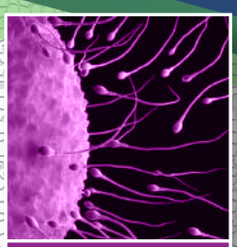
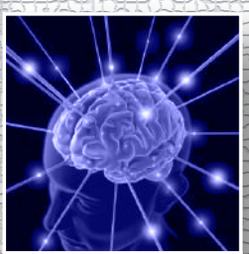
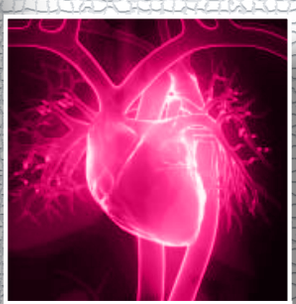
School of Biomedical Sciences
Research Day 2015
cum Cardio-Metabolic Biology Symposium 2015



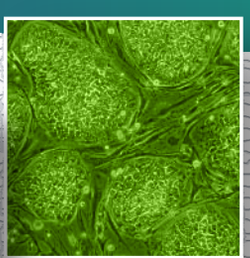
Reproduction, development and endocrinology



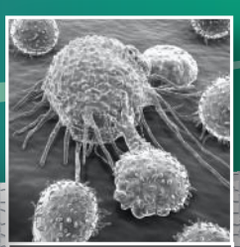
Neurodegeneration, -development and repair



Vascular and metabolic biology



Stem cell and regeneration



Cancer and inflammation

4-5 June 2015

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

香港中文大學
The Chinese University of Hong Kong

CU Medicine HONG KONG
香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

School of Biomedical Sciences Research Day 2015

Members of the Organizing Committee

Professor Andrew M. Chan

Professor Wai Yee Chan

Professor Wing Tai Cheung

Professor Chi Hin Cho (Chairman)

Professor Yu Huang

Professor Xiaohua Jiang

Professor Yiu Wa Kwan

Professor Sidney S.B. Yu

COVER: The five Thematic Research Programs of School of Biomedical Sciences, CUHK
Designed by Ms. Tai Fung Wan, School of Biomedical Sciences, CUHK

CONTENTS

Welcome Message from the Dean of Faculty of Medicine

It is my great pleasure to welcome you to the School of Biomedical Sciences (SBS) sixth Research Day cum Cardio-Metabolic Biology Symposium 2015. This important annual event brings together academics and experts from different fields of the biomedical sciences. It focusses on showcasing and sharing the latest findings from our investigators and serves as a platform for furthering discussions on research themes and directions, and for making useful networks with like-minded individuals.

We are much honoured to have Professor Yan Chen from Shanghai Institutes for Biological Sciences (SIBS), Chinese Academy of Sciences (CAS) and Professor Chris Yun-Fai Lau from University of California, San Francisco, USA as plenary speakers. We are most delighted to learn that a delegation of 6 academics from SIBS led by Professor Yan Chen will join this event. The active contributions from all participants will help pave the way for deepening and broadening collaborations and further strengthening the linkages amongst institutions.

Please join me in thanking members of the Organizing Committee as well as those who provide the background support. Their generous contributions of time and energy and the good cheer in face of brutal deadlines are indispensable ingredients for the continued success of this annual highlight event of the School of Biomedical Sciences.

We hope that at the end of this two-day event, all participants will not only feel inspired and motivated but also renew friendship and commitments for further collaborations to advance the frontiers of biomedical sciences.

Professor Francis K.L. Chan
Dean, Faculty of Medicine
Choh-Ming Li Professor of Medicine & Therapeutics
The Chinese University of Hong Kong

Welcome Message from the Director of School of Biomedical Sciences

I am most delighted to welcome you all to the School of Biomedical Sciences (SBS) Research Day 2015 cum Cardio-Metabolic Biology Symposium 2015.

This is our sixth Research Day since the School established back in 2009. This year the Shanghai Institutes for Biological Sciences (SIBS), Chinese Academy of Sciences (CAS) are joining us to co-organize a symposium on Cardio-Metabolic Biology on Day 1. Prof. Yan Chen, Professor and Director of the Institute for Nutritional Sciences, SIBS will give a plenary lecture at the Symposium. He also leads a delegation of 6 academics to join the Research Day and associated Symposium. It is envisaged that, by sharing the latest research interests and findings, future collaboration could be developed.

On Day 2, we proudly present to you the other plenary speaker – Professor Chris Yun-Fai Lau from University of California, San Francisco, USA.

Being one of our School flagship events, the annual Research Day would not only provide a platform for colleagues to exchange research ideas, but also to showcase our expertise in biomedical research. In this fast changing world, we do encourage our colleagues to stay competitive through sharing the newest discoveries and promoting collaborations with other experts both regionally and internationally.

I sincerely hope that every participant would enjoy and be inspired by the programmes in the coming two days. Meanwhile, I would also like to take this opportunity to thank members of the Organizing Committee and helpers of the Research Day for their thoughtful planning and dedication in making this event a success.

Wai-Yee Chan, Ph.D.
Professor of Biomedical Sciences
Director, School of Biomedical Sciences
The Chinese University of Hong Kong

Biographies of Plenary Speakers

Prof. Yan Chen is a professor and director of the Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China.

Prof. Chen obtained his Bachelor and Master degrees from the Medical School at West China University of Medical Sciences in 1983 and 1986, respectively. He received his Ph.D. from Indiana University School of Medicine in 1994, and conducted his postdoctoral training in UCSD and Salk Institute. Prof. Chen had been working in Indiana University School of Medicine as an Assistant Professor and Associate Professor from 1998 to 2004. Prof. Chen joined the faculty of Institute for Nutritional Sciences at Shanghai, China in 2004.

The research in Prof. Chen's laboratory focuses on applying tools of molecular biology, cell biology and animal models to understand the molecular mechanisms, signal transduction and functional roles in human diseases in the areas of obesity, type 2 diabetes and cancer development. His current research is focused on understanding the molecular mechanisms underlying regulation of metabolism by subcellular compartmentalization and cellular factors, diet- and alcohol-induced fatty liver, and glycogenesis in glucose and lipid homeostasis. In addition, he is also deciphering the cellular and *in vivo* functions of a new tumor suppressor gene. Prof. Chen is highly accomplished with more than 100 peer reviewed papers in high impact journals such as *Nature, PNAS, Mol Cell, J. Hepatology, Diabetes, and Cancer Research.*

Prof. Chris Yun-Fai Lau received his B.A. from the State University of New York, Binghamton in 1973 and his master and doctoral degrees from the University of Texas Health Science Center, Houston, in 1976 and 1979 respectively. He joined University of California as a postdoctoral fellow in 1979 and promoted to Professor since 2001.

For the past twenty years, Prof. Lau has been studying the roles of the Y chromosome genes in human development, physiology and diseases. He an internationally recognized investigator in the field, particularly on male-specific/dominant cancers. The Y-located TSPY gene is the gene for the gonadoblastoma locus on the Y chromosome, responsible for predisposing susceptible germ cells for tumor development. It was initially isolated in his laboratory, and characterized extensively with molecular genetics, genomics and transgenic mouse approaches. TSPY is a putative male-specific oncogene. Significantly, there is a homologue for TSPY on the X chromosome, which is designated as TSPX. TSPX and TSPY originated from the same ancestral gene, but diverged considerably with the evolution of the present day X and Y chromosome respectively. TSPX behaves as a tumor suppressor in many human cancers. Alternatively spliced TSPY and TSPX transcripts could encode proteins with contrasting properties as those for the normal protein. Hepatocellular carcinoma (HCC) is one of the deadliest cancers, and has a male dominance among its patients, especially those chronically infected with hepatitis virus. Since hepatitis C virus infection is extremely high among the Veterans and HCC is a prevalent disease among this population. Understanding the molecular oncogenesis and the male dominance of HCC will be critical for the Veterans Health Administration. Successful implementation of their research programs could shed critical insights on the etiologies, and development of strategies for diagnosis, prognosis and genderized and personalized clinical managements this male-biased cancer affecting thousands of Veterans.

Another project is designed to investigate the role of Y chromosome genes in sexually dimorphic diseases, such as Alzheimer's and Parkinson's diseases, schizophrenia, hypertension, using advanced genomics, molecular genetics and transgenic strategies. Sex differences are most suitable for a near-term implementation of precision medicine, which is a newly developed concept in the medical field, evolved from recent advances in high throughput technologies, such as next generation sequencing and computational tools for large databases. Precision medicine builds a network-based new taxonomy of diseases, in which the individual variability is taken into account for the prevention, diagnosis, prognosis and treatment strategies of each patient.

School of Biomedical Sciences

Cardio-Metabolic Biology Symposium 2015

4 June 2015 (Thursday)

This is a joint meeting with the Shanghai Institutes for Biological Sciences (SIBS), Chinese Academy of Sciences

Cardio-Metabolic Biology Symposium 2015
4 June 2015 (Thursday)

Room G02, Lo Kwee-Seong Integrated Biomedical Sciences Building

09:00-09:10 **Opening Ceremony: Prof. Yan Chen and Prof. Chi Hin Cho**

09:10-09:55 Plenary Lecture by Prof. Yan Chen (Abstract No. PL-01)
"Golgi apparatus-mediated signaling in metabolic control and tumorigenesis"
Chairperson: Prof. Yu Huang

09:55-10:00 **Photo taking**

Time	*Title of Presentation*	*Speaker*	*Abstract No.*
	Session I ***Chairpersons: Ying Yu and Francis F.Y. Lam***		
10:00-10:25	High salt primes a distinct macrophage activation state, M(Na)	Shengzhong Duan	CMB-01
10:25-10:50	Combined simvastatin and 1,25-dihydroxyvitamin D, *in vitro*, provides synergistic bone anabolism of ovariectomized (OVX) rats	Yiu Wa Kwan	CMB-02
10:50-11:15	Amino acids and metabolic signaling	Feifan Guo	CMB-03
11:15-11:40	***Tea Break***		
	Session II ***Chairpersons: Huiyong Yin and Feifan Guo***		
11:40-12:05	EP3 receptor deficiency attenuates pulmonary hypertension through suppressing Rho/TGFβ1 pathway	Ying Yu	CMB-04
12:05-12:30	Nitric oxide-cGMP-PKG inhibits hypertrophy of human embryonic stem cell-derived cardiomyocytes	Lei Sun (representing Xiaoqiang Yao)	CMB-05
12:30-14:00	***Lunch Break***		
	Session III ***Chairpersons: Shengzhong Duan and Yiu Wa Kwan***		
14:00-14:25	CLOCK/BMAL1 regulates circadian change of insulin sensitivity via SIRT1	Qiwei Zhai	CMB-06
14:25:14:50	Protease-activated receptor-1 (PAR-1) activating peptide suppresses ischaemia-induced injuries in rat models of stroke	Francis F.Y. Lam	CMB-07
14:50-15:15	Vitamin D3 and vascular benefits	Yu Huang	CMB-08
15:15-15:40	***Tea Break***		
	Session IV ***Chairpersons: Qiwei Zhai and David C.C. Wan***		
15:40-16:05	Modulation of macrophage function by oxidized phospholipids in low density lipoproteins in the context of atherogenesis	Huiyong Yin	CMB-09
16:05:16:30	FDA-approved drugs as inhibitors of fatty acid binding protein as new drugs for treatment of metabolic diseases	David C.C. Wan	CMB-10

16:30-16:45 ***Closing Ceremony: Prof. Yu Huang***

PL-01

Golgi apparatus-mediated signaling in metabolic control and tumorigenesis

Y. Chen

Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, P.R. China.

