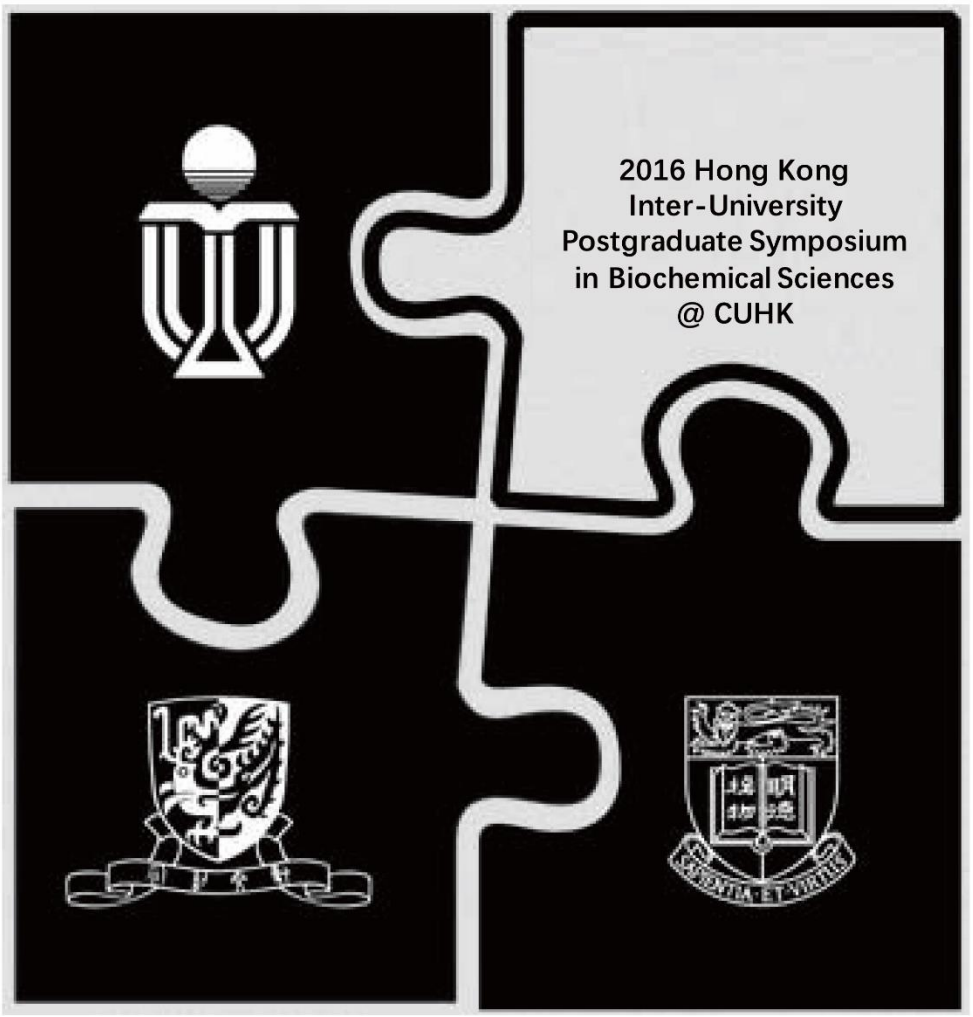
2016 Hong Kong Inter-University

Postgraduate Symposium in Biochemical Sciences



11th June, 2016

Lee Shau Kee Building

The Chinese University of Hong Kong

Hong Kong, CHINA

Symposium Organizing Committee

Professor Ho Yin Edwin CHAN
School of Life Sciences
The Chinese University of Hong Kong

Professor Chun Alex KOON
School of Life Sciences
The Chinese University of Hong Kong

Professor Hiu-Tung Tom CHEUNG
Division of Life Science
Hong Kong University of Science and Technology

Professor Dong-Yan JIN
School of Biomedical Sciences
The University of Hong Kong

Mr. Shao-hong PENG
Postgraduate student representative
School of Life Sciences
The Chinese University of Hong Kong

Miss Lu YUE
Postgraduate student representative
Division of Life Science
Hong Kong University of Science and Technology

Miss Sin-yee FUNG
Postgraduate student representative
School of Biomedical Sciences
The University of Hong Kong

Sponsors

UGC 大學教育資助委員會
University Grants Committee

Chung Chi College, CUHK

Event Schedule

2016 Hong Kong Inter-University

Postgraduate Symposium in Biochemical Sciences

8:15 a.m. – 9:00 a.m.	Registration + Poster Setup
9:00 a.m. – 9:15 a.m.	Opening Remarks
9:15 a.m. – 10:35 a.m.	Student Stage Presentation Session I
	Yip, RKH
	Investigating molecular pathogenesis of Campomelic Dysplasia
	Leung, KY
	Revealing Phylogenetic Differences in Strains using Optical Mapping
	An, Y
	A Molecular Switch that Regulates Cell Fate Choice Between Muscle Progenitor Cells and Brown Adipocytes
	Chen, ZF
	Recruitment of a transcriptional repressor to protein aggregates leads to de-repression of a pro-apoptotic gene activity and contributes to neuronal toxicity in polyglutamine diseases.
10:35 a.m. – 11:35 a.m.	Plenary Talk - Professor Ping-Yee LAW, University of Minnesota, USA
	Morphine regulates NeuroD1 activity and adult neurogenesis: a key to the retention of opiate drug experience?
11:35 a.m. – 12:20 p.m.	Tea Reception + Student Poster Session

12:20 p.m. – 1:20 p.m.	Plenary Talk - Professor Xiaohua GONG, University of California, Berkeley, USA From Intercellular Communication to Mouse Models of Eye Diseases and Beyond
1:20 p.m. – 2:30 p.m.	Buffet Lunch + Student Poster Session
2:30 p.m. – 3:50 p.m.	Student Stage Presentation Session II Wang, T The Microglia Colonization of the Developing Zebrafish Brain Is Dynamic and Driven by Apoptotic Neuron-Released Chemoattractant An, L USP7 Enforces RNF169-dependent DNA Double-Strand Break Responses MA, TC Canonical BMP signaling is required for the maintenance of neural stem cells at cerebellar ventricular zone Chiu, DKC Hypoxia Induces Myeloid-derived Suppressor Cell Recruitment to Hepatocellular Carcinoma Through Chemokine (C-C motif) Ligand 26
3:50 p.m. – 4:30 p.m.	Tea Reception + Student Poster Session
4:30 p.m. – 5:50 p.m.	Student Stage Presentation Session III Lo, CY In-silico screening for inhibitors blocking the assembly of Influenza

	A virus polymerase complex
	Zeng, M
	A new binding site outside the canonical PDZ domain determines the specific interaction between Shank and SAPAP and their function
	Chaudhary, V
	Opposite effect of severe fever-with-thrombocytopenia syndrome virus NSs protein on type I and type II interferon signaling
	Su, YT
	Elucidation of the functional roles of Rho GTPase-activating proteins in adult neurogenesis
6:00 p.m. – 6:15 p.m.	Closing Ceremony + Award Presentation

Abstracts of
Platform Presentation

T01

Investigating molecular pathogenesis of Campomelic Dysplasia

Raymond KH Yip[1], Tiffany YK Au[1], Danny Chan[1] and Kathryn SE Cheah[1]

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

Email: rayyip@connect.hku.hk

Two decades after the discovery that sequence alterations within and around *SOX9* cause Campomelic Dysplasia (CD) - a rare skeletal malformation syndrome characterized by severe bowing of long bones (campomelia), the underlying molecular pathogenesis leading to bone dysmorphism remains unclear. *SOX9*^{Y440X} is the most recurrent mutation identified in CD patients. In *Sox9*$^{+/Y440X}$ mice that recapitulate the human CD syndrome, an expanded population of osteoblasts was detected in the bowed tibiae, suggesting a causative relationship between altered osteogenesis and campomelia. As hypertrophic chondrocytes (HCs) was recently shown as a major source of endochondral osteoblasts, we hypothesized that the CD-associated *Sox9*^{Y440X} mutation may impact this chondrocytes-to-osteoblasts lineage progression in causing campomelia. To test this, we utilized *Col10a1-Cre* to conditionally activate the *Sox9*^{Y440X} mutation in HCs and to trace their fates by lineage analyses utilizing fluorescent protein Cre-reporters. Remarkably, homozygous mutants expressing *Sox9*^{Y440X} specifically in HCs were markedly dwarfed and exhibited congenital anterolateral bowing of tibiae. While the mutant Sox9^{Y440X} protein did not evidently impair or promote HCs differentiation into osteoblasts, it elevated Ihh signaling in the growth plate to induce excessive periosteal ossification. The aberrantly formed periosteal mass in consequence caused mispositioning of the primary ossification center to prompt campomelia. Intriguingly, genetic inactivation of *β-catenin* in the conditional *Sox9*^{Y440X} mutants greatly ameliorated campomelia, implying the phenotype was consequential to a neomorphic function of Sox9^{Y440X} in enhancing β-catenin activity. In conclusion, these findings propose that aberrant periosteal ossification owing to dysregulation of Hh signaling may underlie campomelia phenotype in CD patients.

T02

Revealing Phylogenetic Differences in Strains using Optical Mapping

Leung KY [1], Law YT [2], Chung YL [1], Lai YY [3], Yip YL [4], Kwok PY [3], Ho PL [2] and Chan TF [1]

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

[2] Department of Microbiology, The University of Hong Kong, Hong Kong, China

[3]Cardiovascular Research Institute, University of California – San Francisco, San Francisco, CA, USA

[4] Department of Computer Science and Engineering, The Chinese University of Hong Kong, Hong Kong, China

Email: aldenleung@link.cuhk.edu.hk

Pulse-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST) have been the two major molecular techniques for bacterial strain typing. Optical mapping (OM), a single DNA-molecule imaging technique that captures specific labelling patterns along the DNA molecule, has become an emerging technology. We have performed strain typing by optical mapping on 24 *Staphylococcus aureus* and 24 *Acinetobacter baumannii* clinical isolates. We developed new analytical methods that could differentiate samples down to strain-level resolution, as confirmed by PFGE and MLST results. In addition, our results could reveal structural diversities among different strains, which could not be provided by PFGE or MLST. In summary, we propose that optical mapping could be an alternative technology of choice for microbial strain typing.

T03

A Molecular Switch that Regulates Cell Fate Choice Between Muscle Progenitor Cells and Brown Adipocytes

Yitai An[1], Gang Wang[1], Yarui Diao[1], Mingxi Weng[1], Liang Zhou[2], Kun Sun[2], Tom H Cheung[1], Nancy Ip[1], Hao Sun[2], Huating Wang[2], and Zhenguo Wu[1]

1. Division of Life Science, Center for Stem Cell Research, Center for Systems Biology and Human Health, and the State Key Laboratory in Neuroscience, Hong Kong University of Science &Technology

2. Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong, China

yan@connect.ust.hk

During mouse embryo development, both skeletal muscle and brown adipose tissue (BAT) derive from the same $Pax7^+/Myf5^+$ progenitor cells. However, it is unclear how individual cell fate is selected and stably maintained. Unexpectedly, in cultured *Pax7*-null muscle progenitor cells (MPC) from young mice, several brown adipocyte (BA)-specific genes including *Prdm16* and *Ucp1* and many other adipocyte-related genes were upregulated with a concomitant reduction of key muscle-determination genes, suggesting a cell fate change from MPC to BA. Consistently, freshly-isolated *Pax7*-null but not wild-type MPC formed $Ucp1^+$ BA in culture. Mechanistically, MyoD and Myf5, two master regulators acting downstream of Pax7 in MPC, potently repress *Prdm16*, a master regulator for the brown adipogenic program, via the E2F4/p107/p130 repressor complex. Importantly, inducible *Pax7* ablation in developing mouse embryos promoted BAT development. Thus, the Pax7-MyoD/Myf5-E2F4/p107/p130 axis serves as a critical switch in $Pax7^+/Myf5^+$ progenitor cells to regulate cell fate choice between MPC and BA.

T04

Recruitment of a transcriptional repressor to protein aggregates leads to de-repression of a pro-apoptotic gene activity and contributes to neuronal toxicity in polyglutamine diseases.

Chen ZF[1,2], Peng SH[1,2], Zhang Q[1,2], Rudnicki DD[3] and Chan HYE[1,2]

[1]Laboratory of *Drosophila* Research, School of Life Sciences, Faculty of Science, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China.

[2]Biochemistry Program, School of Life Sciences, Faculty of Science, The Chinese University of Hong Kong, Shatin, N.T., Hong Kon, China.

[3]Department of Psychiatry and Behavioral Sciences, Division of Neurobiology, Program of Cellular and Molecular Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA.

Email: cbestop123@gmail.com

Polyglutamine (polyQ) diseases are a growing group of dominant heritable neurodegenerative diseases caused by the expansion of glutamine-encoding CAG repeats located within the coding region of the affected genes. Expression of the mutant disease genes results in the biosynthesis of elongated polyQ disease proteins. Protein aggregate formation is a pathogenic hallmark of polyQ diseases. Polyglutamine aggregates recruit essential proteins in affected neurons, which leads to the cellular depletion of these aggregate-recruited proteins (ARPs) and results in perturbation of neuronal cell function. Transcriptional dysregulation caused by ARPs has been reported in several polyQ diseases. Here, we demonstrate the sequestration of a transcriptional repressor protein to polyQ aggregates in the brain tissues of polyQ patients. We showed that the depletion of this repressor protein causes the transcriptional de-repression of a pro-apoptotic gene expression in cellular disease models and subsequently leads to neuronal apoptotic cell death. In summary, our investigation unveils a polyQ pathogenic pathway that involves a transcriptional repressor-triggered promotion of apoptotic cell death in polyQ disease. Targeting this novel pathway may be therapeutically beneficial.

T05

The Microglia Colonization of the Developing Zebrafish Brain Is Dynamic and Driven by Apoptotic Neuron-Released Chemoattractant

Jin Xu[1,2], Tienan Wang[1,2], Yi Wu, Wan Jin, and Zilong Wen[1,*]

[1]State Key Laboratory of Molecular Neuroscience, Center for Systems Biology and Human Health, Division of Life Science, the Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, P.R. China

[2]These authors contributed equally to this work.

[*]Correspondence should be addressed to Z.L.W.:

Email: tnwang@connect.ust.hk

Microglia are central nervous system (CNS)-resident macrophages and play important roles in neural development and function. Yet, how microglial precursors born in peripheral hematopoietic organs colonize the CNS remains undefined. Here, by utilizing *in vivo* imaging and genetic manipulation of zebrafish, we showed that microglial precursors enter the optic tectum of the midbrain, where the majority of microglia reside during early development, via the lateral periphery between the eyes and brain and the ventral periphery of the brain in a circulation-independent manner. Intriguingly, the long-term colonization of the optic tectum by microglial precursors requires two distinct processes: homing and settling, both of which are driven by apoptotic neuronal death in the brain. We further showed that the homing process is mediated, at least in part, by lysophosphatidylcholine (LPC) released from apoptotic neurons. Our study reveals that microglia colonization of the developing brain is driven by apoptotic neurons naturally occurred during neurogenesis via secretion of chemoattractant.

T06

USP7 Enforces RNF169-dependent DNA Double-Strand Break Responses

Liwei An[1,*], Yiyang Jiang[2,*], Howin H.W. Ng[1], Jie Chen[1], Qingguo Gong[2,#], Michael S.Y. Huen[1,3,#]

[1]School of Biomedical Sciences, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong S.A.R.;

[2]School of Life Sciences, University of Science and Technology of China, Hefei, China;

[3]State Key Laboratory of Brain and Cognitive Sciences, The University of Hong Kong, Hong Kong S.A.R.

Email: liweian1988@gmail.com

RNF169 encodes a cell intrinsic mechanism that modulates chromatin responses at DNA double-strand breaks (DSBs). To explore how RNF169 is regulated, we mined the RNF169 interactome and have uncovered the USP7 deubiquitylase as a *bona fide* RNF169-interacting factor. Interestingly, USP7 directly interacts with RNF169 at a conserved USP7-binding motif that overlaps with a classical nuclear localization sequence. Accordingly, crystal structures and isothermal titration calorimetric analyses of USP7 ubiquitin-like domains in complex with RNF169 guided the design of a separation-of-function RNF169 mutant, allowing the study of a USP7 binding-defective nuclear RNF169. Finally, genetic ablation or chemical inhibition of USP7 destabilised RNF169, impaired its docking at DSBs, and relieved the RNF169-imposed restriction of DSB repair. Collectively, our findings identify the USP7-RNF169 axis as a regulatory component of the DSB signal transduction cascade.

T07

Canonical BMP signaling is required for the maintenance of neural stem cells at cerebellar ventricular zone

MA Tsz Ching[1], VONG Keng Ioi[1], KWAN Kin Ming[1,2,3]

[1]School of Life Sciences, [2]Centre for Cell and Developmental Biology, [3]State Key Laboratory of Agrobiotechnology (CUHK), The Chinese University of Hong Kong, Hong Kong, P.R. China.

E-mail of the presenting author: charlottetcm@yahoo.com.hk

E-mail of the corresponding author: kmkwan@cuhk.edu.hk

Cerebellum of the central nervous system is responsible for motor coordination and body balance. From embryonic day (E) 10.5 onwards, different types of neurons are sequentially produced from the two cerebellar germinal matrices, namely the anterior rhombic lip (ARL) and the ventricular zone (VZ). The ARL and the VZ are functionally distinct neural stem cell pools responsible for the production of cerebellar glutamatergic neurons and GABAergic neurons, respectively. Canonical BMP signaling was shown to be critical to neurogenesis at the ARL. However, its role in VZ remains uncharacterized. We showed that phosphorylated Smad1/5 were expressed in both the ARL and VZ, suggesting that canonical BMP signaling may regulate VZ neurogenesis. Importantly, conditional knockout of *Smad1/5* in mouse cerebellum resulted in marked reduction in cell proliferation at cerebellar VZ and quicker depletion of cerebellar neural stem cells. Moreover, mRNA expression of cell cycle regulators was dysregulated. On the other hand, we observed reduced expression of junction proteins in the apical endfeet of radial glial cells, which may lead to endfeet detachment from the apical surface in the Smad1/5 mutants. Accordingly, neuronal specification was accelerated, which accounts for precocious generation of Purkinje cells and interneurons in mutants. In summary, we present novel evidence that canonical BMP signaling is important to cerebellar VZ development. Our results suggest Smad1/5 is essential for the maintenance of neural stem cell identity, probably through the regulation on cell cycle progression and apical attachment of radial glial cells.

T08

Hypoxia Induces Myeloid-derived Suppressor Cell Recruitment to Hepatocellular Carcinoma Through Chemokine (C-C motif) Ligand 26

David Kung-Chun Chiu[1], Iris Ming-Jing Xu[1], Robin Kit-Ho Lai[1], Aki Pui-Wah Tse[1], Larry Lai Wei[1], Hui-Yu Koh[1], Lynna Lan Li[1], Derek Lee[1], Regina Cheuk-Lam Lo[1,2], Chun-Ming Wong[1,2], Irene Oi-Lin Ng[1,2], Carmen Chak-Lui Wong[1,2]

[1]Department of Pathology, The University of Hong Kong, Hong Kong

[2]State Key Laboratory for Liver Research, The University of Hong Kong, Hong Kong

Email: kcchiu@pathology.hku.hk carmencl@pathology.hku.hk

A population of stromal cells, myeloid-derived suppressor cells (MDSCs), are present in tumors. Though studies have gradually revealed the pro-tumorigenic functions of MDSCs, the molecular mechanisms guiding MDSC recruitment remain largely elusive. Hypoxia, O_2 deprivation, is an important factor in the tumor microenvironment of solid cancers whose growth often exceeds growth of the functional blood vessels. Here, using hepatocellular carcinoma (HCC) as the cancer model, we show that hypoxia is an important driver of MDSC recruitment. We observe that MDSCs preferentially infiltrate into hypoxic regions in human HCC tissues and hypoxia-induced MDSC infiltration is dependent on hypoxia-inducible factors (HIFs). We further find that HIFs activate the transcription of chemokine (C-C motif) ligand 26 (CCL26) in cancer cells to recruit chemokine (C-X3-C motif) receptor 1 (CX_3CR1)-expressing MDSCs to the primary tumor. Knockdown of CCL26 in cancer cells profoundly reduces MDSC recruitment, angiogenesis, and tumor growth. Therapeutically, blockade of CCL26 production in cancer cells by HIF inhibitor, digoxin, or blockade of CX_3CR1 in MDSCs by CX_3CR1 neutralizing antibody could substantially suppress MDSC recruitment and tumor growth. In conclusion, this study unprecedentedly reveals a novel molecular mechanism by which cancer cells direct MDSCs homing to primary tumor and suggests that targeting MDSC recruitment represents an attractive therapeutic approach against solid cancers.

T09

In-silico screening for inhibitors blocking the assembly of Influenza A virus polymerase complex

Lo CY[1], Yang YH[1], Poon LLM[2] and Shaw PC[1]

1. Centre for Protein Science and Crystallography, School of Life Sciences,

The Chinese University of Hong Kong, Shatin, Hong Kong SAR, China

2. Centre of Influenza Research, School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China

Email: locyedwin@gmail.com

Influenza virus has always been a major threat to humankind, causing sporadic pandemics and recurrent annual epidemics. Moreover, as influenza virus is developing resistance to existing antivirals, it is essential to design new drugs against it. The influenza RNA-dependent RNA polymerase consists of three subunits - PA, PB1 and PB2. By blocking the protein-protein interactions among these subunits, the viral RNA polymerase complex would fail to assemble, thereby inhibiting Influenza virus replication. The co-crystal structure of PA-C terminal and PB1-N terminal was resolved in 2008. It was shown that PB1 binds to PA by inserting a short helix into a hydrophobic core of PA, and the residues at the interacting interface are well conserved within type A Influenza. We employed in-silico screening to identify small molecules that most likely would block the PAPB1 interaction. Compound databases were archived from ZINC (UCSF) and commercial vendors (e.g. SPECS) and then virtually docked to the PA hydrophobic core by Autodock 4.0. Top results were then subjected to post-screening evaluation, including visual inspection by molecular visualization software (e.g. Pymol) and prediction of drug-likeness by Lipinski's rules. After post-screening analysis, we selected ~150 potential hit compounds for primary screening, which involves cytotoxicity assay and ribonucleoprotein (RNP) activity assay. Two hit compounds, compound 221 and 312, were able to inhibit influenza RNP activities and attenuate viral growth. Compound 312 also showed mild effect in vivo using influenza virus infected mouse model. The identification of hit compounds provides the basis for future optimization and lead compound development against influenza virus.

T10

A new binding site outside the canonical PDZ domain determines the specific interaction between Shank and SAPAP and their function

Menglong Zeng

Division of Life Science, State Key Laboratory of Molecular Neuroscience, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, China

Presenting author : Menglong Zeng mlzeng@ust.hk

Corresponding author: Prof. Mingjie Zhang mzhang@ust.hk

Shank and SAPAP are two highly abundant scaffold proteins that directly interact with each other to regulate excitatory synapse development and plasticity. Mutations of *SAPAP*, but not other reported Shank PDZ domain binders, share a significant overlap on behavioral abnormalities with the mutations of *Shank* both in patients and animal models. However, the molecular mechanism governing the exquisite specificity of the Shank/SAPAP interaction is not clear. Here, we discover that a sequence proceeding the canonical PDZ domain of Shank, together with the elongated PDZ BC-loop, form another binding site for a sequence upstream of the SAPAP PDZ binding motif (PBM), leading to several hundred folds affinity increase of the Shank/SAPAP interaction. We provide evidence that the specific interaction afforded by this newly identified site is required for Shank synaptic targeting and Shank-induced synaptic activity increase. Our study provides a molecular explanation on how Shank and SAPAP dosage changes due to their gene copy number variations can contribute to different psychiatric disorders.

