 120 $\times$ TetO and LacO arrays in the genome near the downstream of the two genes, respectively. The expression of TetR-mCherry and LacI-EGFP enable the visualization of the two genes. After that, through the real-time observation of the dynamics of cotranscribed genes in transcription factories, we hope to solve the controversy whether RNA polymerase or DNA template is immobile during transcription. Besides, we want to observe the recruitment of cotranscribed genes into the transcription factories as well as components and biological significances of transcription factories...

PKU-2

## Genome-Wide Evolutionary Analysis of Natural History and Adaptation in the World's Tigers

Yue-Chen Liu, Xin Sun, Carlos Driscoll, Dale Miquelle, Xiao Xu, Paolo Martelli, Olga Uphyrkina, James L. D. Smith, Stephen J. O'Brien, Shu-Jin Luo\*.

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**Supervisor:** Shu-Jin Luo

No other species attracts more international resources, public attention, and protracted controversies over its intraspecific taxonomy than the tiger (*Panthera tigris*). Today, fewer than 4,000 free-ranging tigers survive, covering only 7% of their historical range, and debates persist over whether they comprise six, five or two subspecies. The lack of consensus over the number of tiger subspecies has partially hindered the global effort to recover the species from the brink of extinction, as both captive breeding and landscape intervention of wild populations increasingly requires explicit delineations of the conservation management units. The recent coalescence to a Late Pleistocene bottleneck (circa 110 kya) poses challenges for detecting tiger subspecific morphological traits, suggesting that elucidating intraspecific evolution in the tiger requires analyses at the genomic scale. Here, we present whole-genome sequencing analyses from 32 voucher specimens that resolve six statistically robust monophyletic clades corresponding to extant subspecies, including the recently recognized Malayan tiger (*P. t. jacksoni*). The inter-subspecies gene flow is very low, corroborating the recognized phylogeographic units. We identified multiple genomic regions that are candidates for identifying the adaptive divergence of subspecies. The body size-related gene *ADH7* appears to have been strongly selected in the Sumatran tiger, perhaps in association with adaptation to the tropical Sunda Islands. The identified genomic signatures provide a solid basis for recognizing appropriate conservation management units in the tiger and can benefit global conservation strategic planning for this charismatic megafauna icon.

*PKU-3***Mitochondrial DNA is transported and partitioned via mitochondrial dynamic tubulation at the ER-mitochondria contact sites**

Jinshan Qin, Yuting Guo, Yang Chen, Qian Peter Su, Huiwen Hao, Shujuan Zhao,  
Chong Wang, Dong Li, Li Yu, Yujie Sun

**Supervisor:** Yujie Sun

The replication, partition, and distribution of mitochondrial DNA (mtDNA) are essential for cellular functions. It has been showing that mtDNA synthesis is coupled with mitochondrial fission at Endoplasmic reticulum (ER)-mitochondria contact sites in human cells. Mitochondrial dynamic tubulation is a new defined way of mitochondrial dynamics that the thin, highly dynamic tubules are pulled out of mitochondria by KIF5B. Here, we show that ER tubules were found to play an active role in defining the position of mitochondrial dynamic tubulation. Furthermore, we observe a positive correlation between mitochondrial DNA nucleoids and the initial sites of dynamic tubulation. We propose that through dynamic tubulation, mtDNA synthesized at ER-mitochondria contact sites can be rapidly distributed throughout mitochondria.

*PKU-4***Cap-specific, terminal N6-methylation by a mammalian m6Am methyltransferase**Meiling Zhang, Hanxiao Sun, Kai Li, Dongsheng Bai, Chengqi Yi**Supervisor:** Chengqi Yi

Gene expression can be post-transcriptionally regulated via dynamic and reversible RNA modifications. One representative example is the abundant internal modification N6-methyladenosine (m6A), which has been shown to impact various physiological and pathological conditions. Its study is greatly facilitated by the discovery of its writer, reader and eraser proteins. In comparison to the internal m6A, there exists a terminal modification at mRNA cap, known as N6,2'-O-dimethyladenosine (m6Am). m6Am was first identified more than 40 years ago, and is recently shown to be reversible. However, its methyltransferase is still unknown, significantly hindering the functional study of m6Am.