The orthodox function of Golgi apparatus is the regulation of protein processing and vesicle trafficking. However, whether Golgi apparatus plays a role in cell signaling is not fully appreciated. Through a series of studies in recent years, we have demonstrated that Golgi apparatus plays a critical role in the regulation of cellular signaling by altering subcellular localization of a number of important proteins. PAQR3 is a close homologue of adiponectin receptors PAQR1/2 and exclusively localized at the Golgi apparatus. PAQR3 can interact with Raf1 kinase and sequester Raf1 to the Golgi apparatus and thereby inhibit Ras-Raf-MEK-ERK signaling. Using different mouse models and clinical samples, we revealed that PAQR3 functions as a tumor suppressor in various cancers. PAQR3 has a negative effect on cell proliferation, migration, sprouting, and angiogenesis of endothelial cells. PAQR3 has a functional interaction with p53 in cancer formation and epithelial-mesenchymal transition (EMT). PAQR3 has a suppressive function in A375 human melanoma cells that harbor an oncogenic B-Raf mutation V600E, the most common mutation in melanoma. PAQR3 also has a suppressive activity in chemical carcinogen-induced mitogenesis and tumor formation in mouse skin. Genetic depletion of PAQR3 in mice is able to enhance intestinal tumor formation under the genetic background of heterozygous mutation of tumor suppressor adenomatous polyposis coli (APC). Lately, we found that PAQR3 is frequently downregulated in human gastric liver cancers and correlated with the prognosis of the patients.

PAQR3-modulated signaling also plays an important role in metabolic control. PAQR3 is able to negatively regulate insulin signaling by shunting cytosolic p110alpha of PI3K to the Golgi apparatus and meanwhile competing p85 subunit in forming an active PI3K complex with p110 subunit. In addition, PAQR3 has a modulatory role in obesity. Mice with deletion of PAQR3 are resistant to HFD-induced obesity and hepatic steatosis, accompanied by improvement of insulin resistance and insulin signaling. PAQR3-deleted mice have an increased energy expenditure and physical activity. HFD-induced leptin resistance is revered by PAQR3 ablation. Furthermore, overexpression of PAQR3 reduces leptin signaling while downregulation of PAQR3 enhances leptin signaling in the hypothalamus. Our latest study indicates that PAQR3 has a functional role in cholesterol metabolism. PAQR3 is able to regulate Scap and SREBP2, which are key components in cellular cholesterol homeostasis. PAQR3 overexpression enhanced the cleavage of SREBP, resulting in increased *de novo* synthesis of cholesterol. In PAQR3-downreguated mice and PAQR3-deleted primary hepatocytes, we observed decreased SREBP cleavage. These data indicate that PAQR3 may play an important role in regulating the activity of Scap-SREBP complex, thus leading to regulation of cholesterol homeostasis. Collectively, our studies have pinpointed the important functions of Golgi apparatus in regulating multiple biological activities including tumorigenesis and metabolism control.

CMB-01

High salt primes a distinct macrophage activation state, M(Na)

W.C. Zhang, X.J. Zheng, L.J. Du, S.Z. Duan

Key Laboratory of Nutrition and Metabolism, Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, P.R. China.

High salt is positively associated with the risk of developing hypertension, stroke, coronary heart disease, kidney disease, stomach cancer, type II diabetes and osteoporosis. However, little is known about the mechanisms. In this study, we showed that high salt significantly promoted pro-inflammatory gene and protein expression, while impaired anti-inflammatory gene and protein expression in human monocytes-derived macrophages and mouse bone marrow-derived macrophages. Mechanistically, p38 MAPK/c-Fos/AP1 and ERK/c-Fos/AP1 pathways were shown to mediate high salt-induced pro-inflammatory gene and protein expression. ERK partially mediated the suppression by high salt on anti-inflammatory gene and protein expression. Furthermore, high salt potentiated LPS-induced macrophage activation, M(LPS), while suppressed IL4-induced macrophage activation, M(IL4). The potentiation of M(LPS) was also mediated by p38 MAPK/AP1 pathway and ERK. In a systemic inflammation model induced by LPS, mice in high salt group presented higher concentration of CXCL1 and MIP2a, and more leukocytes including neutrophils and monocytes in blood than mice in normal salt group. Taken together, these results demonstrate that high salt primes a specific and distinct activation state of macrophages, termed as M(Na), with pro-inflammatory property *in vitro* and *in vivo*. Such effects of high salt may be part of the mechanisms by which it promotes hypertension and other diseases.

CMB-02

Combined simvastatin and 1,25-dihydroxyvitamin D, *in vitro*, provides synergistic bone anabolism of ovariectomized (OVX) rats

C.C.W. Poon[1], K.W.Q. Lu[1], Y.F. Ng[4], R.W.S. Li[1,2], D.H.K. Chow[3], L. Qin[3], P.P.Y. Lui[3], S.W. Seto[1,8], S.K. Kong[5], H.P. Ho[6], M.P.M. Hoi[7], S.M.Y. Lee[7], S.M. Ngai[5], S.W. Chan[4], G.P.H. Leung[2], Y.W. Kwan[1]

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[8] National Institute of Complementary Medicine, School of Science and Health, University of Western Sydney, Locked Bag 1797, Penrith, NSW, Australia.

We evaluated the *in vitro* bone anabolic effects of simvastatin (a HMG CoA reductase inhibitor) and 1,25-dihydroxyvitamin D (1,25-$(OH)_2D_3$; the active form of vitamin D), administered alone and in combination, and the underlying mechanisms involved of ovariectomized (OVX) rats (an animal model of human post-menopause-associated osteoporosis). Osteoblasts (bone-building cells) were harvested from control (sham) and OVX rats, incubated with simvastatin (Sim) (1, 3 and 10 nM) and 1,25-$(OH)_2D_3$ (1, 3 and 10 nM), alone and in combination, for 7, 14 and 21 days. Protein expression of bone anabolic markers and bone formation transcription factors (osteocalcin, osteopontin, Runx2, Dlx5, ATF4, type I collagen, OSX and alkaline phosphatase (ALP) activity), and Ca^{2+} deposits of osteoblasts before and after drug treatments were compared. A generalized reduction of the protein expression of bone anabolic markers and bone formation transcription factors was detected in osteoblasts of OVX rats compared with Sham controls. Sim and 1,25-$(OH)_2D_3$, when given alone, elicited a concentration- and time-dependent increase of all bone anabolic biomarkers measured, with a greater magnitude of increase was detected in osteoblasts of OVX rats. Moreover, a combination of Sim (10 nM) and 1,25-$(OH)_2D_3$ (10 nM) consistently elicited the greatest magnitude of increase of bone anabolic biomarkers examined. Thus, our results illustrate the synergistic bone anabolic effects of Simvastatin plus 1,25-$(OH)_2D_3$ combination in treating post-menopause-associated osteoporosis.

Acknowledgement: This project was supported by RGC GRF Grant (2014-2016) (Reference #: 2140841).

CMB-03

Amino acids and metabolic signaling

F.F. Guo

Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, P.R. China.

A disorder in nutrient sensing and metabolism regulation is one of the critical reasons leading to the development of metabolic diseases. Recently, increasing evidence has indicated a role for amino acid in the regulation of insulin sensitivity and lipid metabolism, however, underlying mechanisms are poorly understood. Our lab has focused on studying molecular mechanisms underlying amino acid sensing and metabolism regulation, by using animal and cell culture models maintained on a diet or medium deficient for an essential amino acid leucine, respectively. For the past few years, we have 1) demonstrated that leucine deprivation rapidly decreases fat mass via stimulating lipolysis in white adipose tissue and thermogenesis in brown adipose tissue, which is controlled by a central S6K1/CRH-dependent pathway; 2) found that leucine deprivation also increases insulin sensitivity, which is mediated by mTOR and AMPK signaling pathways; and 3) demonstrated novel functions for amino acid sensing proteins (PRLR etc.) in the regulation of lipid metabolism and insulin sensitivity. Further work will continue to investigate signaling pathways involved amino acid sensing and metabolism regulation in animal and humans and aim to find novel targets for treating metabolic diseases.

CMB-04

EP3 receptor deficiency attenuates pulmonary hypertension through suppressing Rho/TGFβ1 pathway

C.J. Zou, Y. Yu

Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, P.R. China.

Pulmonary arterial hypertension (PAH) is commonly associated with chronic hypoxemia in disorders such as chronic obstructive pulmonary disease (COPD). Prostacyclin analogs are widely used in the management of PAH patients; however, clinical efficacy and long-term tolerability of some prostacyclin analogs maybe compromised by concomitant activation of E-prostanoid 3 receptor (EP3). Here, we found that *EP3* expression is up-regulated in pulmonary arterial smooth muscle cells (PASMCs) and human distal pulmonary arteries (PAs) in response to hypoxia. Either pharmacological inhibition of EP3 or *Ep3* deletion attenuated both hypoxia and monocrotaline-induced pulmonary hypertension, and restrained extracellular matrix accumulation in PAs in rodent models. In a murine PAH model, *Ep3* deletion in SMCs, but not endothelial cells, retarded PA medial thickness. Knockdown of EP3A and EP3B isoforms, but not EP3G, diminished hypoxia-induced TGFβ1 activation. Expression of either EP3A or EP3B in EP3-deficent PASMCs restored TGFβ1 activation in response to hypoxia. EP3A/B activation in PASMCs increased RhoA-dependent membrane type-1-extracellular matrix metalloproteinase (MMP) translocation to the cell surface, subsequently activating pro-MMP-2 and promoting TGFβ1 signaling. Activation or disruption of EP3 did not influence PASMC proliferation. Together, our results indicate that EP3 activation facilitates hypoxia-induced vascular remodeling and pulmonary hypertension in mice and suggest EP3 inhibition as a potential therapeutic strategy for pulmonary hypertension.