T11

Opposite effect of severe fever-with-thrombocytopenia syndrome virus NSs protein on type I and type II interferon signaling

Vidyanath Chaudhary,[1] Shuo Zhang,[2] Kit-San Yuen, [1] Dexin Li,[2] Kin-Hang Kok,[3] Mifang Liang[2] and Dong-Yan Jin[1]

[1]School of Biomedical Sciences, The University of Hong Kong, Pokfulam, Hong Kong
[2]Key Laboratory for Medical Virology and National Institute for Viral Disease Control and Prevention, Chinese Centre for Disease Control and Prevention, Beijing 102206, China
[3]Department of Microbiology, The University of Hong Kong, Pokfulam, Hong Kong

Innate interferon (IFN) response that inhibits viral replication is the first-line host defense against viral infection. To circumvent this response, viruses have developed various counter-measures to antagonize IFN production and/or signaling. Severe fever-with-thrombocytopenia syndrome virus (SFTSV) is an emerging zoonotic pathogen initially identified in China and subsequently found in other parts of the world. SFTSV NSs protein is an IFN antagonist that has been shown to counteract type I IFN induction by targeting TBK1 and IKKε kinases and to impede IFN signaling by interacting with and sequestrating STAT2 in the cytoplasm. In this study, we demonstrated that SFTSV NSs protein suppresses both production and signaling of type I and type III IFNs by preventing STAT1 phosphorylation and activation whereas augments type II IFN signaling. Infection with SFTSV or expression of its NSs protein potently inhibited not only the production of IFN-β induced by double-stranded RNA but also the activation of representative IFN-stimulated genes (ISGs) by IFN-α1, IFN-β, IFN-λ1 and IFN-λ2. In contrast, expression of NSs or infection with SFTSV had no influence on the activation of NF-κB signaling. Co-immunoprecipitation experiments indicated that NSs protein interacts with STAT1 and STAT2. NSs functioned not only to sequester STAT1 and STAT2 in the cytoplasm, but also to inhibit IFN-β-induced phosphorylation at serine 727 of STAT1. However, serine 701 phosphorylation was unaffected. Furthermore, STAT1 protein was inhibited at the transcriptional level. Chromatin immunoprecipitation assay showed that the recruitment of STAT1 and STAT2 to ISRE promoters of ISGs was attenuated when NSs was expressed. IRF1 and CXCl10 expression was further induced as a result of the increase in STAT1 phosphorylation in the presence of both IFN-γ and NSs. Taken together, our findings suggested that SFTSV NSs protein is a viral modulator of IFN signaling that has opposite effect on type I and type II IFN signaling. Mechanistically, NSs might differentially regulate STAT1 and STAT2 activation induced by type I and type II IFNs. Our work provides new knowledge in SFTSV pathogenesis and has implications in the design and development of anti-SFTSV agents and vaccines. This work was supported by RGC-NSFC JRS (N-HKU 714/12), RGC CRF (HKU1/CRF/11G and C7011-15R) and HMRF (HKM-15-M01).

T12

Elucidation of the functional roles of Rho GTPase-activating proteins in adult neurogenesis

Su YT[1,2,3], Ip Jacque PK [1,2,3], Min L [1,2,3], Fu AK [1,2,3], Ip NY*[1,2,3]

[1]Division of Life Science, [2]Molecular Neuroscience Center and

[3] State Key Laboratory of Molecular Neuroscience,

The Hong Kong University of Science and Technology,

Clear Water Bay, Hong Kong, China.

Email: ysuah@ust.hk

Adult hippocampal neurogenesis is the continuous generation of new neurons in the subgranular zone of dentate gyrus throughout life. It is regulated by both intrinsic and extrinsic signals. Dysfunctions of this process may lead to neurological disorders such as autism, epilepsy and schizophrenia. Rho GTPase-activating proteins (Rho-GAPs) are a family of regulatory proteins that bind to activated Rho GTPases, stimulating their GTPase activity. While Rho-GAPs regulate neuronal differentiation, neurite outgrowth, and migration during central nervous systemic development, whether they are important for the regulation of adult neurogenesis remains largely unknown. Accordingly, we investigated the role of a Rho-GAP α2-chimaerin in adult neurogenesis. We found that α2-chimaerin–knockout mice exhibited decreased adult hippocampal neurogenesis. In particular, the effect of α2-chimaerin was mediated through the regulation of neural progenitor cell proliferation, but not differentiation or maturation. Further study involving conditional knockout of α2-chimaerin showed a shift of the division mode of radial glia-like cells/neural progenitors from asymmetric to symmetric division. Furthermore, the dendritic development of adult-born neurons was attenuated in the α2-chimaerin–knockout dentate gyrus. These findings collectively indicate that the Rho-GAP α2-chimaerin is critical for adult hippocampal neurogenesis, suggesting it as a possible target for developing therapeutic treatment of neurological disorders.

Abstracts of
Poster Presentation

P01

The K63-Deubiquitylating Enzyme BRCC36 Limits DNA Break Processing and Repair

Ng HM[1], Wei LZ[2], Lan L[2], Huen MSY[1,3]

[1]School of Biomedical Sciences, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong S.A.R.;

[2]Department of Microbiology & Molecular Genetics,

University of Pittsburgh School of Medicine, Pennsylvania, 15213, USA;

[3]State Key Laboratory of Brain and Cognitive Sciences, The University of Hong Kong, Hong Kong S.A.R..

Email: alicenghoiman@gmail.com

Multi-subunit protein assemblies offer integrated functionalities for efficient cell signal transduction control. One example of such protein assemblies, the BRCA1-A macromolecular complex, couples ubiquitin recognition and metabolism, and promotes cellular responses to DNA damage. Specifically, the BRCA1-A complex not only recognises lys63-linked ubiquitin (K63-Ub) adducts at the damaged chromatin, but is endowed with K63-Ub deubiquitylating (DUB) activity. To explore how the BRCA1-A DUB activity contributes to its function at DNA double-strand breaks (DSBs), we used RNAi and genome editing approaches to target BRCC36, the protein subunit that confers the BRCA1-A complex its DUB activity. Intriguingly, we found that the K63-Ub DUB activity, although dispensable for maintaining the integrity of the macromolecular protein assembly, is important in enforcing the accumulation of the BRCA1-A complex onto DSBs. Inactivating BRCC36 DUB attenuated BRCA1-A functions at DSBs, and led to unrestrained DSB end resection and hyperactive DNA repair. Detailed analysis of BRCC36 at a Fok1-induced DSB also revealed that the K63-Ub DUB is key in its proper configuration and occupancy at the DSB-flanking chromatin domains. Together, our findings uncover a key role of BRCC36 DUB in limiting DSB processing and repair, and illustrate how cells may physically couple ubiquitin recognition and metabolising activities for fine-tuning of DNA repair processes.

P02

Eukaryotic translation elongation factor 1 alpha 1 is Involved in the Nucleocytoplasmic Transport of Proteins Carrying an Expanded Polyalanine Tract

Li L[1,2], Ng KL[1,2] and Chan HYE[1,2]

[1] Laboratory of Drosophila Research, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China

[2] School of Life Sciences, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China

Email: liwenxuetong@126.com

hyechan@cuhk.edu.hk

Polyalanine (polyA) diseases are caused by expansion of translated GCN triplet nucleotide sequences encoding for polyA tract in disease proteins. To date, nine human disorders are found to associate with polyA tract expansion, examples include congenital central hypoventilation syndrome and oculopharyngeal muscular dystrophy. Of note, eight out of nine polyA disease genes encode transcription factors with important roles in development and differentiation. Previous studies demonstrated that wild type unexpanded polyA proteins localized to the cell nucleus while expanded polyA disease proteins primarily localized to the cytoplasm. The mislocalization of expanded polyA disease proteins caused cellular transcriptional dysregulation, which subsequently leads to cell dysfunction. Our laboratory found that expanded polyA domain possesses nuclear export signal activity. By means of glutathione-S-transferase pull-down assay followed by mass spectrometry, eukaryotic translation elongation factor 1 alpha 1 (eEF1A1) was identified as an interacting protein of expanded polyA-containing protein. Knockdown of eEF1A1 expression suppressed expanded polyA protein nuclear export and restored its function. Further, a polyA-SoxNeuro (SoxN) transgenic fly model was established. SoxNeuro is the Drosophila ortholog of the human Sox3 gene, which is a polyA disease gene that associates with mental retardation. The expanded polyA-SoxN protein showed cytoplasmic localization and exerts toxicity in a transgenic Drosophila model. Upon eEF1A1 knockdown, the expanded polyA-SoxN protein was retained in the nucleus and its toxicity was attenuated. Taken together, this study demonstrated that eEF1A1 is a regulator of expanded polyA domain nuclear export, and I established a connection between protein nuclear export and polyA neurodegeneration.

P03

Functional characterization and therapeutic role of Interleukin-1 receptor-associated kinase 1 (IRAK1) in hepatocellular carcinoma

CHENG BYL[1,2], NG IOL[1,2] and LEE TKW[3]

[1]State Key Laboratory for Liver Research,

[2]Department of Pathology, The University of Hong Kong

[3]Deparment of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University

chengyikling.bowie@gmail.com

Hepatocellular carcinoma (HCC) is the second most fatal cancer worldwide. Frequent relapse and drug resistance could be attributed to the existence of cancer stem cells (CSCs) within the tumor bulk. From transcriptome sequencing of 16 pairs of HCC clinical samples, we identified Interleukin-receptor associated kinase 1 (IRAK1) to be significantly upregulated in HCC. IRAK1 overexpression in HCC was further confirmed at mRNA and protein level, and correlated with larger tumor size. Through lentiviral based knockdown and overexpression approaches, we demonstrated that IRAK1 enhanced cell proliferation and promoted CSC properties, including self-renew, *in vivo* tumorigenicity and drug resistance. Pharmacological inhibition of IRAK1 with specific IRAK1/4 kinase inhibitor not only downregulated expression of liver CSC markers including CD24 and CD47; but also consistently suppressed self-renewal, cell proliferation, migration and invasion ability. In addition, IRAK1 inhibition sensitized the cells to doxorubicin and sorafenib treatment *in vitro* through suppression of apoptotic cascade; and reduced tumor volume *in vivo*. Through RNA sequencing analysis by comparing the gene expression profiles between IRAK1 knockdown and control cells, we identified Aldo-Keto Reductase Family 1, Member 10 (AKR1B10) as a novel downstream target of IRAK1. Their interaction was reinforced by the positive correlation between IRAK1 and AKR1B10 expression in HCC samples. Clinically, AKR1B10 was found to be overexpressed in HCC which was associated with poorer patient's survival. Functional analysis demonstrated that knockdown of AKR1B10 offset the IRAK1 induced self-renewal ability via AP-1 complex. Collectively, targeting IRAK1 signaling pathway may be a potential therapeutic strategy against HCC.

P04

Targeting Liver-Tumor Initiating Cells via Hampering the Lipogenesis Pathways through Stearoyl – CoA Desaturase

Kin-Fai MA[1,2], Jessica Lo[1,2], Eunice Yuen-Ting Lau[1,2], Copland John A[3], Irene Oi-Lin Ng[1,2] and Terence Kin-Wah Lee[1,2]

State Key Laboratory for Liver Research[1], Department of Pathology, Li Ka Shing Faculty of Medicine[2], The University of Hong Kong. Mayo Clinic Jacksonville[3], The Department of Cancer Biology, Jacksonville, FL 32224, USA.

Email address: markmkf@hku.hk, tkwlee@hku.hk

Hepatocellular carcinoma is one of the most fatal cancers in the world. Sorafenib is the only FDA approved drug but only extend patients' survival to a median of 3 months. Increasing evidence showed that tumor-initiating cells (T-ICs) are intrinsically resistant to conventional treatments and targeting these cells provides potential therapeutic targets for HCC. For this purpose, we enriched liver T-IC population by serial passages of hepatospheres combined with chemotherapeutic regimens. By cDNA microarray, we found upregulation of stearoyl coA desaturase-1 (SCD1), a key enzyme in lipogenesis, in enriched T-IC population. In HCC clinical samples, 62% of patient samples showed SCD1 overexpression which was associated with shorter disease free survival. Using overexpression and knockdown approaches, SCD1 was found to regulate the traits of T-ICs, including tumorigenicity, self-renewal, drug resistance and expression of liver T-IC markers. Interestingly, SCD1 was markedly upregulated in sorafenib-resistant HCC cells. Pharmacological inhibition of SCD1 not only suppressed self-renewal ability but also consistently enhanced the sensitivity of sorafenib. Using a patient-derived xenograft model (PDTX#1), we found that a novel SCD1 inhibitor (SSI-4) demonstrated maximal growth suppressive effect when combined with sorafenib treatment. Mechanistically, we found that SCD1 maintains the T-IC functions thorough upregulation of ER stress genes including GRP78 and CHOP, as the suppressive effect of T-IC functions upon SCD1 inhibition was rescued by administration of ER stress inhibitor, TUDCA. Collectively, we demonstrated the crucial role of SCD1 in maintenance of liver T-ICs, and targeting SCD1 in combination with sorafenib might be a novel therapeutic regimen against HCC.

P05

Role of a PPARα-regulated sulfotransferase (mL-STL) in embryonic development and bile acid-dependent energy homeostasis

Wang, Kai[1], Cheung Wing Tai[2] and Lee Sau Tuen Susanna[1]

[1]School of Life Sciences, and [2]School of Biomedical Sciences,

The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China

Email: k.wang1102@hotmail.com

Peroxisome proliferator-activated receptor alpha (PPARα) is a ligand-activated transcription factor and a nuclear hormone receptor. PPARα regulates the expression of its target genes and most of them were reported to help maintaining the energy homeostasis during energy deprivation. A new mouse sulfotransferase (SULT) gene, mL-STL, which can be activated by PPARα under energy deprivation, was discovered in our laboratory. mL-STL specifically catalyzed the sulfonation of primary bile acids, which is considered to be one of the reactions to eliminate bile acids. This finding suggests that mL-STL might play a significant role in bile acid homeostasis. Surprisingly, recent evidence also suggests that bile acids act as signaling molecules which can regulate energy metabolism. Thus, we hypothesis that mL-STL, a bile acid-specific SULT, is also essential in maintaining energy balance through the regulation of bile acid homeostasis.

To test our hypothesis, we focused on constructing an mL-STL-null transgenic mouse model. Unexpectedly, no homozygous mutants were found in the newborns from heterozygous mutants intercross, suggesting that mL-STL is required for embryonic development. Our next plan is to explore the mechanism of mL-STL in embryonic lethality. In addition, heterozygous mutant mice will be used for characterizing the role of mL-STL in bile acid and energy balance. This study may help to unveil the role of mL-STL in embryonic development as well as the physiological role of sulfonation in maintaining bile acid and energy homeostasis. [The work described in this abstract was substantially supported by a grant from the Research Grants Council of the Hong Kong Special Administrative Region, China {Project No.: CUHK 14109414}]

P06

PPAR-regulated TCMs as a therapeutic tool to target Metabolic Syndrome

Choy Jonathan,[1] Liu Xing,[1] Ko Ka Shun Joshua,[2] Lau Bik San Clara,[3] Cheung Wing Tai[4] and Lee Sau Tuen Susanna[1]

[1]School of Life Sciences, [3]Institute of Chinese Medicine, and [4]School of Biomedical Sciences, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China.

[2]School of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China.

Email: jonpychoy@gmail.com

Metabolic syndrome (MetS) is defined as a cluster of metabolic risk factors that cause cardiovascular disease. Three different isoforms of the nuclear receptor "peroxisome proliferator-activated receptors" (PPARα, PPARβ, PPARγ) up-regulate genes involved in fatty acid catabolism, adipocyte differentiation and lipoprotein metabolism, and thus ligands of PPAR can be used as therapeutic targets for treatment of MetS. The current therapeutic approach is to search for dual-agonists or pan-agonists (activates all three isoforms) of PPAR, where the synergistic effects of the different PPAR receptors combined with fewer side effects make them superior to their selective mono-agonist counterparts. Because TCMs have a known history of human safety and contain multiple different compounds, they may serve as good candidates to search for dual- and pan- agonists of PPAR for the treatment of MetS. In this study, the aqueous extracts of 10 TCMs, which are widely used to treat hyperlipidemia, have been investigated for a possible mechanism of hypolipidemic effects by acting as a dual- or pan-agonist of the PPAR receptor. The PPAR transactivation activity of the TCM candidates was determined through an *in vitro* luciferase reporter assay. Preliminary results show that several TCMs act as mono- or dual-agonists of PPAR. In future studies, adipocyte differentiation assays will be performed to determine how the aqueous extracts affect adipogenesis, and real-time PCR and Western blot will be conducted to determine whether PPARα target genes are activated. Promising candidates will then be used to develop a TCM formula for dyslipidemic treatment to target MetS. [The work described in this abstract was supported by the Health and Medical Research Fund (Project reference no. 12130531)].

P07

Effects of Oat Beta-Glucan on The Modulation of Gut Microbiome in Rats

Lam KL, and Cheung PCK

Food and Nutritional Sciences Program, School of Life Sciences,

The Chinese University of Hong Kong, Shatin, N. T., Hong Kong SAR

Email: kocaslam@gmail.com

Beta-glucans are complex non-digestible carbohydrates of D-glucose monomers linked by beta-glycosidic linkages. Structurally, beta-glucans are non-starch polysaccharides with repeating glucose residues with a linear chain having none or multiple side branches. Beta-glucans have long been shown to have health promoting effects including anti-tumor, immuno-stimulatory, and anti-obesity. More recently, beta-glucans are considered to have the potential to be used as novel prebiotics. Oat beta-glucan, one of the widely consumed beta-glucans, has a mixed beta-1,3 and 1,4- glycosidic linkages. Previously publications have demonstrated that purified oat beta-glucan gave a slight yet significant increase of *Bifidobacterium* and *Lactobacillus* and a significant reduction of *Enterobacteriaceae*. In this study, oat beta-glucan will be investigated for its prebiotic effect in the modulation of microbiome in rat.

Healthy male SD rats were used as the animal model for the investigation of the effects of microbiome modulation by pure oat beta-glucans used as prebiotics. The animals were randomly assigned to the control and treatment groups, which were administered with PBS and the prebiotics, respectively by intra-gastric gavage. After two weeks of treatment, the caecal contents were collected from the animals for DNA extraction. The 16S rDNA region was sequenced using ion torrent platform. Sequences were then analysed with the QIIME pipeline to find out the alpha and beta diversity. Our current findings show that oat beta-glucan: 1) had no adverse effect on rats in terms of body weight fluctuation; 2) increased the *Lactobacillus* populations; 3) reduced Clostridial population; 4) oat beta-glucan has the potential to be a novel prebiotic that can modulate the gut microbiome in rats, bringing health benefits to the host.

P08

Assembly and maturation of Golgi cisternae

in *Saccharomyces cerevisiae* revealed by electron tomography

Wang PF and Kang BH

School of Life Science, Center for Organelle Biogenesis and Function, State Key Laboratory for Agrobiotechnology, The Chinese University of Hong Kong, Hong Kong, PRC

Email: knightpfwang@gmail.com; bkang@cuhk.edu.hk

S. cerevisiae is the simplest eukaryotic model system and has been an important subject for dissecting the molecular mechanisms of membrane trafficking. Unlike the Golgi apparatus in other eukaryotes in which its cisternae are stacked, individual cisternae of the *S. cerevisiae* Golgi are dispersed throughout the cytoplasm. It was shown that marker proteins of *cis*, medial, *trans*, and the *trans*-Golgi network cisternae characterized in other stacked Golgi apparatus are associated with distinct Golgi cisternae in *S. cerevisiae*. It was also demonstrated that *cis* cisternae gradually transform into trans cisternae in *S. cerevisiae*, providing evidence for the cisternal maturation model. By combined use of electron tomography and immunogold labeling, we are characterizing how a new Golgi cisterna assembly, how *cis* cisternae mature into *trans* cisternae, and how *trans*-Golgi network cisternae arise. We observed clusters of COPII-like vesicles in the vicinity of the endoplasmic reticulum (ER) and dumbbell-shaped fusion intermediates in the clusters in our electron tomograms. Furthermore, we identified stacks of membrane discs labeled by an antibody against Sed5p suggesting that new Golgi cisternae can assemble in groups. The unique organization of the Golgi in *S. cerevisiae* will entail a special mechanism of its intra-Golgi trafficking. Our aim is to characterize the structural intermediates of cisternal assembly and maturation in the *S. cerevisiae* for comparing its mechanism of intra-Golgi trafficking with those of other eukaryotes.

P09

The kinesin motor protein KIF5B regulates RNA trafficking and dendritic spine morphogenesis in hippocampal neuron

Jonathan Hei-Lok Chan[1], Jiandong Huang[1, 2] and Kwok-On Lai[1, 2]

[1]School of Biomedical Sciences, [2]State Key Laboratory of Brain and Cognitive Sciences,
The University of Hong Kong
Email: u3003904@connect.hku.hk (Presenting author)
jdhuang@hku.hk (Corresponding author)
laiko@hku.hk (Corresponding author)

Protein synthesis in neuron can occur locally near individual synapses of the postsynaptic neuron, which may serve to translate synaptic activity into the formation of persistent synaptic connections and mature dendritic spines. To achieve local protein synthesis, specific mRNAs must first be transported to the neuronal dendrites. The anterograde transport of selective cargoes, including mRNAs, protein complexes and organelles to distal dendrites, is carried out by the Kinesin Superfamily (KIFs) of molecular motors. Among them, the three members of the KIF5 family (KIF5A, KIF5B & KIF5C) are present in the ribonucleoprotein complexes (RNPs) that transport dendritic mRNAs. However, it is not clear whether individual KIF5 perform redundant or distinct functions in the transport of RNPs and the regulation of synapse structure. Here we performed time-lapse confocal imaging using the RNA dye SYTO 14 and the Mitotracker CMXRox to monitor the dynamics of RNPs in dissociated hippocampal neurons derived from wild-type or KIF5B heterozygous knockout mice. Consistent with previous studies, majority of the RNPs were stationary, and the motile RNPs exhibited discontinuous movement which was either oscillatory (bi-directional movement over short distances) or unidirectional (moving one direction over long distances). Interestingly, KIF5B heterozygous neurons contained significantly fewer stationary RNPs, and higher proportion of RNPs displayed unidirectional movement. We further found that knock down of KIF5B expression in rat hippocampal neurons using short hairpin RNA (shRNA) led to the loss of mature spines and a significant increase in the number of immature filopodia. These findings suggest that the kinesin KIF5B is crucial for the transport and docking of selective mRNAs, which may subsequently regulate the maturation of dendritic spines.

This study was supported in part by the Research Grant Council of Hong Kong [General Research Fund (GRF) 16100814 and Early Career Scheme (ECS) 27119715].

P10

The anti-aging effect of PCSO-524® shed lights on Alzheimer's disease therapy

Zhu Beika

Supervisor: Karl Herrup

Hong Kong University of Science and Technology

The primary risk factor for neurodegenerative diseases including Alzheimer's disease (AD) is age, thus finding any agent that would slow the aging process would be of great use for AD therapy. In aging cortical neuronal cultures, primary neurons displayed increasing level of the cell cycle protein, Cyclin D. This effect of aging in culture is further exacerbated by the presence of fibrillar amyloid-beta (Aβ) peptide, suggesting that neurons were undergoing an Alzheimer's-like mitotic stress. Based on this study, we used Cyclin D level and the loss of neuronal markers as outcome measures to evaluate drug effectiveness.

PCSO-524®, an extract derived from New Zealand green lipped mussel was used to pre-treat cortical neuronal cultures. This treatment slowed the development of the 'aging' phenotype and significantly blunted the toxic effect of Aβ. We speculate that PCSO-524®'s anti-aging effect might be relevant to its calpain inhibitory and anti-inflammatory abilities. In this model, by blocking activation of calpain, PCSO-524® reduced the cleavage of the calpain substrate, p35 being cleaved to p25. This would have the dual effect of preventing CDK5 hyper-activation and the blocking the activation and altered substrate preference of GSK3β. We have shown that in AD brain, Aβ and other mitogen increased the level of phosphorylated-p38, which would be expected to have the response of preventing several downstream responses, including apoptosis, cell cycle reentry and senescence. We predict that application of PCSO-524® will significantly reduce p38 phosphorylation; future work will test this prediction directly.

Taken together, our data suggest that PCSO-524® displays important anti-aging and anti-amyloid activities – properties that would be of significant value as therapies against Alzheimer's disease.