We identify X as a novel m6Am writer. We first used biochemical purification approaches and mass spectrometry to identify X as a candidate m6Am methyltransferase. We then knocked down gene X and observed reduction of m6Am level, but not m6A level, in vivo. Moreover, we purified recombinant X protein and showed that it can robustly methylate RNA oligos in vitro. Furthermore, we showed that X is specific for the m7G-ppp-Am cap structure. Lastly, we identified the direct RNA targets of X by m6A/m6Am sequencing. Collectively, our results reveal X as a cap-specific, terminal m6Am methyltransferase.

# KUNMING INSTITUTE OF ZOOLOGY

**Kunming Institute of Zoology**

| <b>Title</b>  | <b>Author</b> | <b>Abstract Number</b> |
|---|---------------|------------------------|
| Molecular mechanism of rapidly decreasing blood glucose in Old World fruit bats   | Liu Qi        | KIZ1                   |
| CRISPR-Cas9 knockout library screen for novel regulators of mammary gland stem cells identifies the role of Mcam                    | Yang Xing     | KIZ2                   |
| Identity-by-descent analysis reveals novel susceptibility loci for severe acne in Chinese Han cohort                                | Yang Xingyan  | KIZ3                   |
| Mouse embryonic stem cells have increased capacity for replication fork restart driven by the specific Filia-Floped protein complex | Zhang Weidao  | KIZ4                   |

KJZ-1

## **Molecular mechanism of rapidly decreasing blood glucose in Old World fruit bats**

Liu Qi, Shi Peng

**Supervisor:** Shi Peng

Bats are a unique and enigmatic group of mammals that account for ~1,100 species. Their successful adaptation during evolution most likely result from a variety of complex adaptive traits, such as true self-powered flight and echolocation. Compared to echolocating bats, the nonecholocating Old World fruit bats (family Pteropodidae) exclusively feed on fruits and/or nectar that are rich in diverse sugars, such as fructose, glucose and sucrose.

Fossil and molecular evidence suggests that bats originated in small insectivorous mammals in the early Eocene and that Old World fruit bats were derived from insectivorous bats more than 58 million years ago. Following the divergence in difference from insectivorous diet into carpophagous and/or nectarivorous diet, it's a challenge for Old World fruit bats to face the challenge in regulating glucose homeostasis due to nutrition change from high protein into high sugar. However, we find no significantly higher fasting blood sugar in old world fruit bat than that in insectivorous bats.

We next performed glucose tolerance test experiments to assess the ability of reducing blood sugar of Old World fruit bats by measuring blood sugar after intraperitoneal injection with solution containing the equal glucose mass (2g glucose per 1Kg body weight) and find that Old World fruit bats have significantly higher rates in decreasing blood sugar than insectivorous bats.

Insulin is well known to be a determinant in lowering blood sugar of mammals and thus we first explored the possible factors related to insulin to detect molecular mechanisms of faster decreasing blood sugar in Old World fruit bats. By insulin tolerance test, we didn't find any significant difference in insulin resistance between the old world fruit bats and insectivorous bats. Also, there was no significant difference in anatomical structure of pancreas islet and in insulin concentration between old world fruit bats and insectivorous bats. Detecting positive selection of the genes in insulin signal pathway showed that none of genes displayed the positively selected signals in the group of old word fruit bat. These results strongly suggests that insulin doesn't contribute to faster decreasing blood sugar of old word fruit bats.

We further screened positively selected genes of the old word fruit bats by systematical genomic analysis, and found that the gene SLC2A1 that encoded glucose transporter 1 (GLUT1) was under positive selection. SLC2A1 expresses in almost all tissues and GLUT1 is used to transport glucose across the plasma membranes of mammalian cells independent of insulin. We thus hypothesize that two positively selected amino acids identified in GLUT1 promote uptake of more glucose into cells leading to decrease blood sugar faster in old word fruit bats compared to insectivorous bats. The functional verification for the hypothesis is in progress .

Taken together, we for the first time discover blood glucose decreasing faster in Old World fruit bats than in insectivorous bats, which most likely is derived from adaptation to high-sugar diets during evolution and provide a potential molecular mechanism underlying this phenomenon, probably opening a new window in understanding pathogenic mechanisms and treatment of diabetes in humans.