CMB-05

Nitric oxide-cGMP-PKG inhibits hypertrophy of human embryonic stem cell-derived cardiomyocytes

Y. Wang[1], M. Kong[2], Y. Huang[1], R.A. Li[2], L. Sun[1], X.Q. Yao[1]

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[2] Stem Cell and Regenerative Medicine Consortium, The University of Hong Kong, Hong Kong SAR, P.R. China.

Cardiomyocytes show hypertrophic growth and remodeling in response to prolonged mechanical stress or abnormal neurohumoral activation. Previous studies have showed that nitric oxide (NO), cyclic GMP and protein kinase G (PKG) signaling pathway can inhibit cardiac hypertrophy remodeling process. However, the underlying mechanisms for the anti-hypertrophic effect of NO-cGMP-PKG signals are not fully understood. In the present study, we used human embryonic stem cell-derived cardiomyocytes (hESC-CMs) as the model to explore the mechanism of NO-cGMP-PKG inhibition on human cardiac hypertrophy.

Four hypertrophic indexes were measured, including cell size enlargement, sarcomere organization and expression of cardiac hypertrophy-characteristic genes ANF and ACTA1. Treatment of hESC-CMs with phenylephrine, an alpha-adrenergic receptor agonist, caused marked hypertrophy as indicated by the four hypertrophic index mentioned above. NO, cGMP and PKG were found to inhibit the phenylephrine-induced hypertrophy. Furthermore, suppression of Orai1 expression using dominant-negative construct and siRNA inhibited the phenylephrine-induced hypertrophy, suggesting a positive role of Orai1 in promoting human cardiomyocyte hypertrophy. Importantly, after substitution of endogenous Orai1 with a mutant Orai1, in which a putative PKG phosphorylation site is removed, NO, cGMP and PKG lost their inhibitory effect on cardiomyocyte hypertrophy. Taken together, these data provide conclusive evidence that cGMP and PKG acted through Orai1 to inhibit the phenylephrine-induced hypertrophy in hESC-CMs.

CMB-06

CLOCK/BMAL1 regulates circadian change of insulin sensitivity via SIRT1

Q.W. Zhai

Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, P.R. China.

Epidemic studies show that circadian misalignment, usually resulted from jet lag or shift work, increases the risk of a series of diseases including type 2 diabetes mellitus, which is characterized by insulin resistance. Extensive studies have shown that SIRT1, an NAD^+ dependent protein deacetylase, plays an important role in regulating insulin sensitivity. Interestingly, SIRT1 oscillates in a circadian manner, binds to the heterodimeric core circadian transcription factors CLOCK/BMAL1 and deacetylates BMAL1 thus participating in circadian regulation. However, the underlying mechanism for the circadian expression of SIRT1 is still unclear, and the precise roles of SIRT1 and CLOCK/BMAL1 in the development of insulin resistance induced by circadian misalignment are unknown. Here we show CLOCK/BMAL1 modulates insulin sensitivity by regulating the circadian expression of SIRT1. We found CLOCK and BMAL1 are downregulated under insulin resistant conditions. Knockdown of CLOCK or BMAL1 induces insulin resistance, while their ectopic expression improves insulin sensitivity under insulin-resistant conditions. Moreover, circadian change of insulin sensitivity is impaired in *Clock* mutant, liver specific *Bmal1* knockout or *Sirt1* knockout mice. Further studies show CLOCK/BMAL1 enhances SIRT1 expression, forming a CLOCK/BMAL1-SIRT1 feedback loop, and regulates insulin sensitivity via SIRT1. In addition, constant darkness-induced circadian misalignment in mice decreases BMAL1 and SIRT1 levels, and induces insulin resistance, which can be dramatically reversed by resveratrol. Our findings offer new insights for the coordination of circadian clock and metabolism, and provide a potential application of resveratrol for combating circadian misalignment-induced metabolic disorders.

CMB-07

Protease-activated receptor-1 (PAR-1) activating peptide suppresses ischaemia-induced injuries in rat models of stroke

X. Zhen, E.S.K. Ng, F.F.Y. Lam

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

Background
Ischaemic stroke is one of the leading causes of death and disability worldwide. The role of protease activated receptors (PARs) in this disease is uncertain. In the present study, the actions of a protease activated receptor-1 activating peptide (PAR-1 AP) were investigated in rat models of ischaemic stroke.

Experimental approach
The actions of a PAR-1 AP (SFLLRN-NH_2) were investigated in an *in vivo* rat model of ischaemic stroke induced by middle cerebral artery occlusion (MCAO) and in an *in vitro* model induced by oxygen and glucose deprivation (OGD) in primary cultured rat embryonic cortical neurones.

Results
Rats subjected to MCAO exhibited brain infarct, oedema, and neurological deficit. Rat cortical neurones subjected to OGD showed increased lactate dehydrogenase, caspase-3 activity and TUNEL positive cells, whereas, mitochondrial membrane potential and cell viability were decreased. Furthermore, both models had elevated levels of reactive oxygen species, nitrite, and malondialdehyde, while anti-oxidant enzymes and bcl-2/bax ratio were decreased. These detrimental changes were suppressed by the PAR-1 AP (SFLLRN-NH_2), and the protective actions of this peptide were inhibited by a PAR-1 antagonist (BMS-200261).

Conclusions
The present findings suggest the PAR-1 activating peptide SFLLRN-NH_2 possesses anti-oxidant and anti-apoptotic properties that can suppress ischaemia-induced brain injuries.

CMB-08

Vitamin D3 and vascular benefits

Y. Huang

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An inverse correlation exists between the vitamin D level in the blood and the incidence of heart failure, cardiovascular mortality, and elevation of arterial blood pressure. We provide evidence showing that chronic treatment with calcitriol, an active form of vitamin D, protects against renovascular function in hypertension. The calcitriol-induced protection is likely to be mediated by activation of the vitamin D receptor leading to the down-regulation of the expressions of angiotensin type 1 receptors and NAD(P)H oxidase subunits which in turn prevents the ROS overproduction. The findings in human renal arteries and human endothelial cells are confirmed both by *in vitro* and *in vivo* results in hypertensive rats. Cardiovascular risks increase in the postmenopausal women and vitamin D is supplemented for osteoporosis. Calcitriol restores endothelial function and renal blood flow through normalizing the over-expression of COX-2 and thromboxane-prostanoid receptors in renal arteries during estrogen deficiency in ovariectomized rats. Our studies suggest calcitriol and vitamin D receptor activation as a novel therapeutic strategy to ameliorate vascular dysfunction in cardiovascular and metabolic diseases.

CMB-09

Modulation of macrophage function by oxidized phospholipids in low density lipoproteins in the context of atherogenesis

H.Y. Yin

Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, P.R. China.

Cardiovascular diseases (CVDs) caused by atherosclerosis are the leading causes for mortality and morbidity in developed countries while China, as a developing country, has even higher incidence of CVD than the USA and Japan. The molecular mechanisms of atherosclerosis appear to be multifactual and remain to be clearly defined; mounting evidence suggests that chronic inflammation and oxidative stress play an important role. Atherosclerotic lesion formation resulted from interactions among different cell types including macrophages, endothelial cells, smooth muscle cells, and platelets within the vessel wall. Macrophages are one of the major cell types in the atherosclerotic plaque and early lesion formation originates from phagocytosis of oxidized LDL by macrophage. Recent studies have demonstrated that oxidized phospholipids on low density lipoprotein (LDL) particles play a critical role during the progression of atherosclerosis through affecting the functions of macrophages by oxLDL . However, the exact components in oxidized LDL that may be responsible for these effects have not been clearly elucidated. We identified a novel class of oxidized phospholipid *in vitro* and in human atherosclerotic plaques termed deoxy-A_2/J_2-IsoP-PC due to the presence of a cyclopentenone moiety, the similar structural motif found in cyclooxygenase (COX) - derived prostaglandins, 15-deoxy-PGJ_2 (15d-PGJ_2). These electrophilic compounds are known to have profound biological activities by covalent modification of critical cystein residues in proteins. We chemically synthesized a representative compound 15d-PGJ_2-PC for this novel class of oxidized phospholipids and our preliminary experiments showed that 15d-PGJ_2-PC can activate Nrf2 and PPARs including PPARα, $PPAR_\delta$ and PPARγ, while inhibition of NFκB, suggesting that 15d-PGJ_2-PC may regulate inflammatory response and oxidative stress. Interestingly, these compounds can induce a new phenotype of polarized macrophage, Mox (induced by oxPAPC). Our lipidomic and metabolomic studies demonstrated that this novel Mox phenotype had distinct metabolic features from M1 and M2 phenotypes including arachidonic acid pathways and glycolysis. In summary, our studies demonstrate that these novel phospholipid oxidation products modulate macrophage functions in response to inflammation and oxidative stress in the context of atheroclerosis.

CMB-10

FDA-approved drugs as inhibitors of fatty acid binding protein as new drugs for treatment of metabolic diseases

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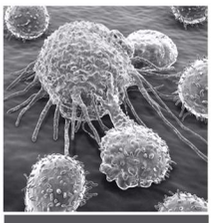
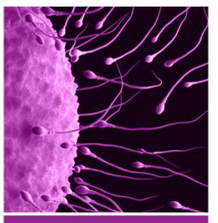
The adipocyte FABP (FABP4) is highly expressed in adipocytes. FABP4 plays an important role in various aspects of metabolic disorders, including insulin resistance, diabetes, and atherosclerosis. Insulin resistance can be observed in high-fat diet-fed mice; deficiency of FABP4 partially protects these mice against the development of insulin resistance. In addition, FABP4-deficient mice exhibit better performances in both insulin and glucose tolerance tests. Apart from genetic approaches, the blockade of FABP4 by small molecules could potentially mimic the phenotype of FABP4-deficient mice. Therefore, pharmacological agents that inhibit FABP4-mediated responses might serve as potential candidates for the treatment of insulin resistance, diabetes, and atherosclerosis. We first identified fluorescein, ketazolam, antrafenine, darifenacin, fosaprepitant, paliperidone, risperidone, pimozide, trovafloxacin, and levofloxacin as inhibitors of FABP4 using molecular docking screening from FDA-approved drugs. Subsequently, the biochemical characterizations showed that levofloxacin directly inhibited FABP4 activity in both the *in vitro* ligand displacement assay and cell-based function assay.

* US provisional patent has been filed. Part of this work has been published in *J Chem Inf Model* in December, 2014 which was listed as the top 1 most read article of the month.