P11

Regulation of Lipid Droplet Expansion by Seipin in *Caenorhabditis elegans*

Cao Z [1], Hao Y [1], Lam WJ [1], Qiu YF [1], Li XS [2], Qu JN [2] and Mak HY [1]

[1]Division of Life Science, The Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong, China

[2]Department of Electronic and Computer Engineering, The Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong, China

Email: zcaoad@connect.ust.hk

Lipid droplets (LDs) are organelles conserved for synthesizing, storing and supplying lipid in eukaryotes. They consist of a neutral lipid core that is surrounded by a phospholipid monolayer. Accumulating evidence suggests that the endoplasmic reticulum (ER) is in tight association with LDs both physically and functionally during the biogenesis and expansion of LDs. Seipin, encoded by the BSCL2 gene in human, is predicted to be an integral membrane protein in the ER. Studies in yeast, *Drosophila*, mice and various cell lines suggest critical roles for Seipin in regulating adipogenesis and LD morphology. However, the exact function of Seipin remains poorly defined. Here we report that a functional GFP fusion protein of the *C. elegans* Seipin ortholog (ceSeipin) is targeted to hitherto uncharacterized cage structures around a subset of LDs. Proper targeting of ceSeipin promotes the expansion of associated LDs. Using genetics, biochemical assays and light microscopy, we have probed the molecular basis of ceSeipin localization. In addition, we are using the CRISPR/Cas9 technique to engineer precise mutations that are predicted to disrupt ceSeipin targeting. Further proteomic studies will be performed to identify interacting partners of ceSeipin.

P12

A potential role of NMDA receptor-dependent expression of Striatin-4 in dendritic spine maturation

Louisa Hoi -Ying Lo, Lianfeng Lin, Quanwei Lyu, and Kwok-On Lai

School of Biomedical Sciences, State Key Laboratory of Brain and Cognitive Sciences, The University of Hong Kong, Hong Kong, China.

Email: lohyl@connect.hku.hk

Most excitatory synapses are located in dendritic spines of the postsynaptic neuron. Immatures spines, such as stubby spines or filopodia, do not possess a distinct spine head, while mature spines appear as mushroom-shaped with large heads, or thin spines with elongated necks and small heads. Spine maturation requires local dendritic protein synthesis in response to synaptic activity. Dysregulated mRNA trafficking and local protein synthesis can lead to altered spine morphology in neurodevelopmental disorders such as Fragile-X syndrome and autism. Nonetheless, the molecular mechanism underlying activity-dependent spine maturation is not fully understood. Striatin-4 (Zinedin) was identified in recent transcriptomic studies as an mRNA transcript present in hippocampal neuropil and putative cargo of RNA-binding protein FMRP. It serves as scaffold protein for signal transduction. Some striatin-interacting proteins, namely mammalian STE20-like protein kinase 3 (MST3) and cortactin-binding protein 2 (CTTNBP2), are encoded by autism risk genes. Despite studies demonstrating Striatin-4 enrichment in dendritic spines, Striatin-4 function in neuron remains unknown. Here we found that Striatin-4 mRNA and protein expression in cortical and hippocampal neurons was regulated by neuronal activity and NMDA receptors. Notably, Striatin-4 was preferentially expressed in mature dendritic spines, and its down-regulation by NMDA receptor antagonist APV switched mature spines to stubby spines and filopodia. Striatin-4 shRNA knockdown in hippocampal neurons led to mature spines loss and increased stubby spines and filopodia proportions, mimicked the spine phenotypes after NMDA receptor blockade. These findings suggest that NMDA receptor-dependent synthesis of striatin-4 is crucial for the dendritic spines maturation in hippocampal neurons.

This study was supported in part by the Research Grant Council of Hong Kong [General Research Fund (GRF) 16100814 and Early Career Scheme (ECS) 27119715].

P13

Characterization of the Regulatory Role of Sma/Mab Pathway by SMA-10/LRIG in *Caenorhabitis elegans*

Luk JCH [1] and Chow KL[1]

[1]Department of Life Science,

The Hong Kong University of Science and Technology, Hong Kong, China

Email: chlukab@connect.ust.hk

The Bone Morphogenetic Protein (BMP) Signaling Pathway is evolutionarily conserved with significant roles in regulations of multiple developmental processes. The regulation of the BMP Signaling Pathway is achieved by modulation of the BMP ligand availability and the receptor replay molecules activity targeting downstream gene regulations. The Sma/Mab signaling pathway is the conserved BMP signaling pathway in *C. elegans* that plays a significant role in the body length regulation. To understand the extracellular regulation of the Sma/Mab signaling, this study focused on *sma-10*, a gene encoding for a leucine-rich repeats immunoglobulin-like domains protein that enhances the Sma/Mab signal. My work aimed to understand expression profile of *sma-10*, to identify the required protein domains of SMA-10 for Sma/Mab signaling regulation and to elucidate the impact of SMA-10 on SMA-6/DAF-4 receptor complex trafficking. Up to this point, we have identified that *sma-10* is constitutively expressed in the pharynx, intestine and hypodermis at steady levels throughout the larval development. We have also demonstrated that all extracellular protein domains of SMA-10 are required while membrane anchorage is dispensable for Sma/Mab signaling regulation. Through tracking the fluorescent-labeled SMA-6 receptors during Sma/Mab signal transduction inside the nematode intestine, we further understood the role of SMA-10 on recycling of SMA-6 receptors and regulation of Sma/Mab signal pathway. (This research is supported by Research Grants Council, Hong Kong.)

P14

Folate Cycle Represents a New Metabolic Vulnerability in Hepatocellular Carcinoma Treatments

Lee D[1, 2], Xu IM[1, 2], Chiu DK[1, 2], Lai RK[1, 2], Wong CM[1, 2], Ng IO[1, 2], Wong CC[1, 2]

[1]Department of Pathology, and [2]State Key Laboratory for Liver Research,

Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong

Email: leed@connect.hku.hk

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related deaths worldwide owing to its late-symptomatic characteristic and mediocre treatment. Metabolic reprogramming emerged as a new hallmark of cancer; understanding its mechanisms is imperative for effective HCC therapeutic development. In folate cycle, a single carbon unit shuttled by a folate derivative through the tetrahydrofolate (THF) backbone in the cytoplasmic and mitochondrial compartment fuel different metabolic processes producing metabolites like NADPH – a major cellular antioxidant, and methionine – precursor of DNA and histone methylation. We found that folate was essential for HCC cell growth. A key enzyme facilitating the folate cycle from the mitochondrial compartment, methylene-THF dehydrogenase 1-like (MTHFD1L), was found to be significantly up-regulated in HCC. Knockdown of MTHFD1L in HCC cells halted NADPH production and the resulting elevation of oxidative stress induced DNA damage and cell cycle delay leading to inhibition of HCC proliferation. Binding of transcription factor Nrf2, potent protector of oxidative stress, and MTHFD1L was confirmed by ChIP assay. Nrf2 over-expression using the CRISPR-activating system in HCC cells further highlighted dependent relationship of Nrf2 and MTHFD1L. MTHFD1L knockdown disrupted the folate cycle as observed from metabolomics studies. Epigenetic alterations in HCC cells were observed in MTHFD1L-knockdown, as the folate cycle produces S-Adenosyl methionine (SAM), precursor for DNA and histone methylation. Therapeutically, folate cycle inhibition via MTHFD1L-knockdown and/or pharmacological induction with anti-folate, methotrexate, led to sensitization of HCC cells towards its conventional targeted-therapeutic agent Sorafenib *in vitro* and *vivo*. Taken together, folate cycle represents is a metabolic vulnerability of HCC for therapeutic targeting.

P15

The functions of tumor driving chromatin remodeler HELLS in liver cancer development

Cheuk Ting LAW, Lai WEI, Felice HC TSANG, Iris MJ XU, Robin KH LAI, Daniel WH HO, Joyce MF LEE, Carmen CL WONG, Irene OL NG and Chun-Ming WONG

Department of Pathology and State Key Laboratory for Liver Research, The University of Hong Kong

Email: ctlawaa0119@gmail.com

Hepatocellular carcinoma (HCC) is the major type of liver cancer and is the 2nd death-causing cancer. HCC development is a multistep process, involving chronic HBV/HCV infection, liver cirrhosis and subsequent HCC formation. This complicated process is orchestrated by the accumulation of multiple genetic and epigenetic alterations. Our research study unearthed that chromatin remodeler HELLS was frequently overexpressed in HCC when comparing to their corresponding non-tumor livers at both mRNA and protein levels. The up-regulation of HELLS was significantly associated with more aggressive clinicopathological features. Moreover, we revealed that aberrant HELLS expression in HCC was conferred by the hyperactivation of its up-stream transcription factor, SP1. Notably, overexpression of HELLS by CRISPR/Cas9 Synergistic Activation Mediator (SAM) system augmented the cell growth and migration abilities of HCC cells. On the contrary, knockdown of HELLS by sh-RNA or genetically knockout by CRISPR/Cas9 system significantly suppressed HCC cell proliferation and migration. Consistently, depletion of HELLS also inhibited in vivo tumorigenicity and lung metastasis in mouse model. Intriguingly, global gene correlation study and metabolic assays uncovered that HELLS is participated in metabolic reprogramming during HCC progression. Inactivation of HELLS restored the mitochondria activity and reduced lactate production in HCC cells. Furthermore, transcriptome sequencing (RNA-seq) analysis revealed that KO of HELLS in HCC cell lines restored the expression of multiple tumor suppressor genes that involved in regulating different cancer hallmark events. . In conclusion, our findings suggested that the frequently up-regulation of HELLS contributes to aberrant epigenetic silencing of tumor suppressor genes and thereby promotes HCC progression.

P16

Development of Loop-Mediated Isothermal Amplification (LAMP) for the Authentication of Transgenic Papaya

But GWC and Shaw PC

School of Life Sciences,

The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China.

Email: gracebut@link.cuhk.edu.hk

Genetically modified (GM) food has become a part of our food life. Artificial manipulation on an organism's genetic material allows introduction of desired traits in organisms. In Hong Kong, over 60% of papaya (*Carica papaya*) available for sell are GM papaya (AFCD, 2015). As more and more consumers prefer organic food, there is a need in rapid identification of transgenic papaya. To improve upon traditional diagnostic polymerase chain reaction (PCR) methods for transgenic papaya, an efficient procedure for authentication of transgenic papaya was developed based on loop-mediated isothermal amplification (LAMP). One of the treats of papaya is due to papaya ringspot virus (PRSV), causing symptoms including ringspot and bumps on fruits and chlorosis in leaves. Transgenic papaya acquire their resistance to PRSV from the inserted fusion gene coding coat protein of cauliflower mosaic virus (CaMV) and PRSV under the control of the CaMV 35S promoter (P-35S). Two sets of LAMP primers designed are based on the transgene P-35S region and the intrinsic Papain gene as internal control respectively. The amplification products could be examined by gel electrophoresis and direct visual detection utilizing DNA binding dyes, SYBR green I. The LAMP detection had shown its sensitivity and accuracy on PCR method and LAMP detection method. Comparing to traditional PCR method, the LAMP detection method provides reliable and rapid screening of transgenic papaya and could be potentially utilized for educational and agricultural purposes. This LAMP detection method is under further streamlined by incorporating experimental steps into a lab-on-a-disc (LOD) to allow efficient on-site detection and results interpretation by laymen.

P17

Maturation of hPSC-CMs in Bioenergetics driven by Oleic Acid and PPARα agonist

Wong N[1], Chow MZ[1], Keung W[1], Li RA[2]

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

[2]Dr Li Dak-Sum Research Centre, The University of Hong Kong - Karolinska Institutet Collaboration in Regenerative Medicine, Hong Kong, China.

Email: nwong729@hku.hk

Human pluripotent stem cells derived-cardiomyocytes (hPSC-CMs) hold great promises for applications in biochemical studies, disease modeling and drug discovery. However, hPSC-CMs display fetal-like cell properties, which limit the translatability of their finding into clinical use. Myocardial substrate metabolism is immature in hPSC-CMs. In mature human adult CMs, more than 95% of ATP formation comes from oxidative phosphorylation, in particular fatty acid β-oxidation, whereas fetal and hPSC-CMs are highly dependent on glycolysis. Since ATP is essential for calcium homeostasis and contractile work, we hypothesize that by up-regulating the metabolic enzymes and fatty acid transporters, hPSC-CMs can derive more ATP and support stronger contractions. Using hES2-CMs to construct engineered 3D human cardiac tissue strips (hCTSs) that allow for measurement of contractility in cardiomyocytes, we have investigated the maturation of bioenergetics in hES2-CMs. Activating the peroxisome proliferator-activated receptors (PPAR) pathway with oleic acid (OA) and the PPAR agonist GW7647, we have shown with qPCR ~ two-fold up-regulation in RNA expression for malonyl-CoA decarboxylase (1.62±0.10) and acyl-Coenzyme A dehydrogenase (2.61±0.12), and a three-fold up-regulation in Enoyl-CoA, Hydratase/3-Hydroxyacyl CoA Dehydrogenase (3.39±0.08) and AMP-Activated Protein Kinase (2.73±0.13). In addition, up-regulation in RNA expression of metabolic proteins is accompanied by a two-fold increase in the developed contractile force (1.90±0.31). Our results show that upon activation of the PPAR pathway in hCTSs, enzymes for oxidative energy metabolism are up-regulated, which aid in supporting higher contractile forces.

P18

Structural and Biochemical Analysis of the Splicing Factor SRSF3

Name of speaker: LONG, Yun Xin Supervisor: NGO, Chi Ki

Email: lyx610@gmail.com

SR proteins are an essential family of splicing factors which serve important roles in both constitutive and alternative pre-mRNA splicing. Classic SR proteins contain one or two N-terminal RNA-recognition motifs (RRMs) and a C-terminal serine/arginine rich domain (RS domain). The RS domain of SR protein acts to promote interaction with other proteins that facilitate recruitment to the site of splicing. In cytoplasm, the phosphorylation of SR proteins is regulated by SR protein kinases (SRPKs). SRPKs phosphorylate SR proteins to a hypophosphorylated state, which will be recognized and translocated into nuclear speckles, from where SR proteins are hyperphosphorylated and consequently released to the sites of active splicing. Extensive investigations have been done on how SRSF1, a prototypic SR protein that contains two RNA recognition motifs (RRMs), is regulated by SRPK1. However, as for SRSF3, another member of the SR protein family, the mechanisms of its phosphorylation by SRPKs is still unclear at this stage. SRSF3 contains only a single RRM followed by the C-terminal RS domain. Comparing with SRSF1, the absence of the second RRM suggests that the recognition and binding of SRSF3 by SRPKs are likely to be different. In this study, we focus on another member of the SRPK family, SRPK2, aiming to investigate the structural and biochemical properties of SRPK2 with its substrate SRSF3, by means of structural studies, localization investigations and biochemical experiments. From localization studies, we found that both phosphorylation and integrality of RS domain have effects on the nuclear localization of SRSF3 constructs. For the structural study, we have purified a complex composed of SRPK2 and SRSF3 truncation constructs. We obtained protein crystals which diffraction at the home source, but whether they are complex crystals remain unknown.

P19

RGS19 upregulates Nm23 metastasis suppressor via transcriptional activation by the cAMP/PKA/CREB pathway

Li YJ [1,2], Tsang TH [1], Cheung YY [1], Park CW [1], Wong YH [1,3]

[1] Division of Life Science and the Biotechnology Research Institute, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong

[2] Department of Ophthalmology, Second Xiangya Hospital, Central South University, Changsha, China

[3] State Key Laboratory of Molecular Neuroscience, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong

Email: ylibz@ust.hk

The Nm23 metastasis suppressor family is involved in diverse physiological and pathological processes including tumorigenesis and metastasis. Although the inverse correlation of Nm23 level with tumor metastasis potential has been widely observed, the mechanisms that regulates of the expression of Nm23 remain poorly understood. Our previous studies have revealed that Nm23-H1/2 isoforms are upregulated by RGS19, a regulator of G protein signaling (RGS) protein which accelerates the termination of G_i signals. Here, we examined the ability of RGS19 to stimulate transcriptional regulation of Nm23 by screening a panel of luciferase reporter genes. Both transient and stable overexpression of RGS19 upregulated the Nm23-H1/2 protein levels and activated several transcription factors including CREB, AP-1 and SRE in HEK293 cells. Interestingly, agents (e.g., adrenergic receptor agonist, forskolin, and PDE inhibitor) that increase the intracellular cAMP level and the phosphorylation of CREB upregulated the expression of Nm23-H1/2 in both HEK293 cells and several cancer cell lines. Conversely, inhibition of protein kinase A (PKA) by H-89 suppressed the phosphorylation of CREB and reduced the expression of Nm23-H1/2. Collectively, these results revealed a PKA-dependent mechanism for controlling Nm23-H1/2 expression, which may provide new approaches to limit cancer metastasis by modulating the expression of the Nm23 metastasis suppressors.

P20

Desktop single molecule sequencing:

Assessment of nanopore sequencing data with the lambda phage genome

Chung YL[1], Chan TF[1,2]

[1]School of Life Sciences, and [2]Partner State Key Laboratory of Agrobiotechnology,

The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China.

Email: clairechung@link.cuhk.edu.hk

Short-read massively-parallel sequencing currently dominate bioinformatics studies, but the short read lengths and amplification biases hinder full sequence resolution. Single-molecule real-time (SMRT) sequencing presents a promising alternative to easily achieve reads over 6kb. The Oxford Nanopore MinION is a desktop SMRT sequencing device. The flowcell contains hundreds of protein nanopores on insulated membranes. When a voltage is applied, single-stranded DNA molecules pass through the nanopores, where nucleotides are identified by current changes. During library preparation, DNA is fragmented into ~8kb and end-repaired. The two DNA strands are ligated into a single strand. Motor and tether proteins control the translocation speed and promotes DNA affinity to membrane. The sequences from two strands are compared to obtain two-directional (2D) reads. The long reads will be useful for scaffolding and *de novo* genome assembly. However, the higher error rate hampers accurate data analysis. To better understand the MinION data nature, we sequenced the 48 kb bacteriophage lambda (NC001416) genome at ~2500× coverage in a 14 h run. We obtained a read length distribution with N50 = 6375. Both sequence lengths and base-calling accuracy decreased along running time, while a voltage correction at 12h boosted the accuracy. Raw reads were aligned to the reference genome with 22.4% mismatch. Our preliminary results agree with reports of similar experiments. By studying the data nature, we expect to improve the base-calling, read correction and *de novo* assembly procedures. Possibilities of other applications, such as for RNA, methylated DNA will also be explored to enhance biological studies.

P21

The role of MMP14 in lineage progression of hypertrophic chondrocytes

TL Chu [1], KY Tsang [1], SW Tsang [1], ZJ Zhou [1], Kathryn SE Cheah [1]

[1]Department of Biochemistry, The University of Hong Kong, Pokfulam, Hong Kong SAR, China

Email: chutl@hku.hk

It is traditionally believed that chondrocytes and osteoblasts are two separate lineages with hypertrophic chondrocytes (HCs) being the terminal stage of chondrocyte differentiation, culminating in apoptosis. However, our previous study shows HCs can contribute to the full osteoblast (Obs) lineage *in vivo*. MMP14 is a transmembrane matrix metalloproteinase responsible for matrix remodeling that is highly expressed at the chondro-osseous junction which coincides with the transition from HCs to Obs. Complete knockout of *Mmp14* in mice results in severe loss of trabecular bone and shortening of limbs. To test whether loss of MMP14 has an impact on the HC to Obs transition, we have employed a genetic recombination approach to track and compare the fate of HCs in wild-type and *Mmp14* conditional and total null mutants. By using *Col10a1-Cre* transgenic mice, we are able to follow the fate of HC descendent cells after conditional ablation of MMP14 in HC descendents. Surprisingly, we found that conditional knockout of *Mmp14* in HC-descendent cells results in increased trabecular bone. Consistent with microCT analysis, increased expression domain of *Col1a1, Opn* and *Mmp13* are observed in *Col10a1-Cre;Mmp14*$^{F/-}$ conditional knockout mutants by *in situ* hybridization. Both complete and conditional deletion of MMP14 activity results in increased number of HC-descendent cells in the trabecular bone. Our results suggest that MMP14 in general negatively regulates HC to Obs transition.

P22

Significance of Epstein-Barr virus encoded Latent Membrane Protein 1-Induced mTOR Signaling in Nasopharyngeal Cells

Zhang J[1], Jia L[1], Lin WT[1], Yip YL[1], Tsang CM[1], Zhou Y[1], Zhu DD[1], Deng W[2], Tsao SW[1]

[1]School of Biomedical Sciences and Cancer Research Centre, [2]School of Nursing, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong, China

Presenter email: junzh128@hku.hk

Nasopharyngeal carcinoma (NPC) is a human malignancy consistently associated with Epstein-Barr virus (EBV) infection. The EBV encoded latent membrane protein-1 (LMP1) is a trans-membrane protein functions as a proto-oncogene by amplifying multiple signaling in cells. The mammalian target of rapamycin (mTOR) is a conserved serine/threonine protein kinase, and emerging evidences show activated mTOR plays multiple functions in biological processes. However, the linkage between LMP1 and mTOR signaling and the potential contribution of mTOR to the pathogenesis and development of nasopharyngeal carcinoma remains poorly understood. Here we report that LMP1 mediates its oncogenic functions by activating the mTOR complex 2 (mTORC2). Our study showed suppression of mTORC2 activity by knockdown of its specific subunit, Rictor, attenuates LMP1-induced cell migration and invasion, as well as epithelial-mesenchymal transition (EMT). Our study further showed LMP1 could increase the secretion of IGF-1 to activate mTORC2. Blockade of the IGF-1 receptor (IGF1R) tyrosine kinase by AG-1024 or neutralization of secreted IGF-1 by its specific antibody could decrease the activation status of mTORC2 induced by LMP1. Finally, we found the transmembrane domains 3-6 (TM3-6) of LMP1 are responsible for the mTORC2 signaling activation, since deletion of TM3-6 could attenuate the secretion of IGF-1 and activation of mTORC2 induced by LMP1, as well as the migration, invasion and EMT of nasopharyngeal epithelial cells. Taken together, in this study, the signaling transduction of mTORC2 activation induced by EBV-encoded LMP1 was defined. Our studies also provide evidences supporting that mTORC2 activation could mediate multiple LMP1-induced phenotypic changes, suggesting a potential strategic treatment for NPC by targeting the mTORC2 signaling.

P23

β-TrCP-mediated ubiquitination and degradation of liver-enriched transcription factor CREB-H

Cheng Y[1], Gao WW[1], Tang HMV[1], Deng JJ[1], Chan CP[1] and Jin DY[1]

School of Biomedical Sciences, The University of Hong Kong

chengyun000@hotmail.com

CREB-H is an endoplasmic reticulum-resident bZIP transcription factor which critically regulates lipid homeostasis and gluconeogenesis in the liver. CREB-H is proteolytically activated by regulated intramembrane proteolysis to generate a C-terminally truncated form known as CREB-H-ΔTC, which translocates to the nucleus to activate target gene expression. CREB-H-ΔTC is a fast turnover protein but the mechanism governing its destruction was not well understood. In this study, we report on β-TrCP-dependent ubiquitination and proteasomal degradation of CREB-H-ΔTC. The degradation of CREB-H-ΔTC was mediated by lysine 48-linked polyubiquitination and could be inhibited by proteasome inhibitor. CREB-H-ΔTC physically interacted with β-TrCP, a substrate recognition subunit of the $SCF^{\beta\text{-}TrCP}$ E3 ubiquitin ligase. Forced expression of β-TrCP increased the polyubiquitination and decreased the stability of CREB-H-ΔTC, whereas knockdown of β-TrCP had the opposite effect. An evolutionarily conserved sequence, SDSGIS, was identified in CREB-H-ΔTC, which functioned as the β-TrCP-binding motif. CREB-H-ΔTC lacking this motif was stabilized and resistant to β-TrCP-induced polyubiquitination. This motif was a phosphodegron and its phosphorylation was required for β-TrCP recognition. Furthermore, two inhibitory phosphorylation sites close to the phosphodegron were identified. Taken together, our work revealed a new intracellular signaling pathway that controls ubiquitination and degradation of the active form of CREB-H transcription factor.

P24

Function of the Hippo signaling pathway in mouse cerebellar development

DONG, Xiao Nan

Supervisor: Prof. KWAN, Kin Ming

The canonical Hippo signaling pathway, which first discovered in *Drosophila*, is known to be a highly conserved pathway regulates tissue growth and organ size by controlling cell fate, proliferation, apoptosis, and cell movement. The mammalian homologs of the *Drosophila* Hippo kinase, Mst1 and Mst2 (mammalian STE20-like 1 and 2) control the activity of the downstream transcriptional coactivators TAZ (transcriptional co-activator with PDZ-binding motif) and YAP1 (Yes associated protein 1), which then control the expression of response genes. The expression level and cellular localization of these factors are important for many early developmental events. Previous studies show that the Hippo pathway plays a crucial regulative role in different organ development, such as in liver, pancreas, kidney, lung and heart, while dysregulation of this pathway leads to tumorigenesis. However, studies focusing on the role of the Hippo pathway in the central nervous system are limited.