KJZ-2

**CRISPR-Cas9 knockout library screen for novel regulators of mammary gland stem cells identifies the role of Mcam**Xing Yang<sup>1,2</sup>, Haibo Xu<sup>1,2</sup>, Baowei jiao<sup>1#</sup><sup>1</sup> State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, 650223, China<sup>2</sup> University of Chinese Academy of Sciences, Beijing, 100049, China.**Supervisor:** Baowei jiao

The mammary gland is a unique organ for postnatal development. Its development is fueled by stem cell self-renewal and differentiation. During the process of development, mammary gland stem cells (MaSCs) give rise to progenitors, which are then differentiated into mature mammary epithelial cells. However, the molecular regulators that orchestrate stem cell renewal, proliferation and differentiation along the mammary epithelial hierarchy remain poorly understood. Using CRISPR-Cas9 technology, we performed a global loss-of-function screen to simultaneously identify novel molecular regulators of MaSCs and mammary gland development. In this article, we found a novel regulator: Mcam. Knock down of this gene impaired stem cell self-renewal and mammary regenerative potential in the *in vitro* mammosphere formation assay and *in vivo* mammary gland reconstitution. This study supported the use of CRISPR-Cas9 knockout library to identify novel regulators of mammary epithelial function and had revealed Mcam could act as a negative regulator of mammary gland development.

KIZ-3

**Identifying and screening the spontaneous Alzheimer's-like Rhesus monkeys**Xingyan Yang, Wenjuan Wu, Minsheng Peng, Quankuan Shen, JiaQi Feng, Wei Lai,

Huilan Zhu, Caixia Tu, Lanying Qin, Yihong Chen, Xiaorong Quan, Donglin Li,

Li He, Yaping Zhang

**Supervisor:** Yaping Zhang

Severe acne is a serious chronic inflammatory skin disease. Although previous GWAS studies have identified numerous common variants associated with severe acne in different populations, these variants just explain a small proportion of the heritability. To explore more details about the genetic basis of severe acne, we perform Identity-by-descent (IBD) mapping in the Chinese Han population based on the dataset of 798700 genome-wide SNPs from 1024 cases and 1029 controls. Comparing the frequency distribution of the IBD segments between the cases and controls, we detect a total of 187 SNPs representing top 0.05% in the permutation as the peak signals. Among of them, five loci (i.e. 3q28, 5q33.3, 11p11.2, 16p13.3, and 16q23) have been reported to be associated with severe acne. Further genotyping of 475 SNPs in 1150 severe acne cases and 802 matched controls and 4405 matched controls, validates *DDB2* (rs10838662,  $P = 7.42 \times 10^{-5}$ ) and *F13A1* (rs435048,  $P = 1.54 \times 10^{-4}$ ) as the candidates risk genes for severe acne in the Chinese Han population. In meta-analysis of the IBD mapping, and independent genotyping, we identify 6p25 referring to gene *F13A1* and 4q23 referring to gene *ADH7*, as the novel susceptibility loci for severe acne.

*KJZ-4***Mouse embryonic stem cells have increased capacity for replication fork restart driven by the specific Filia-Floped protein complex**Bo Zhao <sup>#</sup>, Weidao Zhang, <sup>#</sup>, Yixian Cun, Jingzheng Li, Yan Liu, Jing Gao, Hongwen Zhu, Hu Zhou, Rugang Zhang, Ping Zheng\***Supervisor:** Ping Zheng

Embryonic stem cells (ESCs) are typical model for development research and have great application value in regenerative medicine. But ESCs suffer constitutive DNA replication stress during rapid proliferation, and the genome instability caused by replication stress hampers their applications in regenerative medicine. So, it is very important to understand the regulatory mechanisms of replication stress response in ESCs. We report that mouse ESCs are more efficient than differentiated cells in resolving replication stress. Mouse ESCs can use their specific replication fork complex Filia- Floped to regulate the ubiquitination of helicase Blm by Trim25 on replication fork, which facilitate the stalled replication fork restart to maintain the genomic stability. In this signal pathway, it's indispensable that the serine 151 residue of Filia is phosphorylated in a ATR-dependent manner. Simultaneously, Filia-Floped complex also can regulate the activation of ATR to facilitate the stalled replication fork restart, which is independent with Blm pathway. We draw a conclusion that Filia-Floped scaffold independently regulates ATR activation and Trim25-Blm recruitment to stalling forks in response to replication stress.