Cancer and inflammation

Neurodegeneration, -development and repair



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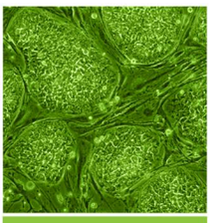
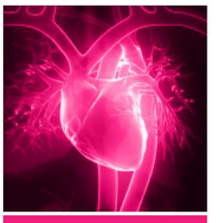


Stem cell and regeneration



Vascular and metabolic biology

School of Biomedical Sciences
Faculty of Medicine
Lo Kwee-Seong Integrated Biomedical Sciences Building
The Chinese University of Hong Kong
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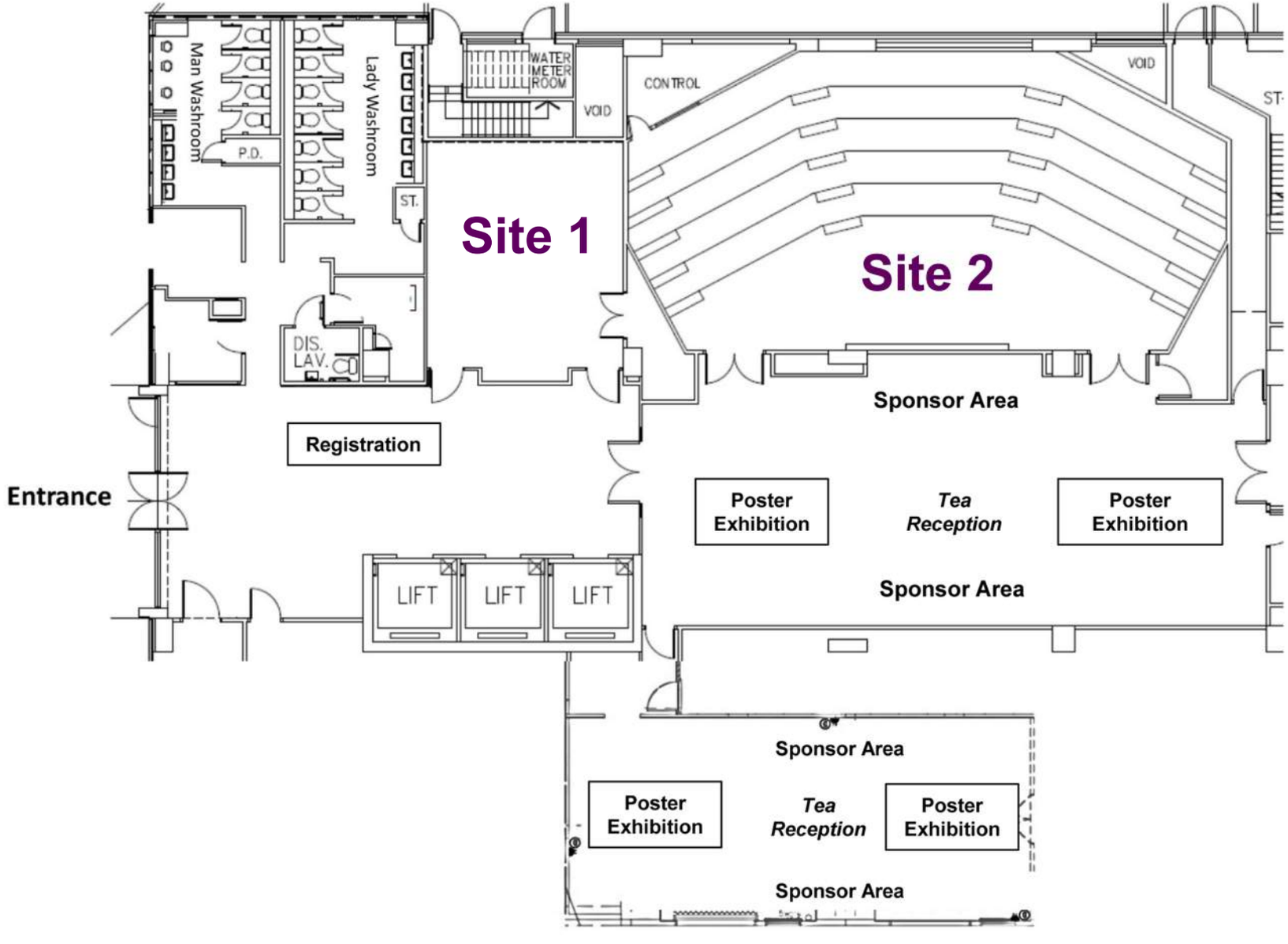
School of Biomedical Sciences

Research Day 2015

5 June 2015 (Friday)

Map of the Meeting Venue
G/F Lo Kwee-Seong Integrated Biomedical Sciences Building



Programme Summary

SBS Research Day 2015
5 June 2015 (Friday)

09:00-09:15 Opening Ceremony: Prof. Francis K.L. Chan (Dean of Faculty of Medicine) & Prof. Wai Yee Chan (Director of School of Biomedical Sciences), Room G02, Lo Kwee-Seong Integrated Biomedical Sciences Building

09:15-09:30 Photo taking

09:30-10:15 Plenary Lecture by Prof. Chris Yun-Fai Lau (Abstract No.: PL-02)
"The cell fate determination functions of SRY in gonadal development and beyond"

Time	*Site 1 (Room G01)*	*Site 2 (Room G02)*
	Session I ***Stem Cell and Regeneration***	***Session I*** ***Neuro-degeneration, -development and Repair***
10:15-10:40	Kenneth K.H. Lee	Wing Ho Yung
10:40-11:05	Xiaohua Jiang	Vincent C.K. Cheung

11:05-11:30 ***Tea Break & Poster Viewing***

	Session II ***Cancer & Inflammation***	***Session II*** ***Neuro-degeneration, -development and Repair***
11:30-11:55	Wing Tai Cheung	Ya Ke
11:55-12:20	Ge Lin	Sun On Chan

12:20-13:30 ***Lunch Break***

13:30-14:30 ***Poster Presentation Session***

	Session III ***Neuro-degeneration, -development and Repair***	***Session III*** ***Neuro-degeneration, -development and Repair / Stem Cell and Regeneration***
14:30-14:55	Christopher H.K. Cheng	Eugene D. Ponomarev
14:55-15:20	Alisa S.W. Shum	William W.J. Lu
15:20-15:45	Helen Wise	Gang Lu

15:45-16:10 ***Tea Break & Poster Viewing***

	Session IV ***Neuro-degeneration, -development and Repair***	***Session IV*** ***Stem Cell and Regeneration***
16:10-16:35	Eric Y.P. Cho	Bo Feng
16:35-17:00	Mary M.Y. Waye	Chao Wan
17:00-17:25	Yechun Ruan	Kingston K.L. Mak

19:00 Conference Dinner (by invitation)

Programme (Site 1)
Room G01, Lo Kwee-Seong Integrated Biomedical Sciences Building

Time	*Title of Presentation*	*Speaker*	*Abstract No.*
	Session I Stem Cell and Regeneration ***Chairpersons: Faye S.Y. Tsang and Kwok Pui Fung***		
10:15-10:40	Role of BRE gene in development and regeneration	Kenneth K.H. Lee	S1-01
10:40-11:05	The role of ccl5/ccr1/ERK axis in dedifferentiation-reprogrammed MSCs: application in cancer targeting	Xiaohua Jiang	S1-02

11:05-11:30 ***Tea Break & Poster Viewing***

	Session II Cancer and Inflammation ***Chairpersons: William K.K. Wu and Franky L. Chan***		
11:30-11:55	*Alu*-derived RNA functions as scaffold for ERK1/2 activation	Wing Tai Cheung	S1-03
11:55-12:20	High risk of liver injury caused by pyrrolizidine alkaloid intoxication and biomarker for its diagnosis and risk assessment	Ge Lin	S1-04

12:20-13:30 ***Lunch Break***

13:30-14:30 ***Poster Presentation Session***

	Session III Neuro-degeneration, -development and Repair ***Chairpersons: Ronald C.C. Wang and Woody W.Y. Chan***		
14:30-14:55	Gene knockout studies in zebrafish provide insights into the evolution of the two gonadotropins and their receptors	Christopher H.K. Cheng	S1-05
14:55-15:20	Perturbation of retinoic acid levels reduces nephron endowment in the offspring of diabetic pregnancy	Alisa S.W. Shum	S1-06
15:20-15:45	The double life of PGE_2	Helen Wise	S1-07

15:45-16:10 ***Tea Break & Poster Viewing***

	Session IV Neuro-degeneration, -development and Repair ***Chairpersons: Richard K.W. Choy and Stephen K.W. Tsui***		
16:10-16:35	Promotion of retinal ganglion cell survival and regeneration by suramin	Eric Y.P. Cho	S1-08
16:35-17:00	Bipolar disorder susceptibility genes	Mary M.Y. Waye	S1-09
17:00-17:25	CFTR interacts with ZO-1/ZONAB in the development of male reproductive tract	Yechun Ruan	S1-10

19:00 Conference Dinner (by invitation)

Programme (Site 2)
Room G02, Lo Kwee-Seong Integrated Biomedical Sciences Building

09:00-09:15 Opening Ceremony: Prof. Francis K.L. Chan (Dean of Faculty of Medicine) & Prof. Wai Yee Chan (Director of School of Biomedical Sciences)

09:15-09:30 Photo taking

09:30-10:15 Plenary Lecture by Prof. Chris Yun-Fai Lau (Abstract No.: PL-02)
"The cell fate determination functions of SRY in gonadal development and beyond"
Chairperson: Prof. Wai Yee Chan

Time	*Title of Presentation*	*Speaker*	*Abstract No.*
	Session I Neuro-degeneration, -development and Repair ***Chairpersons: Edwin H.Y. Chan and Alisa S.W. Shum***		
10:15-10:40	Motor memory formation – the roles of synaptic inputs at apical and basal dendrites of layer 5 neurons in the motor cortex	Wing Ho Yung	S2-01
10:40-11:05	An optogenetic demonstration of motor primitives in the mouse spinal cord	Vincent C.K. Cheung	S2-02

11:05-11:30 ***Tea Break & Poster Viewing***

	Session II Neuro-degeneration, -development and Repair ***Chairpersons: Jerome H.L. Hui and Wing Ho Yung***		
11:30-11:55	Mechanisms of dopaminergic treatment on motor recovery in ischemic stroke injury	Ya Ke	S2-03
11:55-12:20	An antagonist of growth hormone-releasing hormone receptors alleviates experimentally induced ocular inflammation in the rat	Sun On Chan	S2-04

12:20-13:30 ***Lunch Break***

13:30-14:30 ***Poster Presentation Session***

	Session III Neuro-degeneration, -development and Repair/Stem Cell and Regeneration ***Chairpersons: Ge Lin and Arthur F.T. Mak***		
14:30-14:55	Regulation of inflammation in the Central Nervous System: Platelets are primary sensors of neuronal damage	Eugene D. Ponomarev	S2-05
14:55-15:20	Micro & nano structure and mechanics of trabecular bone in elderly osteoarthritis (OA)	William W.J. Lu	S2-06
15:20-15:45	Arise from the dust: features of mesenchymal stem cells and application on neurological disorders	Gang Lu	S2-07

15:45-16:10 ***Tea Break & Poster Viewing***

	Session IV Stem Cell and Regeneration ***Chairpersons: Ping Yuan and Kenneth K.H. Lee***		
16:10-16:35	Stem cell manipulation using CRISPR/Cas9 technology	Bo Feng	S2-08
16:35-17:00	The HIF-α pathway regulates chondrogenesis during cartilage development and repair	Chao Wan	S2-09
17:00-17:25	Roles of Yap1 in chondrocyte differentiation and cartilage diseases	Kingston K.L. Mak	S2-10

19:00 Conference Dinner (by invitation)

PL-02

The cell fate determination functions of SRY in gonadal development and beyond

C.Y.F. Lau

Department of Medicine, University of California, San Francisco, U.S.A.