As a model system to study the central nervous system, cerebellum consists of small number of cell types, and has the well-known functions for motor control, cognition, emotion and language. The mouse granule cell progenitors, which give rise to the most numerous neurons in adult cerebellum, continue proliferation during the early postnatal stage. Until the second postnatal week, they gradually exit the cell cycle and migrate from the external granule layer to form the internal granule layer. Resent studies implicated the Hippo pathway component Yap1 involves in medulloblastoma, a severe cerebellar tumor, which may be resulted in defective regulation of granule cell proliferation and migration. But the detailed role of the Hippo pathway during cerebellar development remains unknown. Through the use of the Mst1/2 conditional alleles with inducible Cre/loxP system, we obtained early postnatal cerebellar granule cells conditional Mst1/2 knockout mice. However, Mst1/2 deletion showed no discernable effect on morphology throughout cerebellar postnatal development. The lack of phenotype may be caused by insufficient Cre recombinase activity. We will optimize the induction of Cre activity, confirm the deletion and further examine phenotype. On the other hand, genes work in parallel to Mst1/2 may play a redundant role during cerebellar development, they will be further studied as well.

P25

Identification of a novel regulatory protein for *Helicobacter pylori* motility

ZHANG Huawei*, Au Wing Ngor Shannon#

School of Life Sciences, The Chinese University of Hong Kong

*: zhw2508@gmail.com. #: shannon-au@cuhk.edu.hk

Flagellar motor is an important virulence factor in bacterial pathogenesis. Several protein factors are reported to associate with motor function by binding to the switch complex, such as YcgR in *S. enteritidis*, EpsE in *B. subtilis*, H-NS and fumarate reductase in *E. coli*. Recently, we have identified spermidine synthase (SpeE) as an interaction partner of motor switch protein FliM in *Helicobacter pylori*. Their interaction was confirmed by pull-down, size-exclusion chromatography and isothermal titration calorimetry. The structure of FliMm-SpeE complex has also been determined by X-ray crystallography. Key amino acids involving in the interaction were identified and confirmed by mutagenesis. *Spee-null* strain was constructed to study the biological roles of their interaction on *H. pylori* motility. The results show that although normal flagellation is detected in *spee-null* strain, but deletion of *spee* results in a reduced motility compared with that of the wild type strain. For the *in vitro* enzyme activity of SpeE, to our surprise, *H. pylori* SpeE has no measurable catalytic activity on spermidine synthesis. The activity assay has further been validated by isothermal titration calorimetry and by using recombinant *E. coli* SpeE as a positive control. Taken together, *H. pylori* may use the alternative pathway for spermidine synthesis. Our findings suggest that SpeE may specifically involve in the motor regulation instead of the spermidine synthesis in *H. pylori*. The results obtained will open up a new regulatory mechanism of flagellar motor.

P26

ATM-dependent Phosphorylation of the Fanconi Anemia Protein PALB2 Promotes the DNA Damage Response

Yingying Guo[1], Wanjuan Feng[1], Shirley M. H. Sy[1,2], and Michael S. Y. Huen[1,2,3]

[1] School of Biomedical Sciences, [2] Centre for Cancer Research, LKS Faculty of Medicine, [3]State Key Laboratory of Brain and Cognitive Sciences, The University of Hong Kong, Hong Kong

Email: yingyingguo0228@gmail.com

The Fanconi anemia protein PALB2, also known as FANCN, protects genome integrity by regulating DNA repair and cell cycle checkpoints. Exactly how PALB2 functions may be temporally coupled with detection and signaling of DNA damage is not known. Intriguingly, we found that PALB2 is transformed into a hyperphosphorylated state in response to ionizing radiation (IR). IR treatment specifically triggered PALB2 phosphorylation at Ser-157 and Ser-376 in manners that required the master DNA damage response kinase Ataxia telangiectasia mutated, revealing potential mechanistic links between PALB2 and the Ataxia telangiectasia mutated-dependent DNA damage responses. Consistently, dysregulated PALB2 phosphorylation resulted in sustained activation of DDRs. Full-blown PALB2 phosphorylation also required the breast and ovarian susceptible gene product BRCA1, highlighting important roles of the BRCA1-PALB2 interaction in orchestrating cellular responses to genotoxic stress. In summary, our phosphorylation analysis of tumor suppressor protein PALB2 uncovers new layers of regulatory mechanisms in the maintenance of genome stability and tumor suppression.

P27

Role of Smad4 in Mouse Abdominal Wall Development

Author: CHOW, Chu Qiao[1]

Supervisor: Prof. KWAN, Kin Ming[1,2,3]

1 School of Life Sciences, The Chinese University of Hong Kong (SLS, CUHK)

2 Centre for Cell and Developmental Biology, The Chinese University of Hong Kong (CCDB, CUHK)

3 State Key Laboratory of Agrobiotechnology, The Chinese University of Hong Kong (SKL of Agrobiotech, CUHK)

Author's email: chowqiao@hotmail.com

The abdominal wall encloses the majority of visceral organs and its malformation is associated with congenital defects such as Prune Belly Syndrome and Pentalogy of Cantrell. Nevertheless, the molecular control of abdominal wall formation is unclear. Previous studies demonstrated that ablation of ectodermal Wnt-signaling led to thinning of ventral abdominal wall. Interestingly, our *En1-Cre* driven *Smad4* knockout mutant displayed similar phenotype, but at a later time point. *Smad4* is the central mediator of TGF-β and BMP signaling, thus implying that these pathways may be involved in the development of ventral abdominal wall and may crosstalk with Wnt-signaling.

At embryonic day E18.5, our *Smad4* mutants showed reduction in size and coverage of the ventral muscle rectus abdominis, as well as loss of dermal and hypodermal components. This phenotype worsened at postnatal and adult stages. Collagen content and number of hair follicles were reduced in the mutant skin. The overall proliferation and apoptotic rates of the AW, however, were unaffected. To investigate the possible interactions between *Smad4* and Wnt signaling, qPCR analysis was performed. Intriguingly, several Wnt ligands and signaling components were up-regulated, whilst a number of Wnt co-receptors were down-regulated. Further analyses are required to decipher the role of Smad4 in regulating Wnt signaling.

P28

Overexpression of CD133 Promotes Tumorigenicity and Suppresses TAX1BP2 in Hepatocellular Carcinoma

Pui Yu SO, Wai Lun LAI, Stephanie MA, and Yick Pang CHING

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

Email: sopuiyu@hku.hk

CD133 is a transmembrane protein that has been identified as a marker for cancer stem cells (CSCs) in liver cancer (hepatocellular carcinoma, HCC). CD133-positive HCC CSCs are more proliferative, tumorigenic and chemoresistant than its counterparts, for which the mechanism remains unclear. To elucidate the function of CD133, we have established inducible stable expression cell lines for CD133. The stable expression of CD133 in these stable expression clones were confirmed using western blotting and the flow cytometry analyses. Furthermore, colony formation assay was performed and the result showed that overexpression of CD133 significantly increased the tumorigenicity of HCC cell line, SMMC-7721. More interestingly, we found that CD133 was a negative regulator of a tumor suppressor protein Tax1 binding protein 2 (TAX1BP2), which is previously identified as an intrinsic block of centrosome overduplication. The expression of TAX1BP2 at both protein and transcript levels was downregulated in sorted CD133-positive cells. As revealed by luciferase reporter assay, the expression of CD133 could significantly suppressed the promoter activity of TAX1BP2, suggesting that CD133 is a suppressor of TAX1BP2 expression. Taken together, our work suggests that CD133 may contribute to tumorigenicity via suppression of TAX1BP2 expression.

P29

Regional Disturbances Around the F508 Residues Exhibited Various Impacts on The function of The Cystic Fibrosis Transmembrane Conductance Regulator

Xinying Chen, Siyu Zhu, Weiyi Xu, Jeng-Haur Chen

School of Biomedical Science, University of Hong Kong

zoechen@connect.hku.hk

Cystic fibrosis (CF) is caused by genetic mutations impairing the function of the cystic fibrosis conductance regulator (CFTR), which is known as ABCC7 forming a unique Cl- channel among other ATP-binding cassette proteins. Over 90% of patients with CF carries a CFTR mutation ΔF508, which has a deletion of the F508 residue in the polypeptide chain and causing great reductions in protein processing and channel activity. However, it is not well understood whether the ΔF508 mutation may disrupt functions of neighbor residues leading to these defects. To address this question, we constructed CFTR mutants with individual deletions on the residues around from the position 491 to 525. Our data demonstrate that mature CFTR with full glycosylation was found abundant in the cells expressing wild-type, ΔV510- and ΔS511-CFTR, whereas other deletion mutants behaved like ΔF508-CFTR mostly expressing immature protein with less glycosylation. Conversely, the alanine replacements at position 503 to 513 showed normal protein trafficking, except of D513A- and N505A-CFTR exhibiting moderate and little expression of mature proteins, respectively. All tested mutants replacing N505 with different side chains had profound deficits in CFTR trafficking, suggesting that the N505 residue is essential for CFTR maturation. Moreover, low-temperature culture, CFTR corrector C18 or combination of two treatments enhanced mature protein expression only in ΔF508- and ΔY512-CFTR among other deletion mutants. Consistently, single-channel studies on deletion mutants ΔF508-, ΔV510-, ΔS511- and ΔY512-CFTR demonstrate that only ΔF508- and ΔY512-CFTR had lower open probability and much prolonged closed time than that of wild-type CFTR. These data suggest that the mutations ΔF508 and ΔY512 may disrupte protein processing and channel activity by similar mechanisms. Our data further suggest that functional defects of ΔF508-CFTR may be originated from regional abnormalities in protein conformation.

P30

Generation of a Chordoma Cell Line: a Model of Human Notochordal Cells?

Guo S [1], Tsang KY [1], Au TYK [1], Tam WK [2], Leung V [2], Cheah KSE [1]

[1]School of Biomedical Sciences, and [2] Department of Orthopaedics and Traumatology, The University of Hong Kong, Hong Kong, China

Email: sguo@connect.hku.hk

The intervertebral disc (IVD), which comprises nucleus pulposus (NP), annulus fibrosus (AF), and cartilage endplate (EP), is sandwiched between vertebral bodies to facilitate back bone movement. Derived from the embryonic notochord, human NP cells are populated by both reminiscent of embryonic notochordal cells (notochordal like cells, NCLs) and chondrocyte- like cells (CLCs) at birth, but the proportion of NCLs rapidly decreases until adolescence, and only CLCs remain in the NP. Chordomas are rare spine tumours that are thought to arise from notochordal remnants because the cells show vacuolated morphology and Brachyury expression. We sought to test the whether chordoma cells could be a model for human notochordal cells that are hard to be obtained from healthy fetuses or childen at early age, and that could be used in cell based functional studies. Here we describe the isolation and characterisation of cells isolated from a chordoma excised from sacrum from a HK patient. Cells that express notochord marker CD24 were manually enriched, followed by confirmation of notochord marker Brachyury through qRT-PCR and immunostaining. Results suggest that the cells isolated from the tumour may resemble notochordal cells. This will pave our way to address differentiation mechanism through genetic manipulation.

P31

Glucose-regulated expression and processing of PDZD2 in mouse islets and rat INS-1E beta cells

So HF [1], Shiu WY [1] and Yao KM [1]

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

Email: dannyso@connect.hku.hk

PDZ domain-containing 2 (PDZD2), a multi-PDZ-domain protein expressed selectively in the beta cells of pancreatic islets, generates its secreted form (sPDZD2) proteolytically by caspase-3. We previously showed that depletion of PDZD2 in INS-1E cells suppressed beta-cell gene expression, and addition of recombinant sPDZD2 ameliorated this knockdown effect. However, the physiological role of PDZD2/sPDZD2 in glucose homeostasis remains unclear. If PDZD2/sPDZD2 plays a role in glucose homeostasis, we expect feedback regulation of PDZD2 expression and processing with changes in extracellular glucose levels. Interestingly, *Pdzd2* mRNA expression was increased by 60% in INS-1E cells grown in low-glucose (2.8mM) medium for 24 hours. Similarly, *Pdzd2* expression was 30% higher when mouse islets were incubated with low-glucose medium, compared to those in high-glucose (13.8mM) condition. In contrast, the level of full-length PDZD2 protein was significantly decreased in INS-1E cells incubated with low-glucose medium, compared to those maintained in high-glucose medium. The discordance in mRNA and protein levels suggested that proteolytic processing of full-length PDZD2 may be enhanced to generate the secreted form. Indeed, a minor but statistically significant increase of sPDZD2 level in low-glucose condition was observed. Co-incubating the cells with exogenous sPDZD2 (0.1nM) at low- or high-glucose medium reduced the magnitude of changes in the mRNA expression of PDZD2, compared to those without sPDZD2 treatment, suggesting a negative feedback mechanism. Taken together, our findings support that the expression and processing of PDZD2 in INS-1E cells and islets is regulated by extracellular glucose levels, hinting at the physiological relevance of PDZD2/sPDZD2 in glucose homeostasis.

P32

Sialyl Lewis^x modulates tumor-mesothelial adhesion for early metastasis

Shan-Shan Li,[1] Carman K. M. Ip,[1] Ayon Ahmed Hassan, [1] Matthew Y. H. Tang,[2] Samuel K. H. Sy,[2] Ho Cheung Shum,[2] and Alice S. T. Wong[1]

[1]School of Biological Sciences and [2]Department of Mechanical Engineering,

University of Hong Kong, Pokfulam Road, Hong Kong.

Email: u3002730@connect.hku.hk

Ovarian cancer is a highly malignant gynecological malignancy with poor prognosis (5-year survival <25%). Over 70% of ovarian cancer patients present advanced/metastatic stages disease at diagnosis, which leads to its high mortality. Unlike most tumors that metastasize via the vasculature, ovarian cancer disseminates within the peritoneal cavity. Key to this is the ability to form successful adhesion to the peritoneal mesothelium. This tumor-mesothelial interaction is mediated by engagement of specialized adhesion molecules that function under ascitic shear stress, but not static condition. However, very little is known about this adhesion mechanism. For this, we have recently identified the highly metastatic population of cancer stem cells (M-CSCs) which have the capability to initiate metastasis. Using a 3D microfluidic platform which most closely mimics tumor behaviour and shear stress in the ascitic environment, we show for the first time that M-CSCs exhibit slower rolling velocity and bind more firmly to the peritoneal mestholium than its non-metastatic counterpart (NM-CSCs). P-selectin expressed by the peritoneum mesothelial is indispensable for this interaction. Moreover, using glycosylation gene profiling, we identify α-1,3-fucosyltransferase V determines Sialyl Lewis^x (sLe^x) synthesis was the carbohydrate group that binds to P-selectin. Specific treatments with sialidase, fucosidase, and RNA-mediated interference of α-1,3-fucosyltransferase V knockdown revealed that sLe^x was critically required for intervening tumor-mesothelium interaction in vitro and in xenograft in vivo. These data shed new light on the significance of sLe^x-P-selectin in early metastasis under shear stress, which may offer opportunities for new therapeutic targets. (This work is supported by RGC grant 17122014).

P33

Bcl3 siRNA dendriplex-, a novel nanomedicine for anti-nasopharyngeal carcinoma *in vitro* and *in vivo*

Jing Ma[a], Ling Peng[b], Siu Tim Cheung[c], Kwok Wai Lo[d], and Alice S. T. Wong[a]

[a]School of Biological Sciences, University of Hong Kong, Pokfulam Road, Hong Kong;

[b]Axi-Marseille University, CNRS, Centre Interdisciplinaire de Nanoscience de Marseille, Marseille, Cedex 09, France;

[c]Department of Surgery and [d]Anatomical & Cellular Pathology, Chinese University of Hong Kong, Shatin, Hong Kong.

E-mail: bluesky5605@163.com

Nasopharyngeal carcinoma is a distinctive type of head and neck cancer with no effective therapy of current treatment. In this study, we aim to investigate the therapeutic potential of amphiphilic dendrimer-mediated Bcl3 siRNA delivery for nasopharyngeal carcinoma. Bcl3 siRNA dendriplexes were formed and characterized for gene knockdown efficiency and MTT assay. The distribution of Bcl3 siRNA dendriplexes was tracked in C666-1 xenografts. The *in vivo* anti-tumor effects were investigated in C666-1 xenografts with complete controls and longtime *i.v.* injection. The anti-tumor effects were also investigated in several patients' xenografts. We showed for the first time amphiphilic dendrimer could form stable dendriplexes with Bcl3 siRNA with almost 80% Bcl3 gene knockdown efficiency and significant cell availability reduction. Using *in vivo* tracking, we demonstrated that Bcl3 siRNA dendriplexes not only distributed in tumor within 30 min, but also lasted for at least 24 h following *i.v.* injection. Bcl3 siRNA dendriplexes could significantly prevent tumor growth in C666-1 xenografts and in patients' derived xenografts xeno-2117 and xeno-C17 without obvious toxicity. These results show that amphiphilic dendrimers were very suitable as Bcl3 siRNA nanocarriers with effective gene knockdown efficiency *in vitro* and significant anti-tumor activity *in vivo*, and thus could develop as a potent nanomedicine for effective and potent nasopharyngeal carcinoma treatment.

P34

Blockade of TGF-β/Smad3 Signaling Pathway Ameliorates Diabetes

Nana Jin[1], Chi-Shing Yu[1], Yongke You[2], Heung Man Lee[2], Ronald CW Ma[2], Huiyao Lan[2], Ting Fung Chan[1]

[1] School of Life Sciences, and Partner State Key Laboratory of Agrobiotechnology, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China.

[2] Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong, China.

Email: jinnana7926@hotmail.com

Diabetes is a group of metabolic disorders in which patients suffered from hyperglycemia chronically. Among 387 million people all over the world who had diabetes, over 90% had type 2 diabetes which begins with insulin resistance. The associated complications are the principal contributors of mortality. Thus, it is critical to understand the underlying mechanisms of type 2 diabetes further to find out the effective therapeutics. As previously reported, TGF-β/Smad3 signaling pathway has an important role in regulating diabetes. However, existing researches are based on low-throughput experimental data, meanwhile regarding diabetic and non-diabetic mice as the same. This work aims to investigate how transcriptome alterations of Smad3 deficiency impact TGF-β/Smad3 signaling pathway to affect the outcomes of diabetes and non-diabetes. 18 RNA samples were from 5 groups of kidney cortex of the mice, including Smad3 WT/KO diabetic/non-diabetic, and Smad3 Hetero diabetic groups. Based on differential expression analysis and Ingenuity Pathway Analysis (IPA), we found PGC-1alpha, which was previously reported having beneficial effects on diabetic mice, showed a significant up-regulation in diabetic samples after Smad3 knocking out, while degree of up-regulation of FXR in diabetic samples in Smad3 wild type was weaken after Smad3 knocking out. This observation was consistent with the prior research that Smad3 acts as a repressor of PGC-1alpha expression. Moreover, cholesterol efflux related genes up-regulated after Smad3 knocking out. These comparative analyses of diabetes versus non-diabetes in mice with Smad3-wild type or Smad3-knock out preliminarily demonstrate the critical role of Smad3 loss in ameliorating diabetes.

P35

The epilepsy and intellectual disability-related gene tbc1d24 encodes a novel synaptic protein that regulates dendritic spine morphogenesis in neuron

Lianfeng Lin[1], Quanwei Lyu[1], Erkang Fei[2], Nancy Y. Ip[2] and Kwok-On Lai[1]

[1]School of Biomedical Sciences, State Key Laboratory of Brain and Cognitive Sciences, The University of Hong Kong

[2]Division of Life Science, State Key Laboratory of Molecular Neuroscience and, Molecular Neuroscience Center, The Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong, China

Email: laiko@hku.hk, linlf@connect.hku.hk

The majority of excitatory synapses are located on dendritic spines of the postsynaptic neuron. Spine formation and turnover is considered as an important mechanism underlying brain development as well as learning and memory. Several missense mutations of the human *tbc1d24* gene have been associated with epilepsy and intellectual disability. However, the physiological role of the TBC1D24 protein remains largely unexplored. Here we report an essential role of TBC1D24 in regulating the density and morphology of dendritic spines in hippocampal neurons. We found that TBC1D24 protein was enriched in the synaptic plasma membrane fraction of adult mouse brains. Immunocytochemistry further revealed that TBC1D24 was present in close proximity to dendritic spines and the postsynaptic scaffold protein PSD-95. Notably, the expression of TBC1D24 in hippocampal neurons was bi-directionally regulated in response to elevation and blockade of neuronal activity. Using short-hairpin RNA (shRNA) to knock down its expression in mature hippocampal neurons, we demonstrated that the maintenance of dendritic spines critically depends on TBC1D24. Moreover, the small GTPase ARF6 was identified as the downstream mediator of TBC1D24 in the regulation of spine morphogenesis. These findings suggest that TBC1D24 is involved in activity-dependent spine morphogenesis in the postsynaptic neuron, and defects in spine development might contribute to the pathophysiology of intellectual disability in individuals harboring the loss-of-function gene mutations.

This study was supported in part by the Research Grant Council of Hong Kong [General Research Fund (GRF) 16100814 and Early Career Scheme (ECS) 27119715].

P36

Effects of the apical Krebs solution on ion transport of cultured pig tracheal epithelia

Xu WY [1,2], Li WNF [1,2] and Chen JH [1,2]

[1]School of Biomedical Sciences, and [2] the University of Hong Kong Shenzhen Institute of Research and Innovation, the University of Hong Kong, Hong Kong, China

Email: WEI.YI.XU@hku.hk

Periciliary fluid (PCF) covering the airway surface is important for maintaining normal epithelial function and mucus properties. However, whether airway epithelia could sense and response to the alterations in PCF is not well understood. To address this question, we added Krebs solution on the apical side of cultured pig tracheal epithelia for incubation of 0, 1, 4, 7, 10 and 24 hr. Ussing chamber method was used to measure the transepithelial short-circuit currents (Isc). Compared to that of the control at 0 hr, Isc at the basal (Isc-Basal) were greatly reduced after 1 hr incubation, gradually decreased from 1 to 7 hr, not apparently changed between 7 to 10 hr and largely recovered at 24 hr. The Isc reductions by amiloride (ΔIsc-Amil), the inhibitor for the epithelial sodium channel (ENaC), were also changed in a pattern similar to that of Isc-Basal. These data suggest that the apical Krebs solution may reduce Isc-Basal by lowering the ENaC activity. However, Isc-Basal and ΔIsc-Amil reductions were attenuated by the pretreatment of epithelia with the AMPK inhibitor compound C and abolished by the ERK inhibitor U0126, suggesting that the ERK-mediated signaling pathway may be the major mediator in this mechanism. Moreover, real-time PCR measurement indicates that the mRNA levels of β- and γ-ENaC were significantly reduced at 7 hr incubation. Our data suggest that the reductions in Isc-Basal and ΔIsc-Amil at 7 hr by the apical Krebs solution may result from activation of the ERK signaling pathway and reductions in β- and γ-ENaC mRNA expressions.

P37

Kinesin-1 regulates extrasynaptic NMDAR targeting and its reduction can confer neuroprotection

Lin RZ[1†], Duan ZG[1†], Fung ML[1†], Wu WT[1], Lo, AC[2], Xia J[3], Huang JD[1]*

[1]School of Biomedical Sciences and [2]Department of Ophthalmology, Li Ka Shing Faculty of Medicine, the University of Hong Kong, Hong Kong, China;

[3]Division of Life Sciences, the Hong Kong University of Science and Technology, Hong Kong, China;

[†]These authors contribute equally.