## **Acknowledgements**

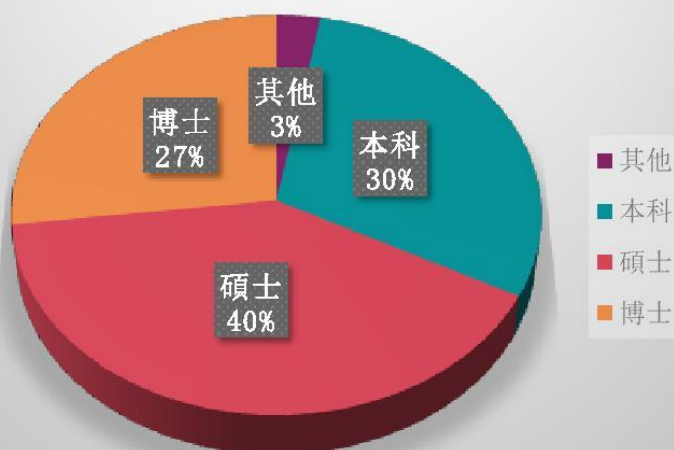
The 2018 SBS Postgraduate Research Day Organizing Committee would like to express its sincere appreciation of the generous support of the following organizations:

- ♦ **Together Sales Department,  
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- ♦ **BioArrow Technology Ltd.**

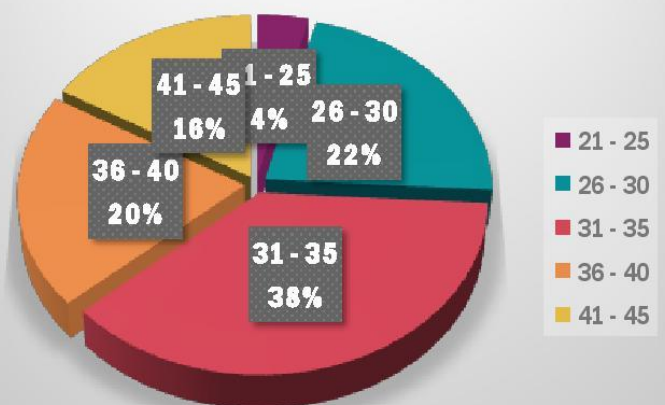
## 卓信團隊簡介

我們為保誠保險有限公司旗下的精英團隊，專注于香港及亞洲的中高端市場，**2017**年榮獲世界華人保險**500**強團隊。整個卓信區域團隊現有顧問超過**160**人，其中本科學歷**30%**，碩士學歷**40%**，博士學歷**27%**。**MDRT**（百万圆桌会员）达成率高达**20%**。

學歷分佈



年齡分布







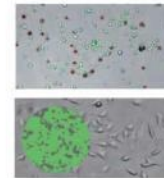
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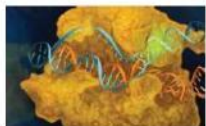


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PG's Orientation Day

Inauguration Ceremony

Christmas Party

Career Planning Talks

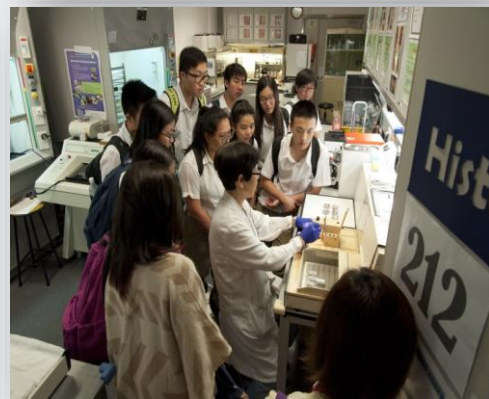
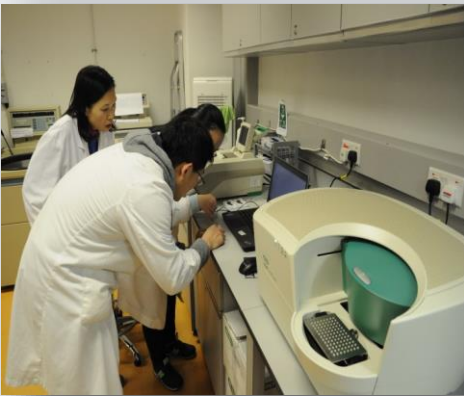
Director's Cup