The sex-determining region Y (SRY) is the testis-determining factor, encoded by the SRY gene on the mammalian Y chromosome. SRY is a founder of a family of SRY-box (SOX) genes, which play key roles in various cell fate determination in numerous cell types and organs during development. SRY and SOX share the conserved high mobility group (HMG) box, but diverse at other domains of the respective molecules. SRY interacts and recruits co-activators and co-repressors to accomplish its gene regulation and chromatin modulation functions. SRY switches on the male sex by determining the fate of the somatic cells to Sertoli cells in the male embryonic gonads, and passes on its functions to the SOX9 factor. Currently the exact mechanisms of SRY and SOX9 actions are uncertain. To identify the target genes for both SRY and SOX9 during gonadal development in the mouse, we have conducted a comprehensive study using advanced genomic strategies. Our results identified numerous SRY and SOX9 targets, many of which are known to be involved in sex determination and differentiation. Bioinformatics and gene ontology analyses showed that SRY binds and simultaneously represses the ovarian and activates the testicular differentiating genes. SRY and SOX9 shares close to half of their respective targets, and bind to the same regions on the promoters of their common target genes. Significantly, many of the targets are involved in Sertoli cell-Sertoli cell junction signaling, important for testis cord formation, the most important early event in testis differentiation. Hence, SRY represses the ovarian genes and switches on the cell fate of the Sertoli cells, which, in turn, form tight junctions important for formation of the testis cord, the earliest vasculature of the embryonic testis. Once accomplished, SRY passes on its functions to SOX9, which continues the regulation of their common targets, and activates its on gene regulatory program beyond SRY actions.

The observation that SRY and SOX9 share close of half of their respective targets suggests that SRY could be an epigenetic modifier for SOX9 actions in other cell fate-determining events, apart from sex determination. Since SRY is only required for sex determination while SOX9 plays crucial roles in the differentiation of numerous other organs, including the brain. Accordingly, if SRY is aberrantly expressed, it could bind to SOX9 targets in these organs and could exert disruptive effects on SOX9 functions. To explore such possibility, we have established a gene activation system, in which a SRY transgene can be activated at a tissue-specific manner in transgenic mice. Our results showed that ectopic activation of SRY impair the development of various vital organs, including the heart, lung, and brain. Transcriptome analysis showed that SRY significantly impairs neurodevelopment in the brain, affecting the cell migration, morphogenesis, behavior, learning, cognition, prepulse inhibition, and neuritogenesis. Since male-biases in both neurodevelopment and neural diseases are frequently observed in various human diseases, including autism, schizophrenia, Hirschsprung, Alzheimer and Parkinson diseases, our results strong suggest that SRY could serve as a male-specific epigenetic modifier in the normal development and diseases of various cell types and organs, including those of the nervous systems.

S1-01

Role of BRE gene in development and regeneration

K.K.H. Lee, C.L.H. Xiao

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The *BRE* (Brain and Reproductive organ-Expressed) gene transcribes a highly conserved protein that is expressed in many tissues, including the adrenal gland, brain, heart, kidney lung, skeletal muscles and reproductive system. In this study, we investigated the function of *BRE* in satellite stem cells during skeletal muscle development and regeneration. We generated *BRE* knockout (BRE-KO) mutant mice that were positively validated at the DNA, mRNA and protein levels. The skeletal leg muscles of these mutant mice were experimental injured with cardiotoxin and allowed to regenerate. We established that accompanying muscle regeneration was impaired when compared with normal wild-type (BRE-WT) mice. In the BRE-KO mice, there were significantly fewer $pax7^{+}$ satellite cells in the injury site and smaller newly-formed myofibers compared with BRE-WT mice.

S1-02

The role of ccl5/ccr1/ERK axis in dedifferentiation-reprogrammed MSCs: application in cancer targeting

R. Chen, X.H. Zhang, F.Y. Yang, L.L. Tsang, H.C. Chan, X.H. Jiang

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

Mesenchymal stem cells (MSCs) are self-renewing, multipotent progenitor cells with potential to differentiate into various cell types. Due to their accessibility and convenient expansion protocols, MSCs have been recognized as promising candidates for cellular therapy in regenerative medicine. However, effective homing and engraftment of transplanted MSCs into the target sites remains a major hurdle as demonstrated by multiple animal studies, which greatly limits their overall effectiveness and clinical use.

Interestingly, previous studies from both our group have demonstrated that after neuronal commitment, differentiated-MSCs can be induced to dedifferentiate and revert back to MSC morphologically under appropriate condition. In addition, we have shown that these dedifferentiated MSCs (De-MSCs) present a variety of distinguishing genetic and phenotypic characteristics distinct from their original counterparts. Strikingly, we have found that De-MSCs express significantly higher levels of chemokines and cytokines, and display enhanced tropism to cancer both *in vitro* and *in vivo*. Furthermore, we have revealed that the enhanced migratory capability of De-MSCs is attributed to upregulated ccl5/ccr1/MAPK pathway. Furthermore, for the first time, we find that histone modification functions as a regulatory mechanism on chemokine inducibility in MSCs. The demonstrated advantages of DeMSCs warrant further investigation into this distinct stem cell population and the detailed mechanisms underlying the dedifferentiation-induced reprogramming process.

S1-03

Alu-derived RNA functions as scaffold for ERK1/2 activation

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Metastasis of tumours are linked to cancer cells undergoing epithelial-mesenchymal transition (EMT), which is regulated by a milieu of chemokines, growth factors and non-coding RNAs. Previously our lab has identified a new member of the GRO chemokine family, and the novel CXC chemokine was named as Tumour-Induced Factor (TIF). The TIF transcript was found to have a long 3'-untranslated region (3'-UTR) that embedded an antisense *Alu* element. Bioinformatics analysis predicts that the embedded antisense *Alu* element is folded into a stem-loop secondary structure, suggesting it might interact with cellular stem-loop binding proteins and modulates cellular activities. To explore the functional roles of the 3'-UTR of TIF, stable CHO cell clones overexpressing a 229 bp 3'-UTR fragment of TIF containing the entire antisense *Alu* element were prepared. Morphological and biochemical studies suggested the stably transfected CHO cells underwent EMT, and displayed a higher levels of phosphorylated extracellular signal-regulated kinase 1/2 (ERK1/2). Unexpectedly, it was found that the overexpressed 229 bp 3'-UTR RNA was tightly associated with non-phosphorylated ERK. Additionally, human epidermoid A431 cancer cells that expressing abundant levels of *Alu* RNAs were found to contain endogenous *Alu* RNA-ERK ribonucleoprotein complexes. Of interest, a conserved ERK binding motif was found in the antisense *Alu* element that was embedded within the 3'-UTR of TIF transcript. These results suggest a possibility that the RNA embedding *Alu* element might function as a scaffold assembling a ribonucleoprotein complex that facilitates phosphorylation of ERK1/2, resulting in the induction of EMT.

 # The authors contributed equally.

S1-04

High risk of liver injury caused by pyrrolizidine alkaloid intoxication and biomarker for its diagnosis and risk assessment

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Diagnosis of drug-induced liver injury (DILI) is a challenge. Pyrrolizidine alkaloids (PAs) are widely distributed in plant kingdom and PA-induced liver injury (PA-ILI) is likely to contribute significantly to DILI. However, currently there is no confirmative diagnostic method for PA-ILI, and thus the risk of DILI associated with PA-ILI is unrecognized. This study aimed to establish and validate blood pyrrole-protein adducts as a specific biomarker of PA-ILI, and also evaluate the incidence of PA exposure in suspected DILI patients. A novel and highly sensitive UHPLC-MS-based method was developed to analyze blood pyrrole-protein adducts in 46 suspected DILI patients, including 15 PA-ILI patients who consumed PA-containing herbs, and 162 healthy subjects. Blood pyrrole-protein adducts were detected in all 15 PA-ILI (100%) and 12 DILI patients with unknown etiology patients (~40%, 12 of 31 patients), but not in all healthy controls. In conclusion, this study confirmed the capability and suitability of blood pyrrole-protein adducts as a specific biomarker for the assistance of confirmative diagnosis of PA-ILI, and also demonstrated PA exposure as one of the important contributors to DILI. Our results also revealed that it is timely for public to be aware of the risk of PA intoxication.

S1-05

Gene knockout studies in zebrafish provide insights into the evolution of the two gonadotropins and their receptors

L.H. Chu, J.Z. Li, Y. Liu, C.H.K. Cheng

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As pivotal regulators in controlling gonadal development and functions, the interaction between the two gonadotropins (FSH and LH) and their receptors (FSHR and LHR) have attracted much research attention in the past two decades. Most of the information came from mammalian studies. However, exactly how the gonadotropins regulate reproduction in non-mammalian species is still far from clear. Using zebrafish as a model, we have systematically knockout the two gonadotropins and their receptors by TALENs. In female zebrafish, the function of gonadotropins is similar to that in mammals where FSH/FSHR is required for oocyte growth, and LH/LHR is indispensable for oocyte maturation and ovulation. Our results also suggest that zebrafish LH could act through FSHR in the absence of LHR. In male zebrafish, in big contrast to mammals, neither FSH nor LH knockout alone affects testis development, but double knockout of these two gonadotropins leads to significant developmental delay of the testis and infertility in male. Both of these canonical and non-canonical functions unveiled in zebrafish reproduction lead us to a fuller understanding of the evolution of gonadotropins and their receptors from fish to mammals.