* To whom correspondence should be addressed. E-mail: jdhuang@hku.hk

Email: duan.neurosci@gmail.com

The cellular response to brain injury mediates the fate of the neuron. Previous studies to identify neuronal responses that could be protective indicated intracellular transport as a potential target, but the underlying mechanisms are still unknown. Here, we showed that a decreased level of kinesin-1, a microtubule-dependent molecular motor, confers neuroprotection by reducing extrasynaptic N-methyl-D-aspartate receptor (NMDAR) targeting and functioning. We found that kif5b, the heavy chain of kinesin-1, was down-regulated by ischemic preconditioning. A loss of 50% of Kif5b protected the neurons against glutamate insult and ischemia provoked neurodegeneration through reducing the level of extrasynaptic NMDARs. Kinesin-1 forms complex with NMDAR *in vivo* and the tail of Kif5b directly binds with the NR2B cytoplasmic tails. Deletion of the binding domain within Kif5b abolished its interaction with NMDAR. Decreased kinesin-1 reduced the formation of this complex *in vivo*, prevented NMDAR concentrating at extrasynaptic sites and inhibited calcium influx mediated by extrasynaptic NMDAR activation to confer neuroprotection. *De novo* upregulation of the reduced Kif5b level abolished such protection effects, while disrupting Kif5b's interaction with NMDAR retained the protection effects. Our findings revealed that kinesin-1 reduction benefits and protects the neurons against neurodegeneration by reducing the cellular response to NMDAR mediated excitotoxic insult, which is likely to be an intrinsic event in the early stage of neurodegeneration. This finding could lead to the development of therapeutic strategies that fine-tune the intracellular transport machinery to postpone or halt neurodegeneration.

P38

Sox9 regulates choroid plexus epithelial polarity and blood-cerebrospinal fluid barrier integrity

Vong Keng Ioi[1], Ma Tsz Ching[1], Gao Caiji[1], Kwan Kin Ming[1,2,3]

[1]School of Life Sciences,

[2]Centre for Cell and Developmental Biology,

[3]Partner State Key Laboratory of Agrobiotechnology (CUHK), The Chinese University of Hong Kong, Hong Kong, P.R. China.

Email of presenting author: kiv1417@gmail.com

Corresponding author: kmkwan@cuhk.edu.hk

The choroid plexus (ChP) is a neurovascular tissue which is an integral part of the blood-cerebrospinal fluid (CSF) barrier. Found predominantly in infants and children, ChP pathologies are therefore detrimental to brain homeostasis and function. Both ChP intraventricular hemorrhage and the highly lethal choroid plexus neoplasms are diagnosed in significant proportions of newborns and children. However, genetic factors and molecular mechanisms contributing to these diseases are poorly understood. We identify that the transcription factor Sox9 is crucial for the proper development and function of ChP. Sox9 was robustly expressed in the hindbrain ChP throughout embryogenesis. Genetic ablation of *Sox9* from the ChP resulted in severe intraventricular hemorrhage, a hallmark of collapsed blood-CSF barrier. Loss of *Sox9* also resulted in aberrant proliferation of the ChP epithelium and therefore ChP hyperplasia displaying histology resembled that in ChP tumors. Interestingly, the apical-basal polarity in *Sox9* mutant ChP epithelium was disrupted. Accordingly, analysis of the CSF composition revealed that protein content in mutant CSF was elevated, suggesting of dysregulated blood-CSF barrier integrity in the *Sox9* mutant. In addition, we found that Sox9 was able to regulate the expression of *Prdm16* which was previously implicated in controlling the size of ChP, suggesting that Sox9 function in ChP was mediated by Prdm16. Thus we provide novel evidence for Sox9 as the master regulator in orchestrating blood-CSF barrier integrity.

Regulation of mammalian hindbrain neural stem cell/progenitor pool maintenance by β-catenin and Suppressor of fused (Sufu)

Michael Wenqi PAN[1], Catherine Hong Huan HOR[1], Chi Chung HUI[2], and Mai Har SHAM[1]

[1] School of Biomedical Sciences, Li Ka Shing Faculty of Medicine,
The University of Hong Kong, Hong Kong, China.

[2] Program in Developmental and Stem Cell Biology,
the Hospital for Sick Children, Toronto, Canada.

Email: wenqipan@hku.hk

Multiple signaling pathways including Shh, Wnt have been reported to regulate the specification, proliferation and differentiation of neural stem/progenitor cells in various neural tube and brain development models. However, how these signals cooperate and interact to achieve their regulatory functions in neural stem cell/progenitor is not clearly described. In this study, using genetic modified mouse models, we aim to show how β-catenin, a signal transducer of canonical Wnt signaling, and Sufu, a negative regulator of mammalian hedgehog signaling, and play convergent yet differential roles in the establishment and maintenance of neural progenitor pools in embryonic hindbrain rhombomere 4 (r4). We found deletion of β-catenin seems not affecting the proper dorsal-ventral patterning of the neural progenitor domains as marked by a series of homeobox genes, while stabilization of β-catenin leads to loss of progenitor markers expressions. In addition, stabilization of β-catenin will cause defective neuronal differentiation and compromised Sox2+ progenitor cell cycle exit, without significantly affecting cell proliferation. We showed that when Sufu is ablated in r4, the Sox2+ neural progenitors become highly proliferative, with a reduction of cell cycle exit, which can be partially restored by removing β-catenin. When Sufu is ablated in β-catenin-stabilized r4, Sox2+ progenitor cell cycle exit defect is further exaggerated compared with β-catenin-stabilized r4. In summary, our current data suggests that β-catenin level is critical for the establishment of neural progenitor domains in r4 and a convergent regulation of neural progenitor cell cycle exit by β-catenin and Sufu.

P40

Analysis of protein sorting at the *trans* Golgi network through STORM super-resolution imaging

Pik Ki Lau, Yan Huang, Teng Zhao, Ying Dai, Shengwang Du, M.M.T. Loy, Yusong Guo

Hong Kong University of Science and Technology

The *trans* Golgi network (TGN) is an essential transport hub in the secretory transport pathway. At the TGN, various cargo sorting machineries function to package specific cargo proteins into distinct transport carriers that are targeted to specific destinations. Protein sorting fundamentally relies on spatial segregation, but it remains largely unclear how proteins that participate the TGN sorting process are spatially related to each other *in vivo*. Here, we utilize 2-color STORM super-resolution localization microscopy (SRLM) to analyze protein sorting at the TGN at high resolution. Using SRLM, we directly visualized the association of specific cargo adaptors with clathrin. We revealed that Golgi-localized Arf proteins are not uniformly distributed on the Golgi, but instead, they are enriched in distinct membrane subdomains. In addition, we observed that a major cargo adaptor at the TGN, adaptor complex-1 (AP-1), is specifically associated with two of the Golgi-localized Arfs, Arfrp1 and Arf1. We also found that AP-1 structure shows distinct spatial relationships with other cargo adaptors. Moreover, we directly observed packaging of a planar cell polarity signaling receptor, Vangl2, in AP-1-decorated structures upon exiting the TGN. These systematic high-resolution imaging analyses indicate that the TGN membrane is mosaic, where different cargo sorting machineries are enriched in different microdomains to mediate sorting of specific cargo molecules. In addition, these analyses also show that some cargo adaptors are adjacent to each other suggesting that they may cooperatively regulate TGN sorting.

P41

KGGSeq: a rapid and robust framework for large-scale whole genome sequencing downstream analysis with integrative functions

Jiang Li[4], Youqiang Song[1,4] , Pak Chung Sham[1,2,3] and Miaoxin Li[1,2,3]

[1] The Centre for Genomic Sciences, the University of Hong Kong, Pokfulam, Hong Kong

[2] Department of Psychiatry, the University of Hong Kong, Pokfulam, Hong Kong.

[3] State Key Laboratory for Cognitive and Brain Sciences, the University of Hong Kong, Pokfulam, Hong Kong

[4] School of Biomedical Sciences, the University of Hong Kong, Pokfulam, Hong Kong.

Email:cougarlj@gmail.com

With the development of next-generation sequencing (NGS) technology, the cost of sequencing has tremendously decreased, which promotes the application of many sequencing methods in genetic studies, disease diagnose and so on. Especially, the whole genome sequencing (WGS) is a very promising method for geneticists because it can provide the comprehensive information of genotypes in both coding and non-coding regions, common and rare variants. However, the Big Data problem emerged at the same time due to the large-scale dataset of WGS and currently no professional tool can solve it at all points. KGGSeq is a software platform for comprehensive downstream analysis of whole-genome sequencing data. It is comprised of six functional modules: quality control, filtration, annotation, pathogenic prediction at variants, pathogenic prediction at genes and statistical tests, which can meet almost all researchers' demands for WGS data analysis. The original bit-block genotype storage mode can save 90% space and be much faster than existing tools. The gap-filled annotation rescue over 1000 exonic variants that are ignored by other tools. Moreover, KGGSeq reads the compressed variant call format (VCF) file by blocked GNU Zip format in parallel and parses the byte input stream directly. A heuristic algorithm is also used to facilitate the extracting information from text files. Finally, we compared KGGSeq with the existing tools by 1000 Genomes Project AFR panel, and KGGSeq always provided a more reasonable annotation and had a higher consistency with dbSNP database.

P42

Dlc1, a Rho GTPase-activating protein, is essential for cranial neural crest development

Yanxia Rao[1], Jessica Aijia Liu[1], May Pui Lai Cheung[1], Lo-Kong Chan[2], Irene Oi-Lin Ng[2] and Martin Cheung[1]

1.School of Biomedical Sciences, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China

2. State Key Laboratory for Liver Research and Department of Pathology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong, China

The neural crest is a transient population of multipotent progenitor cells, which emerges from the dorsal neural tube during early vertebrate development. These cells migrate extensively throughout the body to differentiate into a variety of cell types. The Sox (SRY-related HMG box) family of transcription factor, Sox10, has been implicated in cranial neural crest migration and differentiation via an as-yet unclear mechanism. Here, we revealed that *Dlc1*, a Rho-GTPase-activating protein (RhoGAP) that negatively regulates specific Rho family proteins (RhoA-C), is expressed at the anterior neural plate border region, and later becomes restricted in the premigratory and migrating cranial neural crest cells. Indeed, overexpression of *Dlc1* inhibited RhoA and promoted Rac1 activity consistent with their mutual antagonism that also underlies the transition from neural crest delamination to migration, but the total amount of migratory neural crest cells positive for HNK1 in the transfected cranial region remained unchanged compared to the vector control. By contrast, dominant-negative (DN) inhibition of Dlc1 function in cranial neural tube reduced expression of neural crest specifier, *Sox9*, whereas expression of *Snail2*, *Sox10* and *FoxD3* remained unaltered. In addition, embryos electroporated with DN-Dlc1 exhibited reduced expression of HNK1, and defects in cranial ganglia formation. Furthermore, we found *Sox10* was sufficient to induce ectopic *Dlc1* expression. Consistently, epistasis analysis showed that Dlc1 functions downstream of Sox10 in neural crest migration. Together, our findings reveal an essential requirement for DLC1 to function in the transcriptional cascade downstream of Sox10 to regulate cranial neural crest migration and differentiation likely through the regulation of Rho GTPases activity.

P43

Genetic contribution to intervertebral discs integrity maintenance in mouse model

Chi XIONG [1],Yan LI[1,2], Farroq Muhammad Rai[3], Llinda Sandell[3], Pak SHAM[2], Ying ZHANG[1], You qiang SONG[1], Kathryn S. CHEAH[1], Danny CHAN [1]

[1] Department of Biochemistry, The University of Hong Kong, Hong Kong, China

[2] Department of Psychiatry, The University of Hong Kong, Hong Kong, China

[3] Department of Orthopedic Surgery, Musculoskeletal Research Center, Washington University School of Medicine, US

Email: ssserena@hku.hk

LG/J (good healer) mice display enhanced healing capacity of cartilage, while SM/J are poor healers; according to my preliminary data, in the absence of environmental perturbations, SM/J mice display natural premature disc integrity changes by 4 weeks of age, while LG/J are "protected" at the same age. In SM/J mice, there are significant natural changes within the nucleus pulposus (NP) of the intervertebral discs represented by variations of cell morphology and the extracellular matrix. Given that recombinant inbreeds (RI) of LG/J X SM/J are established, we studied two RIs, LGXSM-6 (good healer) and LGXSM-33 (bad healer), tail discs at 4 weeks of age were obtained from Prof. Linda Sandell's group. The SM/J, LGXSM-6 (RI-6) and LGXSM-33 (RI-33) strains all showed "bad discs" with some differences in disorganization nucleus pulposus and irregular AF-NP boundary. Thus, the genetic traits for articular cartilage repairing appear to be different to the "maintenance" of intervertebral disc structure. Using the available genome-wide SNP genotype data for these four strains. After annotation and filtering, 178 SNPs localized to 87 possible genes were identified. Gene ontology (GO-term) enrichment was performed to identify relationship with biological pathways. Signaling involving ion channels were in the top rank, with VDR featured in many of the pathways. As a prove-of-principle assessment for relevance to the human cohort, we selected 14 top ranking genes for a test association in the human cohort, a total of 600 common variants on these candidate genes were tested. The Q-Q plot of these SNPs shows a clear deviation from the null hypothesis indicating there are more significant associations than expectation within the candidate list than by chance.

P44

Quality control of medicinal decoctions and granules using molecular techniques

Lo YT, Shaw PC

State Key Laboratory of Phytochemistry and Plant Resources in West China (CUHK),

LDS YYC R&D Centre for Chinese Medicine, School of Life Sciences,

The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China

Email: ytlo@link.cuhk.edu.hk

Chinese medicinal materials (CMMs) have been used for therapeutic purposes over thousands of years and the two major ways of CMMs consumption are decoctions and modernized Chinese medicine granules. In terms of authentication, the traditional methods such as organoleptic and microscopic identification are less applicable to them since the organoleptic characteristics are not available. Although chemical authentication still works for processed medicinal materials, chemical profiles are usually similar among related species, also they are subjected to change upon different developmental stages. Molecular authentication is comparatively more accurate as it bases on unique DNA sequences, and it is first stated officially in the 2010 edition of the Chinese Pharmacopoeia; however, it is only applied for crude CMMs but not processed one. This study is thus undertaken to find the availability of DNA present in decoctions and Chinese medicine granules. In this study, decoctions were made by replicating the traditional methods of preparation, respective medicinal granules were also bought from different manufacturers. They were used to (1) compare the efficiency of different DNA extraction methods (CTAB extraction, DNeasy plant mini kit and modified QIAquick Nucleotide Removal kit) by comparing DNA yield, purity and PCR amplificability of the DNA extracted; (2) determine the species identity by using diagnostic primers and DNA sequencing; (3) quantify the herb by using quantitative PCR (qPCR); and (4) determine unknown species identity by adapter ligation-medicated PCR and mini-barcodes. These findings will be useful for developing diagnostic kits and establishing protocols for authorities to further safeguard consumers.

P45

The Roles of Semaphorin 3A and Chondroitin Sulfates on Perineuronal Nets in Regulating the Development of Vestibular Circuitry

P.Y. Kwan [1], C.W. Ma [1], Y.S. Chan [1,2], D.K.Y. Shum [1,2]

[1]School of Biomedical Sciences, and [2]Research Centre of Heart, Brain, Hormone and Healthy Aging, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Sassoon Road, Hong Kong, China

Email: pykwanaa@gmail.com

Perineuronal nets (PN) play an important role on restricting neuronal plasticity during development. Our study of the central vestibular nucleus (VN) found consolidation of PN around GABAergic interneurons as from postnatal day (P)9 of Sprague Dawley (SD) rats. This was accompanied by progressive localization of semaphorin 3A (Sema3A) to chondroitin sulphate moieties (CS) of PN. We hypothesized that PN-CS binding of Sema3A limits the action of Sema3A as a plasticity-inducing factor in the VN.

We tested for structural plasticity in VN explant cultures (P3+up to 31DIV) following treatment with chondroitinase ABC (ChABC) and/or Sema3A. Parallel cultures were fixed for assessment of neurite arborization and growth. Compared with null treatment controls, increase in these parameters suggested involvement of CS and Sema3A in controlling structural plasticity of VN neurons.

To study the impact of PN-CS/Sema3A at the circuit level, the rats were assessed for the emergence of negative geotaxis as a read-out for maturation of the circuit for graviception. We observed negative geotaxis as early as P9, in correlation with consolidation of PN around GABAergic neurons in the VN. ChABC/Sema3A-treated rats showed delayed display of negative geotaxis, similar to effects of bicuculline but contrasting those of muscimol. The delay also correlated with postponed formation of PN on GABAergic interneurons after ChABC treatment, revealing that disturbance of PN consolidation interfered with maturation of the vestibular circuitry for graviception.

We further performed whole-cell patch-clamp recordings of miniature excitatory post-synaptic current (mEPSC) from VN interneurons of P7/P9 rats, control versus those treated with ChABC and Sema3A. We found increase in frequency of mEPSCs, suggesting strengthening of presynaptic signals along extended lengths of dendrites of interneurons in the ChABC/Sema3A-treated VN circuit.

Our results provide evidence for the role of PN-CS-Sema3A in controlling structural and circuit plasticity at the interneuron level with impacts on the developmental display of graviceptive behaviour. [Grant Support HKRGC17125115M & 777911M]

P46

Regulation of pre-mRNA processing in muscle stem cell quiescence and activation

Lu YUE [1], Tom CHEUNG[1]

[1] Department of Life Science, The Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong, China.

Email: lyue@ust.hk

Muscle stem cells, also called satellite cells, are muscle precursor cells that reside between sarcolemma and basal lamina of muscle fibers in adult skeletal muscle. Adult muscle stem cells are maintained in a quiescent state. Upon Injury, they have the ability to proliferate and differentiate into mult-inucleated muscle fibers to regenerate muscle and demonstrate self-renewal to maintain stem cell pool. It was reported that increased MyoD mRNA sequences, one of the myogenic transcription factors critical for satellite cell activation, were detected as early as 6 hour after injury in mononuclear cell in muscle. However, the mechanism of this fast response of MyoD mRNA after injury is unclear. We find out that MyoD transcripts are accumulated in a pre-mRNA form in quiescent satellite cells. Interestingly, MyoD pre-mRNA accumulation decreases in satellite cells after activation and re-increases in satellite cells after certain period of muscle regeneration. Some splicing factors involved in pre-mRNA processing may also play roles in satellite cell quiescence and activation regulation. The accumulation of pre-mRNA and the post-transcription regulation of pre-mRNA processing might suggest a regulatory mechanism controlling muscle stem cell quiescence and activation.

P47

Notch signaling regulates pharyngeal ectoderm development

Li Wang[1], Haoran Zhang[1], Elaine Yee Man Wong[1], Sze Lan Tsang[1], Urban Lendahl[2] and Mai Har Sham[1*]

[1] School of Biomedical sciences and Centre for Reproduction, Development and Growth, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong SAR, China;

[2] Department of Cell and Molecular Biology, Karolinska Institutet, Stockholm, Sweden.

Email: u3003287@hku.hk

During multiple tissues development, Notch signaling pathway plays essential roles in regulating cell proliferation, apoptosis, differentiation and cell fate determination. In human, it has been reported that *NOTCH2* and *JAG1* heterozygous mutations lead to craniofacial Alagille syndrome, while Notch3 is closely related to the lateral meningocele syndrome with defected branchial palate and low-set ear. In mice, haploinsufficiency of Notch1 leads to abnormal cleft palate and fusion of the palatal shelves and tongue, indicating the function of Notch signalling in regulating the craniofacial development. However, despite that Notch signalling has been propoposed to regulate the mesenchymal neural crest and hair cells differentiation in inner ear, whether and how Notch signalling regulate other craniofacial development remains largely unclear. Here, we find that the Notch1 receptor, Jag1 and DLL1 ligands as well as Lfng, Hes1 and Hey1 show specific expression pattern in the pharyngeal ectoderm during different stages. By overexpression of N1ICD in the pharyngeal ectoderm, the epibranchial placodal progenitors loss Ngn2, Islet1 expression and fail to differentiate and delaminate to produce neurofilament. However, the expression of Sox2 and Jag1 is highly elevated in the thickened pharyngeal ectoderm cells, suggesting distinct roles of Notch signalling in regulating the pharyngeal ectoderm cells. Lateral inhibition model is proposed that activation of Notch inhibit the placodal neurogenesis, while promote the spreading of Sox2+ lineages. Our results suggest that Notch signaling level is critical to control the different cell fate decision during the specification of pharyngeal ectoderm and thereby regulate the epibranchial placodal neurogenesis and segmentation of pharyngeal epithelium. These findings provide a possible mechanism underlying the abnormal pharyngeal arch development in Notch mutants.

P48

Cholesterol-lowering activities of short-chain fatty acids in high-cholesterol diet-fed Golden Syrian hamsters

ZHAO YM, CHEN ZY

Food and Nutritional Sciences Programme, School of Life Sciences, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China

Email: davidzhao2147@outlook.com,zhenyuchen@cuhk.edu.hk

Short-chain fatty acids (SCFAs) are the end products from colonic and cecal fermentation of dietary fiber with acetate, propionate and butyrate being the most abundant. SCFAs have been suggested to be partially accountable for the health-beneficial effects of dietary fiber. Previous researchers have shown that dietary SCFAs could suppress the cholesterol synthesis in rat liver. However, the underlying mechanism remains to be established. It is also unknown which short chain fatty acids are responsible for the cholesterol-lowering activity of dietary fiber.

In the present study, five groups of male Golden hamsters were fed a high cholesterol diet (HC, 0.2 % cholesterol added) or one of the four HC diets containing 5 mol/kg diet of acetate, propionate, butyrate, and valeric acid, respectively. After 6-week intervention, it was observed that dietary acetate, propionate, and butyrate but not valeric acid could significantly reduce plasma total cholesterol and non-high density lipoprotein cholesterol. Dietary propionate and butyrate could also significantly lower the ratio of nonHDL-C to HDL-C. Real-time quantitative PCR analysis demonstrated that dietary acetate, propionate, and butyrate upregulated hepatic mRNA expression of sterol regulatory element binding protein-2, LDL receptor, lipoprotein lipase, and downregulated hepatic SREBP1c mRNA expression. Besides, propionate supplementation lead to a higher hepatic abundance of mRNA cholesterol-7a-hydroxylase while dietary acetate downregulated the production of mRNA fatty acid synthase. These SCFA did not markedly affect the mRNA expression of farnesoid X receptor, peroxisome proliferator-activated receptor a in liver, nor the abundance of intestinal mRNA involved in cholesterol absorption such as Niemann-Pick C1 like 1. It was concluded that SCFAs with carbon number 4 orless were hypocholesterolemic. The future research aims to elucidate the mechanisms by which dietary SCFAs could decrease plasma cholesterol.

P49

Functional studies of Smad signaling in Heart Valve development

Liu Pak Lun Baggio [1], Kwan Kin Ming[1,2,3]

1 School of Life Sciences, The Chinese University of Hong Kong (SLS, CUHK)

2 Centre for Cell and Developmental Biology, The Chinese University of Hong Kong (CCDB, CUHK)

3 State Key Laboratory of Agrobiotechnology, The Chinese University of Hong Kong (SKL of Agrobiotech, CUHK)

Email: baggio718@hotmail.com

Heart valve development involves the endocardiac and outflow tract cushion formation which are the future heart valve forming sites for the two sets of atrioventricular valves and the other two sets of semilunar valves respectively. Together with the endothelial-to-mesenchymal transformation (EMT), these processes would bring the primitive heart valve to be formed from embryonic day (E) 8.5-10.5 in mouse. From E10.5 onward, heart valve remodeling begins and it would be involving proliferation, expansion and differentiation of mesenchymal cells, valve maturation and condensation in order to develop functional cardiac valves.

Bone morphogenetic proteins (BMPs) are important for early specification and initiation of EMT in the cardiac cushions. Canonical BMP signaling involves the activation of intracellular R-Smad proteins which are the signal transducers that act as transcription factors. Here, we found that the activated R-Smads (phospho-Smad1 and phospho-Smad5) and their interacting partner co-Smad (Smad4) were expressed at E15.5, E18.5 and adult stages in the heart valve. Using reverse genetic approach through the inducible Cre/*loxP* system, *Smad1* and *Smad5* were inactivated at different stages during heart valve development. In the *Smad1/5* CKO mutants, incorrect positioning and abnormal morphology of heart valve were observed at E18.5 in which inactivation driven by tamoxifen injection at E13.5 and E14.5. Nevertheless, mutants at adult stage with inactivation at six week-old displayed ectopic metaplasia in the aortic vales and as consequences, the *Smad1/5* CKO mutants developed cardiomegaly and ventricular dilation shown by echocardiography and micro-computed tomography (μCT). From the *lacZ* reporter mice, the valvular ectopic metaplasia aroused from the cells with inactivation of *Smad1* and *Smad5*. Together, our data suggest that BMP signaling plays a role at the stage of heart valve remodeling till adult stage apart from mediating EMT and endocardiac cushion formation from E8.5-10.5.