Badminton Competition

Postgraduate  
Research Day

# Glimpse of the SBS Core Laboratories

**Core Lab Highlights:** Induction Training for New Postgraduate Students, Equipment Training for Core Lab Users, New Technology Demo Workshop and Outreach Activities



## State-of-the-art Equipment and Some of Core Lab Work Results

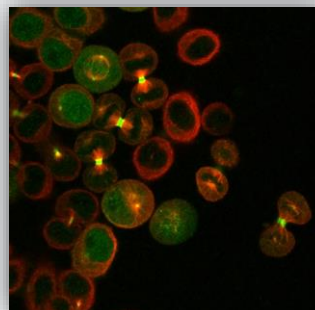
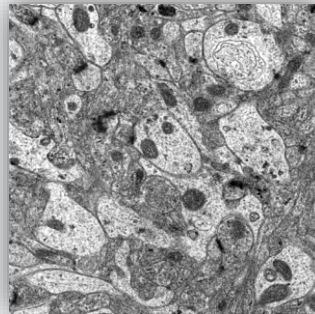
The Ion Chef System & Ion Torrent S5 Sequencer



Seahorse XFe96 Extracellular Cell Analyzer



Centrifugal Concentrator

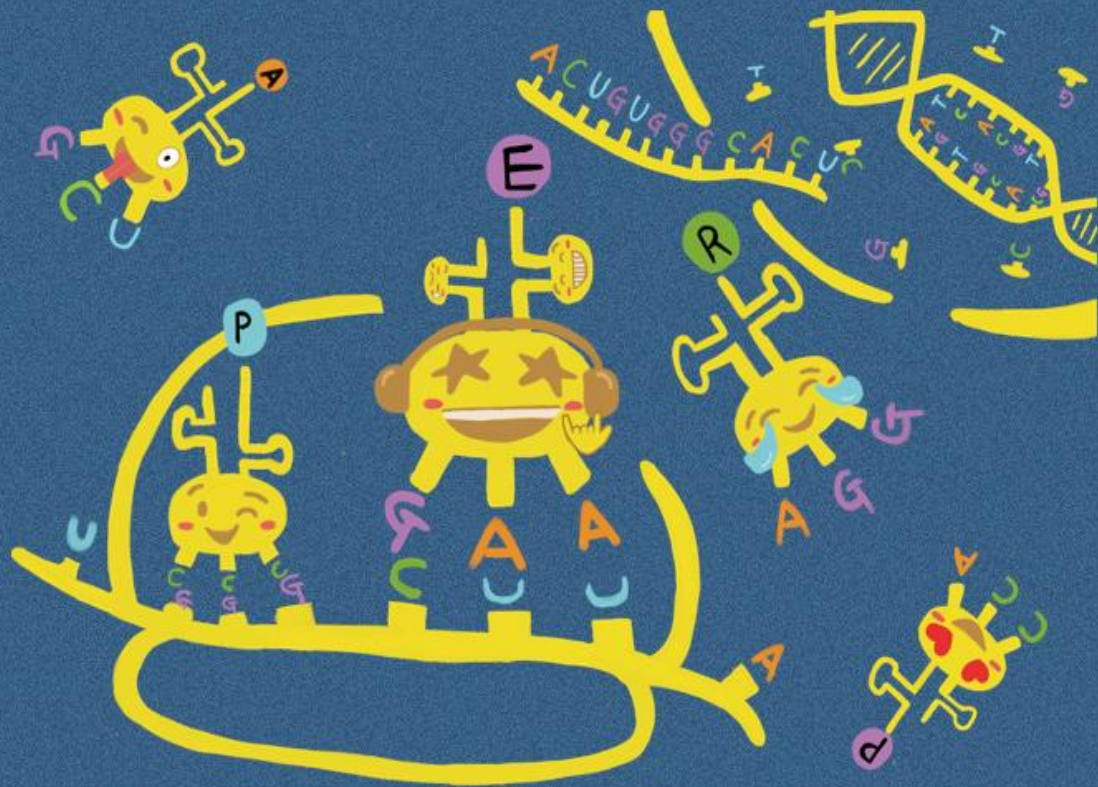


ChemIDoc MP Imaging System



# *Notes*

# *Notes*



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Postgraduate Research Day 2018*

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CANCER BIOLOGY AND EXPERIMENTAL THERAPEUTICS  
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