S1-06

Perturbation of retinoic acid levels reduces nephron endowment in the offspring of diabetic pregnancy

S.T.K. Tam, L.M.Y. Lee, R.C.Y. Kwok, A.S.W. Shum

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Offspring of diabetic pregnancy have reduced nephron mass and are at increased risk of developing chronic kidney disease and hypertension later in life. However, the underlying mechanism by which nephron number is affected remains poorly understood. All-*trans* retinoic acid (RA) is a crucial signalling molecule for nephrogenesis. Embryonic RA is synthesized from vitamin A (retinol) obtained from the mother. Previous studies have demonstrated a positive correlation between maternal circulating vitamin A levels and nephron number. In humans with type 1 diabetes and in our streptozotocin-induced mouse model of diabetes, there is a significant reduction in plasma vitamin A levels. We therefore hypothesize that in embryos exposed to maternal diabetes, there is a reduction of RA levels in the developing kidney, which perturbs nephron formation and leads to reduced nephron mass.

Our preliminary data show that the mRNA level of *Raldh2*, which codes for the major RA synthesizing enzyme in the developing kidney, is significantly lower in the kidney of embryos of diabetic mice in comparison to that of the non-diabetic control. Concomitantly, there is a significant decrease in RA concentrations and down-regulation of the RA-responsive gene *Ret* that plays a critical role in controlling nephron formation. At birth, the number of nephrons in neonates of diabetic mice is significantly less than neonates of non-diabetic mice. These findings are in line with our hypothesis. Further study will be conducted to determine whether normalizing RA homeostasis can restore nephron number. Results of this study may shed light on developing therapeutic treatment for in utero correction of kidney development in the offspring of diabetic pregnancy.

S1-07

The double life of PGE_2

K.H. Tse, K.B.S. Chow, H.Wise

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When Toll-like receptor 4 (TLR4) is stimulated by lipopolysacharride (LPS) in rat dorsal root ganglion (DRG) cells, there is an increase in COX-2 and TNFα mRNA expression. But when DRG cells are stimulated with PGE_2, i.e. the product of COX-2, there was an increase in LPS-stimulated COX-2 but a decrease in LPS-stimulated TNFα mRNA expression. The purpose of the current study was to investigate the EP receptor subtype/s responsible for these actions of PGE_2 and to look at the role of endogenous PGE_2 in neuron-glial communication under normal and pathophysiological conditions. LPS-stimulated COX-2 and TNFα mRNA expression was enhanced by the presence of an EP4 receptor antagonist, implying a role for endogenous PGE_2/EP4-mediated inhibition of LPS signaling. Conditioned medium from DRG neuron-enriched cells (control and heat-shock-treated) also increased COX-2 mRNA in DRG glial cells, and this was further increased by the EP4 receptor antagonist MF498 and decreased to a similar extent by inhibiting TLR4. In contrast, the target of high concentrations of PGE_2 to increase COX-2 mRNA remains uncertain. In conclusion, endogenously-produced PGE_2 has EP4 receptor-dependent anti-inflammatory activity in DRG cells to limit the further production of inflammatory mediators in response to activation of TLR4 receptors.

S1-08

Promotion of retinal ganglion cell survival and regeneration by suramin

A.W.S. Cheung, S.W. Yu, E.Y.P. Cho

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Background
Activation of P2 purinergic receptors by ATP in the damaged central nervous system (CNS) has been associated with neuronal degeneration. In accordance with this, blockage of purinergic receptors is known to afford neuroprotection. Using retinal ganglion cells (RGCs) as an experimental CNS injury model, we have shown that blockage of P2X7 receptors in the retina after optic nerve (ON) injury by specific antagonists protects RGCs from axotomy-induced degeneration. To further explore the role of P2 receptors in CNS neuronal injury and regeneration, the putative non-specific P2 antagonist suramin has been delivered to the retina after optic nerve injury to study its effect on RGC survival and regeneration.

Methods
The ON in adult hamster was damaged to axotomize RGCs, followed by intravitreal injection of suramin or vehicle (saline). RGC survival was quantified at different post-injury times, and the proportion of surviving RGCs that expressed the growth-associated protein GAP-43 – a marker related to regenerative propensity, was determined. RGC regeneration was studied by two means: peripheral nerve (PN) grafting to the cut ON to induce long axon regeneration, or intravitreal PN implantation to induce growth of axon-like processes. Suramin was combined with PN grafting to see whether regeneration of RGCs would be enhanced. The potency of suramin in stimulating RGC regeneration was compared against ciliary neurotrophic factor (CNTF), a growth factor well known to activate RGC regeneration. The role played by retinal glia in suramin's stimulus of RGC survival and regeneration was addressed by documenting reactive changes in astrocytes, Muller cells and microglia.

Results & Conclusion
Suramin was a potent stimulator of RGC survival and regeneration, even more so than CNTF. Enhanced reactive changes (increase in numbers and changes in morphology) were observed in retinal glial cells after ON injury plus suramin injection. Co-application of neurostatin which influenced astroglial but not microglial reaction was found to decrease the stimulus of suramin. These results suggest that suramin stimulate RGC survival and regeneration by acting on both neurons as well as glial cells in the retina.

Supported by GRF grant CUHK463309.

S1-09

Bipolar disorder susceptibility genes

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Bipolar disorder, also known as bipolar affective disorder (and originally called manic-depressive illness), is a mental disorder characterized by periods of elevated mood and periods of depression. In 2008, *DGKH* was reported to associate with bipolar disorder in a genome-wide association study and many polymorphisms located in *DGKH* were found to be associated in a population (of European origin) as reported by the National Institute of Mental Health or a German replicate sample (Baum et al. 2008), such as rs9532988, rs9532989 and rs1012053. Subsequently, Zeng and colleagues also confirmed that *DGKH* was associated with bipolar disorder in a Chinese Han population including 1,139 unrelated bipolar disorder patients and 1,138 ethnically matched healthy controls using tagging single nucleotide polymorphisms (SNPs) strategy (Zeng et al. 2011). In this study, we report a bipolar hypomania case of a 16-year-old Chinese boy who had been alleviated of hypomania and insomnia symptoms upon supplementation of phosphatidylcholine. Following this observation, we investigated whether the potential beneficial effect of phosphatidylcholine supplementation was associated with the fact that the boy carries susceptibility variants in the first intron of *DGKH* for bipolar disorder.

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S1-10

CFTR interacts with ZO-1/ZONAB in the development of male reproductive tract

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Over 97% male patients with cystic fibrosis (CF), a disease caused by mutations in CFTR, are diagnosed with congenital bilateral absence of the vas deferens (CBAVD) and concurrent absence or atrophy of a large portion of the epididymis, which suggests a role of CFTR in the development of these organs, although the underlying mechanism remains unclear. Here we demonstrate that CFTR colocalizes with ZO-1 at the tight junctions in the epididymis, and is expressed before ZO-1 in Wolffian ducts, the embryonic precursor of the epididymis and vas deferens. CFTR interacts with ZO-1 through the PDZ-binding domain. In a three-dimensional epithelial cell culture model, CFTR regulates tight junction assembly and is required for tubulogenesis. CFTR inhibition or knockdown reduces ZO-1 expression and induces the translocation of the transcription factor ZONAB from tight junctions to the nucleus, followed by upregulation of the transcription of CCND1 and downregulation of ErbB2 transcription. The epididymal tubules of cftr(-/-) and cftr(ΔF508) mice have reduced ZO-1 levels, increased ZONAB nuclear expression, and decreased epithelial cell differentiation, illustrated by the reduced expression of apical AQP9 and V-ATPase. This study provides a new paradigm for the etiology of developmental diseases associated with CFTR mutations.

S2-01

Motor memory formation – the roles of synaptic inputs at apical and basal dendrites of layer 5 neurons in the motor cortex

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In the primary motor cortex, distinct streams of information are conveyed by synaptic inputs that terminate at different dendritic sites of pyramidal neurons. As the major output neurons of the motor cortex, how layer 5 pyramidal neurons integrate the synaptic inputs at both proximal basal dendrites and distal apical tuft dendrites in intact brain is not clear. In this study, we examined the properties of motor training induced plasticity in these layer-specific synaptic inputs to layer 5 neurons by chronic field potential recordings from rats. Different synaptic inputs targeting basal and apical dendrites were induced *in vivo* and the potentiation level of synaptic transmission was evaluated. In the initial learning phase, i.e. day 1 to day 3, rats exhibited rapid improvement in single success rate of a forelimb-reaching for food task, which was accompanied by substantial synaptic potentiation on both local basal dendrites. As the motor skill was consolidated gradually during later phase, only the potentiated synaptic input targeting the basal dendrites could be sustained but not those targeting the apical tufts dendrites. Interestingly, the consolidated synaptic potentiation on local basal dendrites was impaired by locally depleting the mesocortical dopaminergic innervation, which also compromised overnight retention of newly learned skill. Our results suggest that motor learning-related synaptic potentiation in basal and apical dendrites of layer 5 pyramidal neurons exhibit different consolidation profiles, implying different functions in motor memory formation. Dopamine plays a critical role in consolidation of synaptic plasticity and motor memory.

This study was supported by HKRGC-GRF grant (CUHK14119214), LKS Seed Fund and LCWIIM Fund.

S2-02

An optogenetic demonstration of motor primitives in the mouse spinal cord

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One line of evidence for the compositionality of the motor system has come from studies in which motor primitives of spinalized preparations were elicited by electrical stimulations. Since electrical currents non-selectively activate all neuronal types, it is impossible to use this approach to identify the neuronal populations responsible for the activation and organization of motor primitives. Recently, optogenetics techniques - methods for activating specific neurons endowed with opsins using light - have offered the possibility of targeting a specific neuronal type in spinal stimulations (eg Caggiano *et al PLOS* 2014). Here, we seek to obtain experimental evidence of spinal modularity using optogenetics. We studied two lines of transgenic mice that express the light-activated channelrhodopsin2 (ChR2) in two distinct but complementary neuronal populations, respectively: the Thy1 line, in which ChR2 is expressed in excitatory neurons excluding motorneurons; and the Chat line, in which the ChR2 is expressed mostly in motoneurons. We hypothesize that light-activated force patterns from the Thy1, but not the Chat, mice would exhibit features characteristic of motor primitives as described in previous studies using the spinalized preparations. In anesthetized mice, a blue laser light was delivered to different loci of the lumbar-sacral spinal cord to elicit forces from the ipsilateral hindlimb. At each locus, a force field was obtained by recording light-activated isometric forces at the ankle at 9-30 positions evenly distributed in the workspace. We observed 5 distinct types of force field in Thy1 mice (N=10), and 4 other types in Chat mice (N=5); in both mouse lines, the clusters of stimulation loci corresponding to the force-field types had a partially overlapping representation along the cord. The Thy1 force fields displayed prominent spatial convergence, with 34% of them showing an equilibrium point (EP) within or just outside of the workspace; 2 spatial clusters of EPs were identified, occupying the dorsal-anterior and ventral-posterior portions of the workspace, respectively. The Chat force fields, on the contrary, were mostly composed of parallel vectors; EPs, found in 20% of the fields, had a much smaller spatial distribution than those of Thy1. In both mouse lines, force fields produced by co-stimulation of two different spinal loci matched very well with the vector summation of the fields elicited separately from the individual loci. We interpret the Thy1 force fields observed to be motor primitives whose flexible linear superposition can give rise to diverse motor patterns. Overall, our findings agree with the classic results of force primitives derived from spinalized frogs (eg Bizzi *et al* 1991), and support that discrete primitives are encoded by partially overlapping groups of spinal interneurons. Our work also demonstrates optogenetics to be a viable approach useful for elucidating the neurobiological basis of motor modularity.