P50

Altered Dendritic Spine Plasticity in a Mouse Depression Model

Ng LHL [1], Huang YH [1], Chang RCC [2,3,4], Lai CSW [1,3,4]

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

[2] Laboratory of Neurodegenerative Diseases, School of Biomedical Sciences, LKS Faculty of Medicine, The University of Hong Kong

[3] State Key Laboratory of Brain and Cognitive Sciences, The University of Hong Kong

[4] Research Centre for Heart, Brain, Hormone and Healthy Aging, LKS Faculty of Medicine, The University of Hong Kong

Email: u3003836@hku.hk

Depressive disorder is a most prevalent psychiatric disorder worldwide and is estimated to be affecting 350 millions of the global population. In the prefrontal cortex (PFC) of depression patients, hypofunction is accompanied with structural deficits, including decreased cell number, neuronal atrophy and decreased number of spine synapses. In rodents, chronic stress exposure induces depressive-like behaviour and results in structural impairment of dendrites of layer 2/3 and 5 pyramidal neurons in PFC, including reduced dendritic spine density and atrophy of apical dendrites. However, it is unclear whether dendritic deficits contribute to depression development. Ketamine, a NMDA receptor blocker, is found to exert rapid, lasting antidepressant effect at a single, sub-anaesthetic dose. Ketamine can also rapidly reverse chronic stress-induced synaptic deficit. Yet, data on the effect of ketamine on dendritic spine plasticity in long-term is lacking. In this study, we used in vivo two-photon transcranial imaging of Thy1-YFP H line mice to investigate dendritic spine plasticity in the chronic restraint stress (CRS) depression model. We found that CRS increased dendritic spine elimination and reduced spine formation of layer V pyramidal neurons in the frontal association cortex. In addition, CRS-induced alterations in spine plasticity precede the onset of behavioural symptoms. Importantly, we found that ketamine treatment counteracted the effects of stress on dendritic spine plasticity.

P51

Construction and characterization of a three-dimensional nanoscale DNA origami box functionalized by a malaria aptamer

Marco S. L. Tang [1], Maia Godonoga [2], Andrew B Kinghorn [1], Yee-Wai Cheung [1], Jonathan Heddle [3] and Julian A. Tanner [1]

[1] School of Biomedical Sciences, Li Ka Shing Faculty of Medicine, University of Hong Kong, Hong Kong, China

[2] RIKEN, Saitama, Japan

[3] Malapolska Centre of Biotechnology, Jagiellonian University, Krakow, Poland

Eamil: szelokt@hku.hk

The DNA origami technique involves assembly of a long DNA scaffold with dozens of hybridizing DNA staples. Here, we present an example of integrating functional DNA aptamers into DNA origamis for molecular recognition at the nanoscale. In this regard, a functional DNA origami nanobox was incorporated with a pair of identical DNA aptamers specific for the malaria biomarker *Plasmodium falciparum* lactate dehydrogenase (PfLDH). The PfLDH aptamer locks the box by forming a DNA duplex with complementary oligonucleotides whereas the box is unlocked through strand displacement triggered by PfLDH binding. The origami box was assembled successfully as characterized by transmission electron microscopy. Moreover, the percentage of closed box correlated with the length of aptamer duplex, demonstrating that the aptamer lock had been incorporated into the correct position and hence kept the box shut. Future work will focus on developing other means of characterizing the box opening dynamics without the need for TEM. The work presented here gives insight into future development of DNA-origami based biosensors, particularly for disease diagnosis.

P52

Investigating the role of Rbfox2 in regulating alternative splicing during muscle stem cell activation

Kangnin LIN [1], Tom CHEUNG[1]

[1] Department of Life Science, The Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong, China.

Email: klin@connect.ust.hk

Satellite cells, or muscle stem cells, are resident somatic stem cells responsible for skeletal muscle regeneration. In resting, uninjured adult muscles, majority of satellite cells are quiescent. Upon stimulation such as acute injury, satellite cells are activated and subsequently proliferate, differentiate and finally fuse to form new muscle fibers. The activation of satellite cell is tightly regulated. Deciphering the mechanism regulating satellite cell activation is needed for understanding the muscle regeneration mechanism. Emerging evidences show that alternative splicing plays important roles in stem cell pluripotency maintenance and cell fate determination but the role of alternative splicing in satellite cell is still largely unknown. To better understand the role of alternative splicing in satellite cell, we investigate the role of Rbfox2 in the regulation of satellite cell activation during acute injury. Previous studies have showed that Rbfox2 regulates alternative splicing in several kinds of stem cells such as embryonic stem cell. In addition, Rbfox2 was also found to be important regulator of myogenic differentiation. We hypothesize that Rbfox2 plays a role in the regulation of alternative splicing during muscle stem cell activation. To investigating the role of Rbfox2, the expression level of Rbfox2 in different stages of postnatal myogenesis was characterized using immunostaining approach. The result showed that Rbfox2 was upregulated after satellite cell activation. To further study the function of Rbfox2, we used siRNA to knock down Rbfox2 in satellite cells and also generate an Rbfox2 conditional know knock out mice line. Some interesting phenotypes have been observed. These results show that Rbfox2 should have interesting function in satellite cell. Our study will help to understand the role of Rbfox2 during satellite cell activation and provide insight as to how transcripts are differentially regulated during satellite cell activation.

P53

Investigation on the Regulation of Dendritic Development in Cerebellar Purkinje Cells

Hu Y[1]; Kwan KM[1,2,3]

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

[2] Centre for Cell and Developmental Biology, The Chinese University of Hong Kong;

[3] Partner State Key Laboratory of Agrobiotechnology, The Chinese University of Hong Kong.

Email: enyahu93@gmail.com

Cerebellar Purkinje cells receive and integrate multiple input signals through spines on the elaborate dendritic arbors for motor coordination. The postnatal dendritic development including primary dendrite growth, spinogenesis and synaptogenesis helps to build up the functional cerebellar circuits. Dysregulation in the Purkinje circuits and degeneration of Purkinje cells are recognized phenotypes found in ataxias, the symptom featured by uncoordinated movement. Our lab was interested in unraveling the detailed molecular mechanisms regulating postnatal Purkinje cell dendritic development; and transcriptional factors, Lhx1 and Lhx5, were previously identified to regulate this process. More specific, decreased dendritic length and higher percentage of immature filamentous spine heads was detected in the *Lhx1/5* conditional knockout mutant Purkinje cells. Currently, we target on identifying downstream factors of Lhx1/5 to regulate Purkinje dendritic development. We focused on Wnt signaling, which regulates hippocampal neuron dendritic growth and spinogenesis through both canonical and non-canonical pathways. Interestingly, down-regulation of Wntless, a conserved Wnt ligand transporting protein, was identified in the *Lhx1/5* mutant Purkinje cells. Therefore, we will continue investigating the relationship between Lhx1/5 and Wnt signaling, and possibly through the role of Wntless in postnatal Purkinje dendrite development.

P54

Aptamer-Directed DNA Tweezers

Simon Chi-Chin Shiu, Yee-Wai Cheung, Roderick M. Dirkzwager, Shaolin Liang, Andrew B. Kinghorn, Lewis A. Fraser, Marco S. L. Tang and Julian A. Tanner

School of Biomedical Sciences, Li Ka Shing Faculty of Medicine,
The University of Hong Kong, Pokfulam, Hong Kong, China.
Email: simon156@hku.hk

DNA tweezers consist of a single-stranded DNA machinery which can switch structure from open to closed states triggered by the presence of target. Because of the known structure of binding between *Plasmodium falciparum* lactate dehydrogenase (PfLDH) and the specific aptamer, different splits were done on the aptamer sequence to choose the pair with highest retention of binding capability. Then, the split aptamer was incorporated to the probing region of the DNA tweezer and split quadruplex was used to generate signal when there was binding. PfLDH was observed to mimic complementary sequence to pull the arms into close proximity and allow the formation of a complete quadruplex. The quadruplex formed interacted with hemin through π-orbital stacking to confer peroxidase activity and oxidize 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) under the presence of hydrogen peroxide to give quantitative absorbance at 420 nm. Due to the intramolecular secondary structure of aptamer and the bulkiness of protein, a series of optimizations were performed on the structure of DNA tweezer to enhance the efficiency of machinery. Our research opens possibilities of using proteins to trigger DNA machine mechanism by aptamer-mediated molecular recognition.

P55

Toward exploring pluripotency landscape through light-induced tuning of Oct4 expression

Weimin Fan[1], Xibin Lu[1], Sihong Li[1], Jianjiang Hu[1], JD Huang[1], KM Yao[1], Wei Huang[1]

[1] School of Biomedical Sciences, LKS Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong, China

Email:wmfan@hku.hk

Oct4 is critical to the pluripotency of embryonic stem cell. According to Hitoshi Niwa's work, either 50% increment or reduction in Oct4 expression would result in stem cell differentiation. On the other hand, Oct4 expression varies from cell to cell in the same population. The variation could be up to 33 fold. The puzzle is that while 50% of increment of Oct4 could leads to differentiation, but under normal condition, the Oct4 expression variation is larger than 50% percent, why some cell with same Oct4 expression in two populations (50% increment and normal condition) would have different cell fate? To have a deeper insight into the influence of Oct4 on embryonic stem cell differentiation tendency, we propose to explore the stem cell pluripotency landscape on Oct4 in a quantitative manner. With synthetic biology tools, we knock down the endogenous Oct4 expression, and quantitatively tune the expression of exogenous Oct4. Surprisingly, Oct4 hyper-expression appeared to be regulated by unknown regulation on mRNA level. Our research might help to reveal the complex regulation of transcription factor network during development.

P56

Role of Orexin in Functional Maturation of Central Vestibular System in Motor Coordination and Spatial Recognition

U.T.F. Lam, Y.S.Chan.

School of Biomedical Sciences, Li Ka Shing Faculty of Medicine. The University of Hong Kong.

Orexin is known to participate in body balance and motor coordination via modulating synaptic transmission in the central vestibular system of adult animals. We hypothesize that orexin also regulates the maturation of vestibular functions during postnatal development. To test whether neonatal perturbation of orexinergic synapses in the vestibular nucleus (VN) exerts any effect on the acquisition of vestibular-related behaviors, we blocked or activated orexin receptors in the VN of postnatal day (P) 1 rats by implanting drug-containing Elvax slice onto the dorsal surface of VN for slow release of the drug to the underlying VN. Pharmacological intervention was achieved by loading the slices with orexin, orexin receptor antagonist (SB334867) or orexin receptor agonist ([Ala11, D-Leu15]-orexin-B). Specific behavioral tests including negative geotaxis (a graviceptive response), surface righting and air righting were performed on these rats at different postnatal stages until adulthood. Neonatal treatment with orexin receptor antagonist accelerated acquisition of negative geotaxis, surface righting, as well as air righting in the course of early postnatal development. In contrast, neonatal treatment with orexin or its receptor agonist delayed acquisition of negative geotaxis and surface righting. Adult rats pretreated at neonatal stage with orexin receptor agonist or antagonist were further tested for (i) spatial navigation by dead reckoning test; (ii) motor coordination by rotarod test and balance beam test. Treatment with orexin receptor antagonist enhanced the performance of dead reckoning while treatment with agonist impaired the performance of both dead reckoning and motor coordination. Taken together, our findings demonstrated that orexinergic modulation in the VN impacts developmental refinement of neural circuit for vestibular-related behaviors. [Supported by N_HKU735/14]

P57

Elucidating the role of Pabpn1 in mRNA alternative polyadenylation during satellite cell activation

Kim S.W. Lam[1] , Tom H. CHEUNG[1]

[1] Division of Life Science, The Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong

Email: swlam@ust.hk

Satellite cells are muscle stem cells that are primarily quiescent in resting muscle. Upon injury, satellite cells will activate, proliferate, and differentiate to repair the damaged muscle. The quiescent state is a tightly regulated process. Dysregulation of the quiescent state will result in a depletion of the stem cell pool and impairment of muscle regeneration. Previously, our group has identified that microRNA pathway as an essential regulation to maintain satellite cell quiescence. As binding sites of microRNAs are on the 3'UTR of mRNAs, the susceptibility of mRNA to microRNA regulation would depend on lengths of 3'UTRs. Therefore, we hypothesized that alternative polyadenylation plays an important post-transcriptional regulation to maintain satellite cell quiescence. Alternative polyadenylation involves a number of proteins. In particular poly(A) binding protein nuclear 1 (PABPN1) has been implicated as an important regulator of polyadenylation site selection. To better understand the role of PABPN1 in mRNA alternative polyadenylation, the expression level of PABPN1 *in vivo* in uninjured and regenerating muscle was characterized. In addition, the expression level of PABPN1 was also investigated *ex vivo* on satellite cells associated muscle fibers. It was found that PABPN1 was highly expressed in regenerating muscle and activated satellite cells. The result of this project will shed lights on how alternative polyadenylation regulates muscle stem cell function.

P58

Multifunctional drug M30 mitigates neurodegeneration and depressive-like behaviors induced by corticosterone in rats

Lam CS[1], Tipoe GL[1, 2], Wong JKC[3], Youdim MBH[4], Fung ML[1,2*]

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

[2]Research Centre of Heart, Brain, Hormone & Healthy Aging, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR

[3]Department of Pharmacology & Pharmacy, The University of Hong Kong, Hong Kong SAR

[4]Eve Topf Center for Neurodegenerative Diseases Research, Faculty of Medicine, Technion-Israel Institute of Technology, Haifa 31096, Israel

Email: lamsing@connect.hku.hk

Hypercortisolemia has been shown to induce pathogenic cascades leading to hippocampal atrophy and depression in both clinical patients and experimental animals. Monoamine oxidases (MAO), the downstream target of glucocorticoid, mediate the turnover and availability of monoamines and enzymatically produce hydrogen peroxide as a by-product. Yet, the pathophysiological role of MAO in monoamine deficiency and neurodegeneration induced by hypercortisolemia remains elusive. M30, a selective brain MAO inhibitor and an iron-chelating antioxidant, exerts neuroprotective effects in neurodegenerative disease models. We aimed to examine the antagonistic effect of M30 against neurodegeneration and depressive-like behaviors induced by corticosterone (CORT). Adult male Sprague-Dawley rats were given subcutaneous CORT injections with or without simultaneous M30 treatment for 2 weeks. CORT-treated rats exhibited behavioral despair and anhedonia in forced swimming test and sucrose preference test. Chronic CORT treatment results in remarkable increases in the levels of MAO activities, serotonin turnover, oxidative stress, neuroinflammation and apoptosis in the hippocampus when compared with the control group. Moreover, CORT treatment activated the cytokine-responsive serotonin and tryptophan catabolic enzyme indoleamine 2,3-dioxygenase (IDO-1) resulting in serotonin depletion. Furthermore, CORT treatment reduced spine densities of pyramidal CA1 and CA3 neurons. Remarkably, M30 administration effectively prevented depression in rats and deleterious alterations in the hippocampus induced by CORT. To conclude, our findings provide evidence that M30 mitigates the depressive-like behavior induced by CORT via targeting MAO-A overactivation that significantly contributed to oxidative stress, neuroinflammation, IDO-1 activation, serotonin deficiency and neurodegeneration.

P59

Elucidating the role of DLC1 in Metastatic Melanoma

Yang Xintao[1] Cheung Martin[1]

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

Email: xintao@hku.hk

Deleted in liver cancer 1 (DLC1) is a Rho GTPase-activating protein (RhoGAP) that is generally recognized as a tumor suppressor. Whether it functions, as a tumor suppressor in melanoma is largely unknown. In this project, we revealed that cytoplasmic DLC1 was detected in human metastatic melanoma cells characterized by dermal invasion. More importantly, we found SOX10, a member of SOX family transcription factor which is crucial for melanoma growth and progression, was initially localized in the nuclei of benign melanocytic nevus and became co-localized with DLC1 in the cytoplasm of metastatic melanoma, suggesting a correlation of their expression and possible functional interaction with melanoma progression. In addition, there are five DLC1 isoforms corresponding to different transcript variants due to alternative splicing. qPCR and Western blot analysis demonstrated that transcript variant 2 was predominantly expressed in panel of melanoma cell lines compared to other variants, indicating that DLC1 isoform 2 could play a role in the development of melanoma. To investigate this issue further, loss-of-function approach using lentiviral-mediated expression of shRNA targeting *DLC1* transcript variant 2 in melanoma cell lines is ongoing to investigate its functional requirement for melanoma growth, tumorigenicity and metastasis. By far, our pilot findings suggest a unique paradigm for DLC1 in metastatic melanoma.

P60

The role of RGS20 in cell cycle regulation and angiogenesis

Manton LEUNG[1], Astrid S. ALIM[1], Richard XU[1], and Yung H. WONG[1]

[1]Division of Life Science, State Key Laboratory of Molecular Neuroscience, and the Biotechnology Research Institute, HKUST, Clear Water Bay, Kowloon, Hong Kong, China

Email: mhleungab@ust.hk

G-protein coupled receptors (GPCRs) are involved in numerous cell signaling pathways including cell proliferation and angiogenesis. Regulator of G-protein signaling (RGS) proteins help to terminate the GPCR-induced cellular signals by accelerating the GTPase activity of the G alpha subunits. RGS20 is considered as a potential metastatic marker, as it is up-regulated expression level in primary human squamous cell carcinoma and metastatic melanoma (Nadiminty et al. 2010, Prostate 70: 276-287; Riker et al. 2008, BMC Medical Genomics 1: 1). The metastatic phenotype may be caused by deregulation of cell cycle. In our study, cell cycle related pathways, INK4/cyclin/CDK and PTEN/AKT, were examined for their possible involvement downstream of RGS20. Apart from proliferation, RGS20 may play a role in blood vessel formation. Treatment of VEGF in human umbilical endothelial cells can significantly increase RGS20 expression in mRNA level (Gerritsen et al. 2003, British Journal of Pharmacology 140: 595-610), indicating a potential role of RGS20 in vessel dynamic. Highly vascularized tumor xenografts induced by NIH3T3/Ras^{GV}/RGS20 have been reported (Wang et al. 2013, Cancer letters 339:33-41) which provides supportive evidence for the association between RGS20 and angiogenesis. For better understanding of the RGS20 action in angiogenesis, we profiled a series of angiogenic genes, including cytokines and growth factors, using a targeted cDNA array.

P61

The roles of *Irx3* and *Irx5* genes in inner ear patterning

LIU Yuchen[1], Wang Boshi[1], Elaine Wong[1], Chi-Chung Hui[2] and Mai Har Sham[1]

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

[2]Department of Molecular Genetics, University of Toronto, Toronto, Canada

Email: liuyuchenjulia@gmail.com

The *Iroquois* genes encode homeodomain transcription factors that are essential in multiple aspects of embryogenesis in metazoans, especially in the tissue patterning. For example, *Irx3* and *Irx5* could cooperatively regulate cardiac morphogenesis and limb bud progenitor specification. Our previous study indicated that both *Irx3* and *Irx5* are expressed in the developing inner ear. Their expression profiles were broad and overlapped at early stage in the otic vesicle, and gradually restricted to the non-sensory region of the cochlear epithelium. Lack of *Irx3* and *Irx5* lead to the fusion of vestibular and cochlear sensory organs and the loss of ductus reuniens that connect the saccule and cochlear duct. Considering the region specific expression pattern of *Irx3* and *Irx5*, and the sensory organ patterning defects in *Irx3/5*$^{-/-}$, we hypothesis that *Irx3* and *Irx5* contribute to inner ear neurosensory patterning and auditory sensory organ development. Here we show that deletion of *Irx3* and *Irx5* result in expansion of *Lfng* and *Jagged1* positive neuro-sensory competent domain, restriction of *Lmx1a* and *Tbx1* expression region and increased *NeuroD* positive delaminating neuroblast. However, the dorsal otic marker *N-Myc* and *Hmx2* are not affected, suggesting *Irx3* and *Irx5* could repress neural-sensory fate and help to maintain proper sensory/non-sensory boundary in otic vesicle, but not regulate otocyst dorsal-ventral axis patterning. In addition, *Irx3/5*$^{-/-}$ displayed ectopic neural-sensory competence and continuous neurogenesis in GER region of cochlear epithelium, causing abnormal and bigger auditory spiral ganglion. Furthermore, the malformed spiral ganglion forms a lump near the basal cochlear epithelium and cannot generate radial bundle that innervate mechonosensory auditory hair cell. Our results indicate that *Irx3* and *Irx5* contribute to negatively regulate neuro-sensory competence and neural fate commitment in otic epithelium.

P62

Quantitative analysis of myosin-driven apical constriction in delaminating neuroblasts

Yanru An[1,2], Guosheng Xue[1], Toyotaka Ishibashi[1], Chris Doe[3], Yan Yan[1,2]

[1]HKUST, Division of Life Science, Hong Kong, China, [2]HKUST, Center for Systems Biology and Human Health, Hong Kong, China, [3]University of Oregon, Institute of Neuroscience, Eugene, OR

Email: yanab@connect.ust.hk

The epithelial to mesenchymal transition (EMT) process is important for organ formation, tissue homeostasis and tumor metastasis. In our project, we use the formation of neuroblasts, which develop into *Drosophila* embryonic ventral nerve cord, as a model to study EMT events. For each proneural cluster in the neuroectoderm, mediated by the lateral inhibition, only one cell undergoes EMT and becomes neuroblast, while the surrounding cells remain as epithelial cells. Apical constriction is a key event during EMT, and we aim at dissecting how apical constriction is driven by the actin-myosin network in a single cell delamination event.

Through imaging live embryos, we noticed that myosin dynamics is distributed across the apical surface of both the delaminating neuroblasts and their neighboring non-delaminating cells. Correlation analysis indicated that medial myosin contractions correlate with apical area constriction for both presumptive neuroblasts and their neighbors; however, the medial myosin contractile pulses showed higher amplitudes and frequency in the presumptive neuroblasts. To specify the role of junctional myosin, we depleted the medial myosin pulses by injecting embryos with low-dose CytoD. Single presumptive neuroblasts still underwent apical constriction but would reflex back and unable to decrease their apical area in the end. Quantitative analysis showed that the junctional myosin intensity increase precedes apical area decrease in one round of apical constriction in these cells. Based on the results, we propose that the junctional myosin plays a role in initiating apical constriction and the medial myosin might function to stabilize the cell shape in the delaminating neuroblasts.

P63

Precise Control of Gene Expression via Synthetic Gene Circuits

LZ LIU[1§], YF Zhao[1, 2], JD Huang[1*]

1) School of Biomedical Sciences, The University of Hong Kong;

2) Department of Physics, The University of Hong Kong;

*Correspondence should be addressed to JDH jdhuang@hku.hk

§ Email of presenting author: liulz@hku.hk

Precise control of interesting gene expression is required to investigate the role of a gene playing in various biological processes including cell fate decision, stress response. Moreover, in the emerging field of synthetic biology, the ability to precisely control gene expression is important to achieve controllability, stability, and modularity of the synthetic gene circuits, which guarantee the elegance of the gene circuits in biotechnology, biomedical and industrial applications.

To better understand the design principles of synthetic gene circuits, we proposed to investigate how to fine-tune dose-response curve and level of gene expression noise (cell-to-cell variation). Specifically, we studied how the intracellular level of a transcriptional repressor, the number of the transcriptional repressor binding site in the regulated promoter and transcriptional autoregulation influence the shape of dose-response curve and gene expression noise.

Our data suggested that in a non-autoregulation scenario, increase of the intracellular level of the transcriptional repressor, reduced response sensitivity of the circuit to inducer while moderately enhanced noise level at low expression phase. Then, we demonstrated that we could achieve fine-tuning of gene expression noise by mean of changing the promoter structure. In addition, the data showed that transcriptional negative feedback reduced the abruptness of the transition and attenuated gene expression noise. The promoter structure influenced behaviours of autoregulation gene-circuits but almost did not influence behaviours of non-autoregulation gene-circuits. Specifically, in an autoregulation circuit, an increase of the number of repressor binding site moderately reduced the respond sensitivity of the circuit to inducer, however, escalated gene expression noise at low and high expression phases.

The results of this study could be useful for rational design synthetic gene circuits, to achieve desired sensitivity of the gene circuits, and control the cell-to-cell heterogeneity.