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S2-03

Mechanisms of dopaminergic treatment on motor recovery in ischemic stroke injury

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The development of effective treatment for ischemic stroke is a vexing challenge for centuries, especially for chronic patients where neuroprotective and thrombolytic agents are no longer appropriate. Recent clinical studies have shown that administration of dopamine agonists or restoring central dopamine level via levodopa, can substantially improve motor performance in stroke patients. However, the mechanisms of dopaminergic treatment on motor recovery have not been thoroughly explored. In our study, we tested the hypothesis that levodopa acts by enhancing cortical neuroplasticity to promote functional recovery. In rodent model of focal ischemic stroke induced by photothrombosis, we assessed the integrity of cortical dopaminergic system and found that it is significantly disrupted by focal stroke. Through a 3-week daily treatment of levodopa, we recapitulated its beneficial effects on sensorimotor function by subjecting the animals to various motor tasks, including open-field test, rotarod performance test, forelimb food retrieval task, limb-use asymmetry test and horizontal ladder test. Further examination of neuronal connectivity and synaptic protein expression revealed that perilesional tissue remodeling occurs following stroke and is modulated by dopamine. With tantalizing evidence that dopamine replacement can enhance functional recovery by coalescing connections across the cortex, we demonstrated the modulating dopaminergic transmission is a promising therapeutic strategy for stroke rehabilitation.

S2-04

An antagonist of growth hormone-releasing hormone receptors alleviates experimentally induced ocular inflammation in the rat

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Growth hormone-releasing hormone, growth hormone, insulin-like growth factor-1 (GHRH-GH-IGF1) axis exerts regulatory effects on immunity. Its involvement in ocular inflammation is still to be investigated. Here we studied this signaling in endotoxin-induced uveitis (EIU) generated by lipopolysaccharide (LPS). GHRH receptor (GHRHR) protein was expressed in the pituitary, retina, iris-ciliary body (Iris-CB) and cornea of normal rats, and was elevated in Iris-CB after LPS insult, in parallel with up-regulation of GHRH signaling genes Pit-1, GHRHR, SV1, GHRH and GH. Elevation of GHRHR and GH receptor (GHR) were found on Iris-CB epithelium, and GHRHR was confined to the infiltrated macrophages and leukocytes in aqueous humor but not on those in stroma. Treatment with GHRHR antagonist decreased LPS-stimulated surges of GH and IGF-1 in aqueous humor, and reduced cell infiltration and secretion of inflammatory biomarkers. Our results suggest that inflammation in Iris-CB activates GHRH signaling, GHRHR may affect maturation and migration of immune cells that contributes to EIU pathogenesis. Blocking GHRHR function alleviates inflammation in the iris-CB by reducing infiltration of macrophages and leukocytes, and production of TNF-α, IL-1β and MCP-1 into the aqueous humor. In conclusion, we show that the GHRH-GH-IGF1 signaling pathway is involved in acute ocular inflammation, and GHRHR antagonist may serve as a potential therapeutic agent for treatment of this eye disease.

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S2-05

Regulation of inflammation in the central nervous system: platelets are primary sensors of neuronal damage

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Inflammation in the central nervous system (CNS) is a complex process with a high social and economic impact worldwide. Currently there is no effective therapy for prevention and treatment of CNS inflammation that accompany many neurodegenerative diseases including traumatic brain injury (TBI), multiple sclerosis (MS) etc. Platelets respond to a vascular damage, but their role in the neurodegenerative and neuroinflammatory diseases is not well known. We have previously found that administration of brain lipid rafts induced a massive platelet activation and degranulation. The brain-specific gangliosides were specifically recognized by the platelets and this recognition occurred during disruption of blood brain barrier, a hallmark of CNS inflammation. We compared inflammatory response in the CNS of wild-type vs. $ST3^{-/-}$ mice that lack of major brain-specific gangliosides. Our study revealed that level of microglia activation and leukocyte infiltration was substantially lower in $ST3^{-/-}$ animals in both MS and TBI models. Further implications of pathogenic and regulatory roles of platelets and their direct interactions with neuronal cells and autoimmune CD4 T cells will be further discussed. Our study determines a new role of platelets as “innate immune cells” that directly recognize a neuronal damage and contribute to inflammation in the CNS.

S2-06

Micro & nano structure and mechanics of trabecular bone in elderly osteoarthritis (OA)

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Subchondral bone cyst (SBC) is a major radiological finding in knee osteoarthritis (OA), together with joint space narrowing, osteophyte and sclerotic bone formation. The presence of subchondral bone cyst (SBCs) may be associated with the severity of pain, and it is also speculated that the presence of SBCs might increase intraosseous pressure of subchondral bone. Yet the exact effect of SBC on the structural and mechanical properties trabecular bone, which provides the support to overlying articular cartilage, remains to be elucidated. Therefore, this study aimed to investiate the micro as well as nano structure and mechanical competence of trabecular bone of knee OA in presence or absence of SBC. A total of 20 postmenopausal women (54-87 years old) with the late-stage of primary knee OA were recruited in this study. Tibial plateau specimens were collected during joint replacement surgery. The samples were grouped for comparison according to presence or absences of SBC in micro-CT images. The specimens were processed for micro-CT and mechanical testing using. The bone volume fraction (BV/TV, %) was significantly higher in knee OA specimens in presence of SBC (32±7%) in comparison with those in absence of SBC (16±5%, $p<0.001$). Meanwhile there were more plate-like trabecular bone surrounding SBC (0.78±0.61) than those without SBC (1.81±0.28, $p<0.001$), which was indicated by structure model index (0~3). Furthermore, the trend in conversion of rod-like (close to 3) towards plate-like trabeculae was noticed in different locations of knee OA specimens with SBC formation. Trabecular bone around SBC presented higher modulus compared with those without SBC ($p=0.034$). The stiffer trabecular bone in presence of SBC correlated with its plate-like morphology. The presence of SBC was associated with conversion of trabeculae towards plate-like morphology together and the increase of mechanical competence in advanced knee OA.

S2-07

Arise from the dust: features of mesenchymal stem cells and application on neurological disorders

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Mesenchymal stem cells (MSCs) are adult stem cells that can be isolated from a wide array of adult tissues including bone marrow, adipose tissues and umbilical cords. They have the capabilities for self-renewal and can be expanded *ex vivo* without any apparent modification in the phenotype or loss of function. They are capable of differentiating into osteoblasts, chondrocytes, myoblasts, adipocytes, and neuron-like cells. Furthermore, emerging studies have shown that MSC has immunomodulatory properties, and can produce immunoregulatory molecules. It has been observed that MSCs interact with immune cells in a variety of ways including suppressions of cell proliferation of T cells, B-cells and dendritic cells, and modulation of their cytokine production. They also can serve as gene therapy carrier for brain tumor treatment due to the migration and immunosuppressive properties. These useful features facilitate neurological disorders based on different pathogenesis. Current strategies for their isolation, expansion, characterization, and neuronal differentiation as well as their latest applications on neurological disorders based on mentioned features will be discussed. The long term survival, migration property and differentiation possibility in pathological brain will be demonstrated.

S2-08

Stem cell manipulation using CRISPR/Cas9 technology

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Embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC) are valuable tools for studying the early embryonic development, for modeling human diseases, and for developing regenerative medicine. However, due to the limited understanding of the molecular mechanisms underlying the pluripotency, and a lack of suitable tools for precise genome editing in human pluripotent stem cells, the application of these cells is largely hindered.

Recently, we have investigated the potential of newly developed TALE and CRISPR/Cas9 technologies for modulating the expression of pluripotency genes as well as for genome editing. First, we systematically investigated the potential of TALE- and Cas9-based transcription factors (TF) in activating the stringently silenced pluripotency gene Oct4 (Pou5f1) in mouse and human somatic cells. With a number of TALEs and sgRNAs targeting various regions in the mouse and human Oct4 promoters, we found that the most efficient TALE-VP64s and highly effective sgRNAs targeted around -147 to -80 bp upstream of the transcription start sites (TSS) to induce high activity of luciferase reporters. When multiple TFs were applied simultaneously, significant transcriptional synergy was observed. The optimized combinations of TALE-VP64s could enhance endogenous Oct4 transcription up to 30-fold in mouse NIH3T3 cells and 20-fold in human HEK293T cells; and more importantly, the enhancement of OCT4 transcription ultimately generated OCT4 proteins.

Furthermore, we also initiated a study to explore the potential of CRISPR/Cas9 technology for precise genome editing in human and mouse ESCs. We have established a couple useful cell lines and obtained preliminary results to document the potential of using this tool for ESC study. This work is currently in progress.