P64

Phage infection affects the central carbon metabolism of the marine cyanobacterium *Prochlorococcus*

Xingqin Lin and Qinglu Zeng

Division of Life Science, The Hong Kong University of Science and Technology,

Clear Water Bay, Hong Kong, China

Email: xlinae@ust.hk

In cyanobacteria, CO2 is fixed in the Calvin cycle by reacting with ribulose 1,5-biphospahte (RuBP). Glucose is synthesized in the Calvin cycle and can be consumed in the oxidative pentose phosphate pathway (PPP) to produce NADPH and ribose 5-phospahte, which serves as building blocks of nucleotides. Several genes involved in the light reaction of photosynthesis and PPP are found in viruses (cyanophages) that infect the marine cyanobacterium *Prochlorococcus.* However, none of the Calvin cycle genes exist in cyanophages. In addition, many cyanophages contain a Calvin cycle inhibitor gene *cp12*. We hypothesized that during infection cyanophages use these host-like metabolic genes to redirect carbon flux to PPP for phage DNA synthesis rather than carbon fixation. Here, we used *Prochlorococcus* strain MED4 and T4-like myovirus P-HM2 as a model system to start exploring this hypothesis. We showed that the ATP/ADP ratio of the infected cells was comparable to that of the uninfected cells, which is consistent with previous studies that light reactions of photosynthesis are sustained during infection. Compared to uninfected control, the carbon incorporation rate of infected cells dropped dramatically. In agreement with this lower carbon fixation, RuBP amount decreased during infection. Meanwhile, fructose 6-phosphate (F6P), a key intermediate used as indicator of carbon flux in PPP previously, increased significantly in the infected cells, suggesting higher carbon flow in PPP during P-HM2 infection. Our results reveal that host carbon flux is redirected from Calvin cycle to PPP when infected with myovirus, which might confer a fitness advantage for phage progeny production.

P65

Roles of Neuronal and Astrocytic Proheparanase in Regulating Synaptic Strength in Rat Hippocampus

Chow GWH [1], Cham WC [1], Chan YS [1,2] and Shum DKY [1,2]

[1]School of Biomedical Sciences, and [2]Research Centre of Heart, Brain, Hormone and Healthy Aging, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong, China

Email: george.chow@hku.hk

Following our report of neuronal expression of heparanase, we pursued the role of heparanase in synaptic plasticity. With hippocampal cells in culture, phorbol ester treatment stimulated secretion and pericellular retention of proheparanase. Synaptosomes prepared from hippocampal slices revealed clustering of proheparanase with syndecan-3 and AMPA receptors. Exogenous proheparanase triggered internalization of AMPA receptors as shown in hippocampal cell culture, lowered synaptic strength and decreased long-term potentiation as shown with hippocampal slice preparations. The results support our hypothesis of a role of proheparanase in regulating surface AMPA receptor level at the synapse. We then tested for sources of proheparanase in the synaptic environment. With highly purified hippocampal neurons forming networks at around 21 DIV and low-dose NMDA (glutamate analogue) to induce LTD chemically, we observed twice as much proheparanase in serum-free medium as that of controls. Similar treatment of purified astrocytes in culture showed proheparanase in medium 5-fold that of controls. We tested if proheparanase is released into the extracellular environment via exosome. The exosome fraction was recovered from the spent medium of astrocyte cultures following differential centrifugation and then ultracentrifugation. Indeed, proheparanase was detectable in the exosome fraction as marked by immunopositivities of Lamp1 and Alix. The results suggest synaptic spillover of glutamate triggers peri-synaptic astrocytes to respond via proheparanase secretion which then regulates peri-synaptic AMPA receptor level as a strategy in the regulation of synaptic strength.

P66

S-Nitrosylation of XIAP at Cys 213 of BIR2 domain impairs XIAP's anti-caspase 3 activity.

Weiwei Wu[1], Oi Wan Wan[1] and Kenny K. K. Chung[1]

[1]Division of Life Science, State Key Laboratory of Molecular Neuroscience, The Hong Kong University of Science and Technology, Hong Kong, China.

Email: wwuab@connect.ust.hk

XIAP is the most widely expressed IAP and plays an important role in regulating cell survival. XIAP contains three baculoviral IAP repeats (BIRs) domain and a RING domain. BIR domains provide XIAP anticaspase activities whereas RING functions as an E3 ligase. S-nitrosylation is a reversible post-translational modification of proteins by adding a nitric oxide to the thiol group of cysteine residue, which regulates a number of cellular processes. Our previous study found that XIAP can be S-nitrosylated at BIR domain and affect its anti-caspase activity. However, another study suggested S-nitrosylation of XIAP is through the RING domain, which affect its E3 ligase activity. In order to figure out the mechanism through which S-nitrosylation affect XIAP's anti-apoptotic function, We performed mutagenesis study and found that Cys213 of BIR2 is the critical cysteine residue for XIAP S-nitrosylation. Amino acid substitution of Cys213 diminished the S-nitrosylation of XIAP. The mutant protein XIAP-C213H was not affected by NO treatment in its anti-apoptosis function. These results confirmed the importance of one critical cysteine residue (Cys213) in XIAP S-nitrosylation and S-nitrosylation-induced loss of anti-caspase function. These findings will help understand how nitrosative stress contributes to PD pathogenesis through affecting prosurvival proteins such as XIAP.

P67

Differential sulfation of chondroitins in the brain extracellular matrix regulates neuroplasticity

Hau WF, Chan YS, Shum DKY

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

Email: hauwf14@hku.hk

Chondroitin sulfate (CS) is repeatedly reported to be a barrier for neurite outgrowth. However, emerging evidence shows that sulfation of CS may alter its biological functions. Three sulfation sites have been found in the repeating disaccharide units of mammalian CS: C-2 of glucuronic acid, C-4 and/ or C-6 of N-acetylgalactosamine. We hypothesized that differential sulfation of chondroitin leads to plastic event in the establishment of neural circuitry. To address this, we assessed the change, if any, in abundance of the sulfated CS disaccharides changed in the rat brain during postnatal development. Notably, we found decrease in mono-6-sulfated moieties (C6S) decrease, accompanied by increase in mono-4-sulfated moieties. Secondly, during post-traumatic vestibular compensation in adulthood, we detected increase in C6S in the rat brainstem supporting that sulfation of chondroitin changes in coordination with plastic changes in the brain. Thirdly, we recovered CS from the brain of rats at P0, P14 and adult age was biotinylated to allow immobilization onto streptavidin-coated dish. Anti-CS56 signal was detected in the dish after the treatment and it can be removed by chondroitinase. Assessment of the neurite growth pattern of cortical neurons seeded on immobilized CS is in progress.

P68

The role of Hedgehog receptor Cdo in controlling cochlear hair cell and pillar cell formation

Y. Liang[1], E.Y.M. Wong[1,2], H. R. Caro[1], Y. Liu[1], R. S. Krauss[4], P.X. Xu[3,4], C.C. Hui[5], M.H. Sham[1,2]

[1]School of Biomedical Sciences and [2]Centre for Reproduction Development and Growth, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China

[3]Department of Genetics and Genomic Sciences and [4]Developmental and Regenerative Biology, Mount Sinai School of Medicine, New York, USA

[5]Program in Developmental and Stem Cell Biology, The Hospital for Sick Children, Toronto, Canada

Email: lyujian@connect.hku.hk

Cdo (Cell adhesion molecule-related, down-regulated by oncogenes) is a novel receptor of the Hedgehog (Hh) pathway. Mutations in *Cdo* cause holoprosencephaly, a human congenital anomaly defined by forebrain midline defects prominently associated with diminished Hedgehog pathway activity. *Cdo* function as acomponent and target of the Hh signalling and feedback network. *Cdo* enhances Shh signalling by acting as co-receptors with *Ptch1,* or via regulation of *Gli* transcription factors. A proper balance of *Gli* repressor and activators is required to mediate Hh signalling druing inner ear morphogenesis.

Cdo homozygous knockout mice have profound hearing loss. However, the role of *Cdo* in inner ear development is still unknown. To understand the function of *Cdo* receptor in the modulation of Hh signaling in mammalian inner ear development, we present the differential expression pattern of *Cdo* in the developing mouse inner ear. We found that the expression of *Cdo* at E12.5 marks the prospective organ of Corti, but by E16 *Cdo* is down-regulated in hair cells and becomes restricted to supporting cells, suggest that *Cdo* may have distinct roles in molecular pathways that direct cells towards different cell fates in cochlea. Besides, the otic vesicle-derived inner ear structures are under-developed, with reduced proliferation and premature cell cycle exit during prosensory specification and ectopic hair cells formation in the *Cdo* mutants. It is possible that *Cdo* in Hh signaling are required for inhibiting cells from differentiating into hair cells and specifying progenitor cells to generate the distinctly fated cell populations in the inner ear.

P69

In silico structure-based screening and characterization of influenza virus inhibitors targeting cap-binding domain of PB2

Ming Liu[1], Chun-Yeung Lo[1], Lit-Man Poon[2] and Pang-Chui Shaw[1]

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

[2] School of Public Health, The University of Hong Kong, Hong Kong, China

Michelle_ming@163.com

Influenza A virus has long been one of the major threats to the health of the community. Although currently there are antivirals for the treatment of influenza infection, drug resistance is emerging rapidly amongst circulating influenza strains. Consequently, effective anti-influenza drugs with novel targets are needed. By in silico structure-based screening targeting the cap-binding domain of influenza A/Victoria/3/1975(H3N2), PDB ID:2VQZ), around 50 compounds out of 200,000 were selected, obtained and tested. Compound 225 exhibited inhibitory effect against the influenza RNP transcriptional machinery and were also capable of inhibiting the replication of influenza (A/WSN/1933/H1N1) with an IC50 of 3.8 μM. Referring to the interaction mode between 225 and the cap-binding site, 8 analogs were designed and tested. This study highlights the finding of a compound with a unique scaffold that has inhibitory towards a novel drug target, which provides us with valuable information for the design of inhibitors against influenza virus.

P70

Upregulated biosynthesis of Norepinephrine and MAOA expression mediates the oxidative stress and cell death in SH-SY5Y cells

Jing-Jie Li[1], Chun-Sing Lam[1], George L. Tipoe[1,2], Man-Lung Fung[1,2]

[1]School of Biomedical Sciences; [2]Research Centre of Heart, Brain, Hormone and Healthy Aging,

Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, P.R. China

Email: lijingjiejie@hotmail.com

Obstructive sleep apnea (OSA) is a major breathing disorder affecting 5-7% of adult population. Repeated blockade of the upper airway causes intermittent hypoxia (IH), leading to oxidative stress and inflammation. In addition, elevates levels of norepinephrine (NE) were found in OSA patients. Dopamine beta-hydroxylase (DBH) and tyrosine hydroxylase (TH) are two crucial enzymes required for NE biosynthesis. The NE homeostasis is maintained by activities of NE transporter (NET) for the reuptake and monoamine oxidase–A (MAOA) for the deamination of NE and also serotonin (5-HT), which produces H2O2 as a byproduct. MAOA is a target for antidepressant and depression is one of the comorbidities commonly reported in OSA patients. Moreover, tryptophan, a precursor of 5-HT, is catalyzed by 2,3-dioxygenase (IDO) that its expression is responsive to oxidative stress and inflammation. Studies were performed on cultured SH-SY5Y cells that constitutively express MAOA but not the MAOB subtype. Cells were treated with IH (repeated episodes of hypoxia at 0.5% or 1.5% oxygen for 4h followed by 21% oxygen for 4h) in the presence or absence of NE at concentrations of 0.01 μM and 0.1 μM. We found that cell viability (detected by MTT assay) was significantly decreased in a dose-dependent manner following IH treatment for 24h. M30, an inhibitor of MAOA with antioxidant properties, dose-dependently attenuated the lowered viability induced by IH. Also, IH treatment elevated the expression level of DBH and p-TH (Ser40), which were reversed by antioxidant 5 mM N-Acetyl-L-cysteine (NAC) or 2 μM calcium channel blocker nifedipine (NIF), suggesting that the upregulated NE biosynthesis induced by IH is mediated by ROS and calcium influx. Besides, the protein levels of MAOA and IDO expressions were increased by exposure to IH or NE for 48h, which were antagonized respectively by M30 or 10 μM clorgyline, a selective

inhibitor of MAO-A. Blockade of NET with desipramine (0.1 μM) attenuated NE-induced MAOA upregulation, indicating an involvement of NE reuptake. Finally, NE treatment significantly increased the ratio of GSSG/GSH with decreased the expression of superoxide dismutase (SOD2) and catalase, which were ameliorated by 10 μM clorgyline. Our results suggest that NE biosynthesis is upregulated by IH causing an increased expression of MAOA, which plays a significant role in oxidative stress and cell death induced by chronic intermittent hypoxia.

P71

Gamma-secretase cleaves stromal interaction molecule 1 induces capacitative calcium entry deficits in familial Alzheimer's disease

TONG Chun-Kit Benjamin[1,2], LAI Kwok-On[1] and CHEUNG King-Ho[1,2]

[1]School of Biomedical Sciences, LKS Faculty of Medicine, University of Hong Kong, Hong Kong SAR, China.

[2] HKU-Shenzhen Institute of Research and Innovation, Shenzhen, Guangdong, China.

Email: ckben@hku.hk

Alzheimer's disease (AD) is the most common form of dementia and mounting evidence suggests calcium (Ca^{2+}) disruption is its proximal pathogenic origin. Ca^{2+} dysregulation observed in cells expressing familial Alzheimer's disease (FAD)-causing presenilins (PS) has been attributed to the exaggerated Ca^{2+} release and the attenuated store-operated Ca^{2+} entry (also known as capacitative Ca^{2+} entry, CCE). Several mechanisms have been proposed for the exaggerated Ca^{2+} release, yet the underlying molecular mechanisms for attenuated CCE remain elusive.

In this study we employed Ca^{2+} imaging, FRET microscopy, in situ proximity ligation assay, in vitro γ-secretase cleavage assay and primary neuronal culture to delineate the mechanism for CCE attenuation and its linkage to AD pathology. We showed that the attenuation of CCE depends upon PS-associated γ-secretase activity. PS1 and STIM1 interact in human neuroblastoma SH-SY5Y cells, and mutant PS1 enhances γ-secretase cleavage of STIM1 in the transmembrane domain that has high similarity with amyloid precursor protein. Furthermore, FAD PS1-induced CCE attenuation destabilizes mature dendritic spines that are rescued by γ-secretase inhibition or overexpression of STIM1. Our results suggest a molecular mechanism of CCE deficits in which FAD-mutant PS1 enhances γ-secretase cleavage of STIM1, reducing recruitment of Orai1 that results in impaired CCE. These findings indicate a physiological role of PS1/γ-secretase in modulating the availability of STIM1 for CCE, and suggest that identification of STIM1 as a substrate of γ-secretase provides a novel therapeutic target for the treatment of AD.

P72

Regulation of Ryanodine Receptor Type 2 Single Channel Activities by Cyclic-ADP-ribose and Calcium

LEE Shuk-Kwan[1], TONG Chun-Kit Benjamin[1] and CHEUNG King-Ho[1,2].

[1]School of Biomedical Sciences and [2]Research Centre of Heart, Brain, Hormone and Healthy Aging, LKS Faculty of Medicine, University of Hong Kong, Hong Kong China.

Email: ckben@hku.hk

Mobilization of intracellular calcium (Ca^{2+}) by second messengers, including the inositol-trisphate ($InsP_3$), cyclic ADP-ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP), triggers signaling cascade that controls life and death. It is well-established $InsP_3$ binds to $InsP_3$ receptor to release Ca^{2+} from the endoplasmic reticulum (ER). Recently nuclear membrane electrophysiology demonstrated NAADP activates Ca^{2+} release from the two-pore channel (TPC). Although cADPR has been suggested to mobilize ER Ca^{2+} through the activation of ryanodine receptor (RyR), direct evidence showing the activation of RyR by cADPR in native ER membrane is lacking. In this study, we applied nuclear membrane electrophysiology on a tetracycline-induced stable mouse RyR2 receptor expressing HEK293 cell. We characterized the electrophysiological properties and the ligand regulations of RyR2 single channel using the nuclear membrane patch-clamp technique. Using 140 mM potassium ion (K^+) as the charge carrier, we demonstrated that both caffeine and cADPR activate RyR2 single channel from isolate nuclei with conductance of about 625 pS. The detected RyR2 single channel traces show a linear current-voltage relationship in symmetrical K^+ solutions and channel open probability displays [cADPR] denpendency that peaked at 100 nM. Furthermore, in saturating [cADPR], RyR2 channel activities display cytoplasmic [Ca^{2+}] dependence. Taken together, nuclear membrane electrophysiological approach provides a novel approach to characterize the biophysical properties of RyR and both cADPR and Ca^{2+} are physiological ligands that regulate RyR channel activities.

P73

A positive feedback loop between PIM2 and TNFα contributes to hepatocellular carcinoma tumorigenesis and progression

Xuming Tang[1], Tingting Cao[2], Yun Zhu[1], Liyi Zhang[2], Jinna Chen[2], Xinyuan Guan[2], Jiandong Huang[1]

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

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

Email: xmtang@hku.hk

Up-regulation of Pim-2 proto-oncogene, serine/threonine kinase (PIM2) was found in many kinds of human malignancies. In this study, we characterized the roles of PIM2 in the pathogenesis and progression of hepatocellular carcinoma (HCC). Up-regulation of PIM2 was frequently detected in HCC patients and significantly correlated with HCC recurrence ($P = 0.014$) and poor prognosis ($P = 0.013$). Functional studies showed that PIM2 overexpression could promote HCC cell proliferation, cell migration and invasion, resistance to chemotherapy and tumor formation in nude mice, all of which could be effectively inhibited when PIM2 was silenced with short hairpin RNA (shRNA). Mechanistic studies revealed that PIM2 overexpression could activate NF-κB signaling pathway through up-regulating phosphorylation level of RIPK2. Furthermore, we found TNFα, a pro-inflammatory cytokine, could stimulate the expression of PIM2 in HCC cells and PIM2 overexpression in HCC cells could in turn up-regulate the expression level of TNFα. Besides, the mRNA levels of PIM2 and TNFα in HCC patients are closely correlated (Pearson $r = 0.5332$, $P < 0.0001$). We also found that PIM kinase inhibitor AZD1208 treatment could effectively attenuate HCC cells' tumorigenic ability both in vitro and in vivo. Collectively, our study revealed a positive feedback loop between an inflammatory factor and a proto-oncogene that contributed to the tumorigenesis process of HCC, and PIM kinase inhibition may serve as a therapeutic target in the treatment of HCC.

P74

Ephs and ephrins in the Enteric Nervous System Development

Zhining Li[1], Carly Leung[1], Hon Man Sit[1] and Mai Har Sham[1]

[1]School of Biomedical Sciences, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong SAR, China.

Email: ning3178@hku.hk

Transmembrane tyrosine kinase receptors Ephs and their membrane-bound ligands ephrins are involved in controlling cellular behavior. Interactions of Ephs and ephrins affect cytoskeleton dynamics and mediate various cellular responses, including repulsion and adhesion of migratory neural crest cells. Eph/ephrin signalings also affect cell-matrix interactions through crosstalk with the integrin signaling pathway. By performing time-lapse live-cell imaging in ENS defect model $Sox10^{NGFP/+}$, we observed that Sox10 $^{NGFP/+}$ ENCCs formed abnormal aggregates and displayed abnormal interactions on extracellular matrix with enhanced adhesion properties. RNA-seq analysis of wild type and $Sox10^{NGFP/+}$ ENCCs showed that over 2000 genes were differentially expressed, including several Ephs/ephrins, which was confirmed by qRT-PCR analysis. In order to dissect their functions in ENS, we have investigated the expression patterns of Ephs and ephrins in E10.5 to E14.5 developing mouse gut by immunofluorescence analysis. The EphA2/A4 and ephrinA4 were expressed in gut mesenchyme and $Sox2^{+}$ endothelial cells while ephrinA1 was strongly expressed in mesenchyme but hardly detectable in endothelium. They also co-expressed with Sox10 in ENCCs. EphB1/B2 were detected in the mesenchyme and $Sox10^{+}$ ENCCs from E12.5. Both ephrinA5 and ephrinB2 were specifically detected in $PGP9.5^{+}$ neuronal lineage at E12.5 and E14.5. The differential expression of Ephs and ephrins in different cells in the gut suggest that they are required for ENS development and may be involved in cellular interactions during ENS development. Therefore, we hypothesize that Ephs and ephrins could be involved in ENS development and could be candidate Hirschsprung disease genes.

P75

Raising and lowering the 'alarmone' in bacteria

Ning YANG[1], Shujie XIE[1] and Rory M. Watt[1]

[1] Faculty of Dentistry, The University of Hong Kong, Hong Kong, China

Corresponding author email: rmwatt@hku.hk

Bacterial cells synthesize phosphorylated guanine molecules known as alarmones in response to adverse extracellular conditions, such as amino acid starvation. There are three known alarmone species synthesized by bacteria: guanosine 5'-triphosphate, 3'-diphosphate (pppGpp), guanosine 3', 5'-bis(diphosphate) (ppGpp), and guanosine 5'-monophosphate, 3'-diphosphate (pGpp). Small alarmone synthase (SAS) proteins, which are present in certain Gram-positive bacteria such as *Staphylococcus aureus*, have been shown to synthesize pppGpp, ppGpp and pGpp. Bifunctional Rel (RelA/SpoT) proteins have the ability to synthesize, as well as hydrolyze (p)ppGpp molecules. However, the roles of SAS proteins remain to be fully elucidated. Furthermore, the proteins responsible for the hydrolysis or biochemical interconversion of pppGpp, ppGpp and pGpp molecules, across bacterial species, also remain to be fully established. Here, we report the biochemical characterization of the bifunctional Rel protein from *S. aureus* (SA-Rel). We compare and contrast its biochemical activities against the two SAS proteins that are also encoded by *S. aureus* (SA-RelP, SA-RelQ); as well as Rel and SAS proteins encoded by other bacterial species. We show that the full length SA-Rel protein efficiently synthesizes pppGpp and ppGpp, but does not synthesize pGpp. Other bifunctional Rel proteins share this property. In contrast, SA-RelP and SA-RelQ can synthesize pppGpp, ppGpp and pGpp alarmones. Furthermore, we reveal that the catalytic N-terminal domain of SA-Rel has the ability to efficiently hydrolyze pGpp to GMP and diphosphate. In conclusion, our results suggest that SAS proteins may be primarily responsible for pGpp synthesis in many Gram-positive bacterial species, and bifunctional Rel proteins may be responsible for its degradation.

P76

Immunoglobulin-like domains of PECAM-1 contribute to trans-homophilic interaction

Menglong Hu[1], Quan Hao[1,*]

1. School of Biomedical Sciences, University of Hong Kong , Laboratory Block, 21 Sassoon Road, Pokfulam, Hong Kong, China

(menglong.hu@gmail.com)

Platelet–endothelial-cell adhesion molecule-1(PECAM-1) plays important roles in cell adhesion and communication. It acts as a significant intermediary of tight cellular junction between adjacent surfaces of endothelia and most embryonic cells through its homophilic binding pattern. Heterophilic interaction with those candidates (CD177, glycosaminoglycans, CD38 and integrin $\alpha_V\beta_3$) happens in processes like leukocyte migration and tumor cell invasion. However, the conformational change of PECAM-1 mediated endogenous and exogenous signal transmission remains to be elaborated, and the mechanisms of homophilic and heterophilic binding pattern are still unclear. Here, we resolved the crystal structure of PECAM-1 Ig-like domain 1-2 trans-dimer without glycosylation. Both Ig-like domain 1 and 2 share the classical Ig domain structure which is composed of two layers β-sheets with seven antiparallel β-strands, and are anchored by a pair of cysteines that consist of a disulfide bond. And the trans-dimer of Ig domain 1-2 is resulted from hydrophobic interfaces on Ig-like domain 1 and 2 separately. The status of dimer in solution also indicate the homophilic binding pattern of PECAM-1. In addition, cell adhesion assay shows few differences on PECAM-1 derived cellular junction between proteins with and without glycosylation on first three putative glyco-sites of PECAM-1, which announces mutations on glyco-sites for crystallographic study does not affect original status of dimmer. Our finding shows the details of how PECAM-1 assemble with each other among adjacent endothelia cells and form a barrier between hematologic system and tissues. This project is supported by RGC grant (HKU 766412).

P77

Biochemical Characterization of a PPX/GppA Exopolyphosphatase Protein from *Zymomonas mobilis*

Tang NY[1], Lu B[1], Bartlam M[2] and Watt RM[1]

[1] Faculty of Dentistry, The University of Hong Kong, Hong Kong, China

[2] College of Life Sciences, Nankai University, PR China

Email: pnytang@hku.hk

Inorganic polyphosphate (poly-P) molecules are linear chains of 3 to *ca.* 1000 (ortho)-phosphate units, linked by 'high- energy' phosphoanhydride bonds. Poly-P is found in all cells in nature. In prokaryotes, poly-P is essential for normal growth, stress-adaptation and survival; and consequently its intracellular levels are closely regulated. In most prokaryotes, the degradation of cellular poly-P is catalyzed by members of the highly-conserved exopolyphosphatase (PPX/GppA) protein family. The majority of PPX/GppA protein homologues characterized to date efficiently hydrolyze poly-P chains from their termini, liberating phosphate units. However, some PPX/GppA homologues have negligible poly-P hydrolyzing activities, and appear to function primarily as nucleotide 5'-phosphohydrolases. Here, I report the biochemical characterization of the 508aa PPX/GppA protein from the ethanol-producing bacterium *Zymononas mobilis* subsp. *mobilis* NCIMB 11163 (ZmPPX). ZmPPX possesses efficient exopolyphosphatase activities against a range of poly-P chain lengths. Incubation of ZmPPX with nucleotide substrates showed that ZmPPX also has notable ATPase, GTPase and pppGpp 5'-phosphohydrolase activities. The removal of the N-terminal 29aa residues [ZmPPX(30-508)] did not drastically affect its exopolyphosphatase activities, but completely abrogated its pppGpp 5'-phosphohydrolase activities. In contrast, the alanine-replacement of a highly conserved glutamate residue (ZmPPX-E137A) led to a near-complete loss of all hydrolytic activities. These results suggest that residues 1-29 may play an important role in the binding of nucleotide substrates, or in the modulation of active site architecture.

P78

Uncovering the role of *csf1rb* in hematopoietic development

Shuting WU, Jin XU, Rongtao XUE

Division of Life Science, State Key Laboratory of Molecular Neuroscience

Center of Systems Biology and Human Health, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, PRC

Email: swuah@connect.ust.hk

Hematopoiesis is the formation of all the cellular components in the blood system. This process is basically conserved in all vertebrates; it involves two waves: an embryonic transitory primitive wave generating only erythroid and myeloid cells, and later a definitive wave in which the multipotential self-renewing hematopoietic stem cells (HSCs) can generate and replenish all mature blood cells throughout the adulthood. During differentiation, these HSCs firstly specify into lineage-specific progenitors through cell fate commitment and later differentiating into all mature blood lineages. This process is highly hierarchical and finely regulated, and researchers have long been interested in understanding the underlying mechanisms. One of these signaling pathway, colony-stimulating factor-1 (CSF-1) and its receptor CSF1R, is important for the mononuclear phagocyte lineage such as macrophages in mice, and both nullizygous mice show defects caused by the absence of several tissue-resident macrophages including osteoclasts and microglia. There are two orthologous of *csf1r* in zebrafish, *csf1ra* and *csf1rb*. While previous loss-of-function study on *csf1ra* recaptured the phenotype of myeloid cells deficiency in mutant mice, the role of *csf1rb* remain unclear. Interestingly, our recent work found that *csf1ra* and *csf1rb* are differentially expressed *in vivo*, and knock out of *csf1rb* impairs the development of definitive hematopoietic stem and progenitor cells (HSPCs), as well as its numerous progenies, which has not been observed in the mutant mice. The fully characterization of the role of csf1rb will uncover previously unknown involvement in the hematopoietic development and provide a vivid example to dissect the contribution of whole-genome duplications (WGD) to the functional evolution of vertebrate orthologs.

P79

Sufu regulates the cell cycle exit and differentiation of cochlear hair cell progenitors

Chin Chung Ho[1] , Boshi Wang[1] , Elaine Y. M. Wong[1] , Yuchen Liu[1] , Chi-Chung Hui[2] and Mai Har Sham[1]

1 School of Biomedical Sciences, The University of Hong Kong, Pokfulam, Hong Kong, China

2 Program in Developmental and Stem Cell Biology, The Hospital for Sick Children, Toronto, Canada

Email: cedricho@hku.hk

Cochlear hair cells, which are essential for hearing, are derived from Sox2-positive progenitors in the prosensory domain. The progenitors exit the cell cycle from E12.5 to E14.5, and start to differentiate from E14.5. The progenitors at the apical region become post-mitotic but remain undifferentiated for more than 3 days. It has been suggested that the timing of differentiation is regulated by Sonic Hedgehog (Shh) signaling. However, the functions of Shh signaling downstream mediators, Sufu and Gli factors, in hair cell differentiation are still unclear. We showed that cochlear hair cell differentiation in *Pax2*-cre; $Sufu^{flox/flox}$ mutant mice was delayed. Since this mutant had reduced Gli3 repressor level, $Gli3^{P1\text{-}4/P1\text{-}4}$ and $Gli3^{\Delta 699/\Delta 699}$ mutants were examined. $Gli3^{P1\text{-}4/P1\text{-}4}$ mutant with downregulated Gli3 repressor level exhibited delayed hair cell differentiation. On the other hand, in $Gli3^{\Delta 699/\Delta 699}$ mutants with high level of Gli3 repressor, hair cell differentiation was accelerated. It suggests that Sufu positively regulates hair cell differentiation by increasing Gli3 repressor level. As the timing of cell cycle exit determines the number of postmitotic progenitors that are available for differentiation, it is important to investigate whether Sufu regulates cell cycle exit of prosensory cells. By BrdU labeling, it was found that, in *Pax2*-cre; $Sufu^{flox/flox}$ mutant, there were some BrdU-positive cells in the prosensory domain at the basal region of the cochlear duct at E14.5 while the prosensory cells at basal region of control cochlear duct already exit cell cycle. Our data suggest that Sufu positively regulates both cell cycle exit and hair cell differentiation.

P80

The role of Smad4 in regulating muscle stem cells in vivo

Chenghao Situ[1], Gang Wang[1], Han Zhu[1], and Zhenguo Wu[1]

1. Division of Life Science, Center for Stem Cell Research, Center for Systems Biology and Human Health, and the State Key Laboratory in Neuroscience, Hong Kong University of Science &Technology

csitu@ust.hk

Muscle stem cells, also termed satellite cells, are crucial for skeletal muscle growth and egeneration. In healthy adult muscle, satellite cells are quiescent but poised for activation. During injury-induced muscle regeneration, most activated satellite cells transiently re-enter the cell cycle to proliferate and subsequently exit the cell cycle to differentiate, while some satellite cells return to the quiescent state to maintain the stem cell pool. It remains unclear how each step is precisely regulated. Previous cell culture-based studies indicate that BMP signaling negatively regulate myogenic differentiation. Its roles in vivo at different developmental stages remain to be elucidated. As Smad4 is a core factor in the BMP/TGFbeta pathway, to address its role in vivo, we generated a satellite cell-specific, tamoxifen-inducible mouse knockout strain (i.e., Smad4 f/f: Pax7CreER). Upon injury, the adult KO mice showed severely impaired regeneration, underscoring Smad4's important role in muscle regeneration. Furthermore, the smad4 deficiency did not affect satellite cells' maintenance, activation and proliferation, but the smad4 KO satellite cells differentiated precociously, which is consistent with the results in vitro. We propose that Smad4 promotes satellite cells expansion by inhibiting premature differentiation, which is essential for muscle regeneration. To understand how Smad4 inhibits differentiation, we are planning to identify genome-wide Smad4 target genes by RNA-Seq. In addition, we will also examine the role of Smad4 in embryonic and neonatal muscle development and satellite cell formation. Our work would provide a better understanding of the role of Smad4 in regulating muscle satellite cells in vivo.

P81

Cdk5-mediated phosphorylation of liprinα1 is critical for synaptic plasticity

Huang HQ[1,2,3], Lin XC[1,2,3], Liang ZY[1,2,3], Zhao T[4], Du SW[4], Loy M. MT[4], Lai KO[1,2,3,†], Fu AK[1,2,3], and Ip NY[1,2,3,*]

[1]Division of Life Science, [2]Molecular Neuroscience Center, [3]State Key Laboratory of Molecular Neuroscience, 4Department of Physics,
The Hong Kong University of Science and Technology,
Clear Water Bay, Hong Kong, China
Email: xlinal@ust.hk

The precise regulation of synaptic plasticity is crucial for proper nervous system functioning. For example, changes in the molecular composition of the postsynaptic region regulate synaptic plasticity, which underlies learning and memory. Recent evidence suggests that such changes in postsynaptic composition can be regulated by the serine/threonine kinase, cyclin-dependent kinase 5 (Cdk5). To better understand how Cdk5 regulates synaptic plasticity, it is important to identify Cdk5 substrates and characterize their functions. Liprinα1, a member of the liprinα family, interacts with the LAR family of transmembrane protein tyrosine phosphatases, which are important for synaptic maintenance. Here, we found that liprinα1 is highly expressed in postsynaptic regions and can be phosphorylated by Cdk5. Knockdown of liprinα1 in hippocampal neurons by shRNA significantly reduced dendrite numbers and dendritic spine protrusion density, which can be rescued by the overexpression of RNAi-resistant wild-type liprinα1. Importantly, liprinα1 phosphorylation dramatically reduced dendritic spine density and impaired their maturation. Blockade of liprinα1 phosphorylation by a small interfering peptide promoted spine morphogenesis and maturation. Furthermore, the treatment of acute mouse hippocampal slices with the interfering peptide enhanced synaptic plasticity, as demonstrated by enhanced long-term potentiation. These findings collectively reveal an important role of the Cdk5-mediated phosphorylation of liprinα1 in hippocampal synaptic function and plasticity.

P82

Elucidating the Functional Implications of Polyphosphate-Protein Interactions

Khong ML [1], Li L [2] and Tanner JA [1]

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

[2]Neuroscience Research Institute, Peking University.

Email: meilikhong@gmail.com

Composed of tens to hundreds phosphate residues linked together by high-energy phosphoanhydride bonds, inorganic polyphosphate (polyP) is a simple polymer found in all living cells. PolyPs are present endogenously as a free molecule and/or complexing with another molecular species as means to exert its physiological roles. Our aim is to determine significant polyP-protein interactions to understand the cellular functions of polyP in osteoblasts. By cross-linking polyP to biotin and immobilising polyP via biotin-streptavidin interactions, an affinity chromatography approach identified candidate proteins that interact specifically and functionally with polyP. Proteins identified included chaperones and proteins involved in gene transcription and cargo trafficking. Independent binding experiments confirmed that cyclophilin B (CypB), a chaperone involved in protein folding activity and extracellular matrix (ECM) organisation, interacts with polyP specifically and at relatively high affinity. Recombinant human CypB was expressed and purified for biochemical assays. These assays revealed that long-chain polyP inhibits the peptidyl-prolyl cis-trans isomerase activity of CypB more effectively than that with cyclosporin A, a well-characterised cyclophilin inhibitor. Coupled with future co-localisation studies and assays to validate the intracellular effects polyP exerts on CypB activity, we will be able to have a clearer understanding as to whether the functional effect polyP has on CypB activity is significant for crosstalk between polyP, chaperone-mediated protein folding and ECM physiology.

P83

Deregulation of RNA N6-adenosine methylation contribute to human carcinogenesis

Mengnuo Chen, Wei Lai, Chuck-Ting Law, Felice Ho-Ching Tsang, Iris Ming-Jing Xu, Joyce Man-Fong Lee, Carmen Chuk-Lui Wong, Irene Oi-Lin Ng, Chun-Ming Wong.

The State Key Laboratory for Liver Research, Department of Pathology, Li-Ka Shing Faculty of Medicine, The University of Hong Kong

Email:cmnhku16@hku.hk

Epigenetic alterations profoundly contribute to human carcinogenesis. Traditionally, epigenetic researches primarily focus on DNA methylation, histone modifications and chromatin remodeling. Recently, emerging evidences suggest that diverse and revisable chemical modifications on RNAs, also known as "epi-transcriptome", represent a new layer of epigenetic regulation. N-6-methyladenosine (m6A) is the most abundant modification on eukaryotic mRNA. m6A modification is involved in regulating mRNA stability, alternative splicing, and translation efficiency. However, the roles of m6A deregulation in human carcinogenesis remain to be explored. By whole-transcriptome sequencing (RNA-Seq), we first identified that METTL3, the major m6A methyltransferase, was significantly upregulated in multiple human cancers. Functionally, we proved that knockdown of METTL3 remarkably inhibited cancer cell proliferation, migration and colony formation in vitro and substantially suppressed tumorigenicity in nude mice. On the contrary, using CRISPR-dCas9 activation system, we demonstrated that overexpression of METTL3 significantly increased cell proliferation, promoted colony formation on soft agar and augmented in vivo tumorigenicity. To delineate the underlying molecular mechanisms, we interrogated the transcriptome changes of two METTL3 knockdown cell lines by RNA-Seq. To this end, we identified SOCS2, a prominent tumor suppressor, as a down-steam target of METTL3 mediated m6A modification. We found that SOCS2 contains two conserved m6A enrichment sites located at its 5' and 3'-UTR regions. Knockdown of MELLT3 impaired m6A mediated mRNA degradation and thereby stabilized SOSC2 mRNA. Our findings suggested that deregulation of METTL3 and its associated m6A modification could contribute to human carcinogenesis through epigenetically controlling the stability and expressions of critical tumor suppressor genes.

P84

Identification of long non-coding RNAs Involved in Glycine max (Soybean) Nodule Function Via RNA-Seq

Mui Z, Lin X, Wong WHJ, Ku YS, Wong FL, Chan TF, Lam HM

School of Life Sciences
The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China
E-mail: zeta.mui@gmail.com

Nodulation is an intricate symbiotic process of which the symbiotic bacteria rhizobia infects root hairs of legumes to form a nitrogen-fixing organ. During such organogenesis, root cells undergo extensive transcriptomic changes a physiological changes to prepare cells for the housing of rhizobia, such as dedifferentiation of root cortical cells and cellular division. ENOD40, an early nodulin and nodulation-specific long non-coding RNA (lncRNA) was discovered to have functions in initiation of nodulation, inducing cortical cell dedifferentiation and division. ENOD40 is also expressed in mature nodules in soybean, suggesting that ENOD40 might have a potential function in mature nodules. In light of discoveries of lncRNAs with functions in plant development, gene and epigenetic regulation, phosphate homeostasis and its observed expression in mature nodules, an RNA-seq experiment is designed to uncover more lncRNAs that might have functions in mature nitrogen-fixing nodules in soybean, enabling the selection of candidates for further functional study. Ribosomal RNA-depleted RNA samples of C08-R4 nodules and remaining roots were sequenced with 3 biological replicates. Paired-end sequencing reads were trimmed, and were use to perform de novo transcriptome assembly and reference-assisted assembly with the soybean William 82 reference genome. After filtering assembled transcripts with protein-coding domains using BLASTx to the NR database and differential gene expression calling, 325 putative lncRNA candidates were found with tissue-specific expression.

P85

Synergism between the pharmacological inhibition of PARP1 and ATM involves targeting major DNA damage repair pathways

Joyce P.Y. MAK and Randy Y.C. POON

The Hong Kong University of Science and Technology

joycemak@ust.hk

Poly(ADP-ribose) polymerase 1 (PARP1) plays indispensable roles in both DNA single-stranded break (SSB) repair and alternative non-homologous end-joining (Alt-NHEJ) for DNA double-stranded break (DSB) repair. Because of this, inhibitors of PARP1 (including the FDA-approved Olaparib) are being used to target cancers deficient in the homologous recombination repair (HRR) such as advanced BRCA1/2-mutated ovarian cancer. Similar to BRCA1/2, ATM plays a critical role in HRR, and can be targeted using small-molecule inhibitors such as KU-60019. As both PARP1 and ATM are highly expressed in nasopharyngeal carcinoma (NPC), we studied the potential synergism between Olaparib and KU-60019 in NPC cell lines. We found that the combined treatment of Olaparib and KU-60019 led to an accumulation of DNA SSBs and subsequent generation of DSBs, which in turn activated the G_2 DNA damage checkpoint and apoptosis. Interestingly, a PARP1 inhibitor that does not induce the so called "PARP trapping" (Veliparib) did not show synergism with KU-60019, suggesting that PARP trapping rather than the inhibition PARP1 catalytic activity alone is crucial for synthetic lethality with HRR inhibition.

P86

Bigelovin induced apoptosis in colorectal cancer through extrinsic pathway

Li MY[1], Yue GGL[2], Tsui SKW[1], Tan NH[3], Lau CBS[2], Fung KP[1,2].

[1]School of Biomedical Sciences; [2]Institute of Chinese Medicine &State Key Laboratory of Phytochemistry and Plant Resources in West China,
The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong;
[3]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China.
Email: limingyue1028@163.com

Colorectal cancer (CRC) is one of the most malignant cancers with high mortality. Despite the effectiveness of the current chemotherapy, the high incidence of side-effects (up to 98%) severely affect normal life. Traditional Chinese Medicine is proven to be a rich source of anti-tumor drugs. Previous studies demonstrated that bigelovin, a sesquiterpene lactone isolated from *Inula helianthus-aquatica*, has anti-tumor, anti-inflammatory, and anti-angiogenic activities. However, no study has been reported on the effect of bigelovin and its underlying mechanisms on colorectal cancer. In this present study, *in vitro* assays including MTT, Annexin V/PI double staining, Western blot and *in vivo* studies in HCT 116 tumor-bearing nude mice were used to evaluate the effects of bigelovin on CRC. From the results, bigelovin could dose- and time-dependently suppressed the growth and colony formation, and induced apoptosis in human colon cell lines HT-29 and HCT 116. Further study showed that extrinsic pathway played essential roles in bigelovin-induced apoptosis, and death receptor 5 (DR5) was one of bigelovin targets in CRC. In murine model, bigelovin treatment (5, 10, 20 mg/kg; every two days for ten times; intraperitoneally) could inhibit tumor growth. Bigelovin (20 mg/kg) demonstrated significant tumor inhibition and fewer side effects than conventional FOLFOX (containing oxaliplatin, folinic acid and 5-fluorouracil) treatment. In addition, *in vivo* data confirmed that bigelovin-induced apoptosis was through up-regulation of DR5. In conclusion, this is the first study on the effects of bigelovin on CRC, the promising results in tumor-bearing mouse model suggested the potential of bigelovin in CRC treatment.

P87

Restoration of autophagic flux improves endothelial function in diabetic mice

Lei Zhao, Jian Liu, Li Wang, Jiang-Yun Luo, Ling-Shan Gou, Chi Wai Lau, Xiao Yu Tian, Yu Huang

School of Biomedical Sciences and Institute of Vascular Medicine, Chinese University of Hong Kong, Hong Kong, China

Email: zhaoleieva@gmail.com

Endothelial dysfunction contributes to the development of atherosclerosis and vascular complications in diabetes mellitus, however, the mechanisms underlying endothelial dysfunction are incompletely understood. Growing evidence reveals that autophagic flux plays an essential role in maintaining normal proper vascular function. Alterations in autophagic flux are increasingly being implicated in disease processes that include diabetes and atherosclerosis. However, the role of autophagy in endothelial cell is poorly understood. Therefore, the present study aims to investigate the role of autophagy in endothelial dysfunction in diabetes. We observed increased accumulation of P62 and LC3 in aortas of diabetic mice compared with non-diabetic mice. Blocking the terminal stage of autophagy with chloroquine increased LC3 and p62 in aortas from diabetics to a lesser extent than aortas from controls, indicating autophagic flux was reduced in diabetes. Reduced autophagic flux was closely associated with increased mitochondrial reactive oxygen species production, a marked reduced of agonist-stimulated endothelial nitric oxide (NO) bioavailability and acetylcholine-induced endothelium-dependent relaxations (EDRs). We identified that the transcription factor EB (TFEB), a master regulator of lysosomal biogenesis and autophagic flux, nuclear translocation was reduced in db/db mouse endothelium. Most interestingly , overexpression of TFEB restored autophagic activity, attenuated mitochondrial ROS production and improved endothelial function in db/db mice. The present results suggest that inadequate autophagy contributes to endothelial dysfunction in diabetes mellitus. Restoring autophagic flux reduces mitochondria ROS and thus improves endothelial function, suggesting that the restoration of autophagy may represent another novel therapeutic strategy for treating diabetic vasculopathy.

P88

Bone morphogenic protein 4-Smad induced up-regulation of platelet-derived growth factor AA impairs endothelial function

Weining Hu, Chi-Wai Lau, Xiaoyu Tian, Yu Huang

Institute of Vascular Medicine and School of Biomedical Sciences, Chinese University of Hong Kong, Hong Kong, China.

Email：weining.hu@gmail.com

yu-huang@cuhk.edu.hk

Bone morphogenic protein 4 (BMP4) is an important mediator of endothelial dysfunction in cardio-metabolic diseases, whereas platelet-derived growth factors (PDGFs) are major angiogenic and pro-inflammatory mediator, although the functional link between these 2 factors is unknown. The present study investigated whether PDGF mediates BMP4-induced endothelial dysfunction in diabetes mellitus. We found that PDGF-AA impaired endothelium-dependent vasodilation in aortas and mesenteric resistance arteries. BMP4 upregulated PDGF-AA in human and mouse endothelial cells, which was abolished by BMP4 antagonist noggin or knockdown of SMAD1/5 or SMAD4. BMP4-impared relaxation in mouse aorta was also ameliorated by PDGF-AA neutralizing antibody. Tail injection of Ad-Pdgfa-shRNA ameliorates endothelial dysfunction induced by Bmp4 overexpression (Ad-Bmp4) *in vivo*. Serum PDGF-AA was elevated in both diabetic patients and diabetic *db/db* mice compared with nondiabetic controls. Pdgfa-shRNA or Bmp4-shRNA adenovirus reduced serum PDGF-AA concentration in *db/db* mice. PDGF-AA neutralizing antibody or tail injection with Pdgfa-shRNA adenovirus improved endothelial function in aortas and mesenteric resistance arteries from *db/db* mice. The present study provides novel evidences to show that PDGF-AA impairs endothelium-dependent vasodilation and PDGF-AA mediates BMP4-induced adverse effect on endothelial cell function through SMAD1/5-and SMAD4-dependent mechanisms. Inhibition of PDGF-AA ameliorates vascular dysfunction in diabetic mice (supported by RGC-GRF, RGC-CRF and NSFC).

P89

Focal TLR4 activation mediates disturbed flow-induced endothelial inflammation and dysfunction

Qu D, Lau CW, Wang L, Xu J, Song WC, Tian XY, Huang Y

School of Biomedical Sciences and Institute of Vascular Medicine, Chinese University of Hong Kong, Hong Kong SAR, China

Email: irisqu@link.cuhk.edu.hk

The endothelium governs the health of blood vessels, while its dysfunction plays a critical role in both the initiation and progression of cardiovascular diseases including atherosclerosis. Disturbed flow of blood at arterial branches and curvatures directly modulates endothelial physiology and predispose the region to endothelial dysfunction and development of atherosclerotic lesions. Emerging experimental and clinical evidences show that activation of the Toll-like receptors (TLRs), in particular TLR4, contributes to vascular inflammation and atherosclerosis. However, whether TLR4 participates in the disturbed flow-induced endothelial dysfunction and inflammation has not been studied yet.

An expression profile and functional assessment of TLRs have been provided, demonstrating that TLR4 was the dominant among TLR family members in vascular endothelial cells. Upon TLR4 activation, MyD88-dependent pathway mediated expression of a serial of inflammatory cytokines, chemokines and adhesion molecules, and impaired endothelial function. TLR4 expression and its downstream inflammatory signaling were elevated in response to disturbed flow in aortic arch compared and thoracic aorta and in partial carotid ligation models, which were inhibited in the $Tlr4^{mut}$ mice. The same results were also observed in the *in vitro* flow settings in endothelial cells. Of several endogenous TLR4 ligands examined, fibronectin containing its extra domain A (FN-EDA) expressed by endothelial cells was identified to be increased by disturbed flow and directly interact and activate TLR4.

Our results demonstrates for the first time an indispensable role of TLR4 in disturbed flow-induced endothelial inflammation which may serve as the critical initiating step in atherogenesis, and pinpointed FN-EDA as an endogenous mediator that transfers the haemodynamic force to TLR4 activation. This study emphasizes distinct endothelial phenotypes exposed to different blood flow patterns and focal alterations of microenvironment that niches pathogenesis of atherosclerosis.

(Supported by RGC-CRF and NSFC)