S2-09

The HIF-α pathway regulates chondrogenesis during cartilage development and repair

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Articular cartilage, a highly organized avascular connective tissue, has a limited intrinsic repair capacity following trauma or degeneration with aging. Hypoxia is a hallmark for chondrogenesis during embryonic development or regeneration. Hypoxia functions as a stimulus for initiation of gene programs regulating chondroprogenitor cell proliferation, differentiation and metabolism. The chondrogenic cells are readily located in a hypoxic microenvironment during cartilage development or repair. Hypoxia inducible factor-alpha (HIF-α) is the key transcription factor to response to the oxygen availability of the cells during development and repair. However, the role of the HIF-α pathway in cartilage development and repair remains to be elucidated. In the present study, we examined the effects of conditional deletion of HIF-1α in the condensing mesenchyme using the Cre/loxp strategy. Our results indicate that deletion of HIF-1α in the mesenchyme impairs joint development. Deletion of HIF-1α decreases the colony forming efficiency of mesenchymal stem cells (MSCs) and impairs their chondrogenic differentiation potential. Based on a cellular phenotypic screening assay, prolyl hydroxylase inhibitor deferoxamine (DFO) was identified as an HIF-α activator. DFO increases the proliferation of primary chondrocytes and the colony forming efficiency of chondroprogenitor cells. DFO promotes chondrogenic differentiation indexed by upregulation of chondrogenic marker genes expression and increased proteoglycan synthesis in the micromass cultures. In the three dimensional (3D) bioscaffold culture, the expression of Sox9 and collagen type Ⅱ is upregulated when treated with DFO. 3D complexes containing DFO enhances the migration and engraftment of chondroprogenitor cells during cartilage repair in a mouse osteochondral defect model. Histological scoring indicates that the 3D complexes containing DFO significantly enhances articular cartilage repair. Our results suggest that activation of HIF-α promotes articular cartilage repair through coordinating MSCs migration, differentiation and engraftment.

S2-10

Roles of Yap1 in chondrocyte differentiation and cartilage diseases

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Skeletal degenerative diseases such as osteoarthritis and osteoporosis are very common in the aged population in Hong Kong and Mainland China. However, current therapeutic interventions are not satisfactory due to incomplete understanding of the molecular mechanisms for bone and cartilage formation and homeostasis. Recently, Hippo pathway is found to be implicated in the control of organ size and tissue regeneration. However, its role in the skeletal tissues has not been fully elucidated. Here, we focus on Yap1, a central mediator of Hippo pathway, to investigate its function in chondrocyte differentiation and cartilage and bone maintenance. We found that Yap1 regulates multiple processes during chondrocyte maturation, which includes cell commitment, proliferation and differentiation during endochondral ossification. In addition, our *in vivo* mouse models demonstrated that Yap1 is also involved in the maintenance of cartilage integrity and bone regeneration. These findings are highly valuable for further development of new therapeutic strategies for patients suffering with skeletal degenerative diseases. We will discuss in details the possible molecular interactions of Hippo pathway with other signaling networks in cartilage and bone homeostasis. We will also delineate examples of possible skeletal diseases that may be correlated to the dysregulation of the Hippo pathway.

Poster Presentation Session

5 June 2015 (Friday) 13:30 – 14:30

*****Presenting authors please be available by your poster for answering questions*****

Title of Presentation	*Abstract No.*
Orphan nuclear receptor TLX functions as a potent suppressor of oncogene-induced senescence in prostate cancer via its transcriptional co-regulation of CDKN1A ($p21^{WAF1/CIP1}$) and SIRT1 genes D.L. Wu, S. Yu, L. Jia, C. Zou, Z.Y. Xu, L.J. Xiao, K.B. Wong, C.F. Ng, F.L. Chan	**CI-01**
PTEN mutants found in autism have distinct biochemical properties C.W. Wong, P.M.Y. Or, A.M.L. Chan	**CI-02**
Enhancer of Zeste Homolog 2 inhibits miR-34a expression in human pancreatic ductal adenocarcinoma by inducing the formation of heterochromatin C.H. Li, Z.G. Xiao, Y.C. Chen	**CI-03**
Oncogenic cell cycle-related kinase promotes tumor immune escape via modulation of myeloid-derived suppressor cells in hepatocellular carcinoma J.Y. Zhou, A.S.L. Cheng	**CI-04**
TCP-1 as a diagnostic agent and drug delivery tool for colorectal cancer in humans L.F. Li, L. Lu, J. Shen, Z.J. Li, S.S.M. Ng, C.H. Cho	**CI-05**
Epigenetic loss of Cadherin-1 (CDH1) correlates with multidrug resistance in human hepatocellular carcinoma cells L. Jiang, J.Y.W. Chan, K.P. Fung	**CI-06**
Suppression of human mast cell activation by matrix fixed osteopontin H.Y.A. Lau, C.W. Ng	**CI-07**
Effects of peptides derived from bovine and human lactoferrins on activities of three key HIV-1 enzymes J.H. Wong, Y. Wang , D. Ip , W. Pan, Y.S. Chan, X. Dan, R.C.F. Cheung, D.C.C. Wan, T.B. Ng	**CI-08**
Potentiating effects of plectasin-derived fragments, cathelicidin and fragments, and lactoferricin with the antibiotic imipenem against drug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* J.H. Wong, E. Chan, C.H. Cho, T.B. Ng	**CI-09**
Effects of antimicrobial peptides and fragments derived from them on the pathogens *Helicobacter pylori* and *Candida albicans* J.H. Wong, Y. S. Chan, C.H. Cho, T.B. Ng	**CI-10**

Title of Presentation	*Abstract No.*
The draft genome, transcriptome and microbiome of dermatophagoides farinae reveal a broad spectrum of dust mite allergens T.F. Chan, K.M. Ji, A.K.Y. Yim, X.Y. Liu, J.W. Zhou, R.Q. Li, K.Y. Yang, J. Li, M. Li, P.T.W. Law, Y.L. Wu, Z.L. Cai, H. Qin, Y. Bao, R.K.K. Leung, P.K.S. Ng, J. Zou, X.J. Zhong, P.X. Ran, N.S. Zhong, Z.G. Liu, S.K.W. Tsui	**CI-11**
Role of CFTR in insulin secretion: implications in diabetes J.H. Guo, Y.C. Ruan, H.C. Chan	**RDE-01**
MicroRNA in cell fate determination L. Li, S. Gu, Y.K. Suen, H.H. Cheung, D.D. Cao, W.Y. Chan	**RDE-02**
Semaphorin 3A signaling during the entry of sacral neural crest cells into the hindgut of mouse embryos C.F. Wang, W.Y. Chan	**RDE-03**
Modeling Werner syndrome and autism spectrum disorder with iPSC H.H. Cheung, X. Liu, W.Y. Chan	**RDE-04**
Molecular mechanisms underlying the anti-inflammatory effect of carbon monoxide in human bronchial epithelial cells R.G. Zhang, G.J. Lau, M.Y. Hao, A.W.M. Chow, W.C.Y. Yip, W.H. Ko	**RDE-05**
Epigenetic and transcriptional dynamics in neonatal spermatogonial stem cell development J. Liao, S.H. Ng, W.Y. Chan, T.L. Lee	**RDE-06**
Fibroblast growth factor 21: a potent metabolic regulator in the protection against pancreatic islet dysfunction and type 2 diabetes mellitus W.Y. So, P.S. Leung	**RDE-07**
The role of L3mbtl2 in renal ischemia/reperfusion injury H.H. Huang, W.J. Liu, Y. Wang, Y.S. Zhao, Y. Xia	**RDE-08**
Regulation of lipid homeostasis by mammalian TRAPP complex C.M. Li, S. Zhao, S.S.B. Yu	**RDE-09**
Genetic disruption and molecular study on an atypical small GTPase M.X.M. Luo, C.M. Li, G.K.Y. Siu, S.S.B. Yu	**RDE-10**
Heat shock 70kDa protein 5 (Hspa5) is essential for pronephros formation by mediating retinoic acid signaling W.L. Shi, C.D. Wang, S.M. Sperber, Y. Chen, Q. Zhou, G. Xu, Y. Deng, H. Zhao	**RDE-11**

CI-01

Orphan nuclear receptor TLX functions as a potent suppressor of oncogene-induced senescence in prostate cancer via its transcriptional co-regulation of CDKN1A ($p21^{WAF1/CIP1}$) and SIRT1 genes

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Oncogene-induced senescence (OIS) is an important tumor-suppressing mechanism to prevent both premalignant transformation and cancer progression. Overcoming this process is a critical step in early cancer development. The druggable orphan nuclear receptor TLX (NR2E1) is characterized as an important regulator of neural stem cells and also implicated in the development of some brain tumors. However, its exact functional roles in cancer growth regulation still remain unclear. Here we report that TLX can act as a promoter of tumorigenesis in prostate cancer by suppressing OIS. We determined that TLX exhibited an increased expression in high grade prostate cancer tissues and many prostate cancer cell lines. Functional studies revealed that TLX could perform an oncogenic function in prostate cancer cells, as its knockdown triggered cellular senescence and cell growth arrest *in vitro* and *in vivo*, whereas its overexpression promoted the malignant growth of prostate cancer cells. Furthermore, enhancement of TLX activity, by either ectopic expression or ligand stimulation, could potently prevent doxorubicin-induced senescence in prostate cancer cells and also allow prostatic epithelial cells to escape OIS induced either by activated oncogene $H\text{-}Ras^{G12V}$ or knockdown of tumor suppressor *PTEN*, via a mechanism of direct but differential transcriptional regulation of two senescence-associated genes, repression of *CDKN1A* and transactivation of *SIRT1*. Together, our present study shows for the first time that TLX may play an important role in prostate carcinogenesis through its suppression of OIS, and also suggest a potential therapeutic significance of targeting the senescence-regulating TLX in prostate cancer.

CI-02

PTEN mutants found in autism have distinct biochemical properties

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Autism Spectrum Disorders (ASD) are a collection of neurocognitive deficits characterized by the lack of social interaction and language skills. The human tumor suppressor gene *PTEN* encodes a lipid phosphatase and is mutated and inactivated in 1% of ASD. In ASD patients, germline missense mutations are found in different regions of *PTEN*. These patients have diverse clinical features. This allows the fine "mapping" of PTEN functions relevant to ASD pathogenesis. We have analyzed 14 such ASD mutants for their biochemical properties. *PTEN* ASD mutant ectopically expressed in a PTEN-null prostate cancer cell line, PC3, have drastically different protein expression levels suggesting some ASD mutation may cause changes in protein stability. Using p-Akt as a readout, all ASD mutants have reduced lipid phosphatase activities. Heightened PI3-K>Akt signaling has been described in *Pten* knockout mice harboring autistic behaviors. Interestingly, the I135R mutant activated the Akt to a level greater than control cells. Thus, some PTEN ASD mutant may have dominant acting properties. By GFP-fusion tagged and immunofluorescence analysis, *PTEN* mutants showed diverse ability in membrane and cytosolic localizations. In addition, a panel of 12 autism cases with mental retardation and overgrowth from local population was screened for *PTEN* mutation. Two mutations in the coding exons were identified. A T>C transition mutation in codon 101 altering the isoleucine to threonine, and a single G insertion in codon 109 resulting in a frame shift mutation and translational termination.

CI-03

Enhancer of zeste homolog 2 inhibits miR-34a expression in human pancreatic ductal adenocarcinoma by inducing the formation of heterochromatin

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MicroRNA-34a (miR-34a) is frequently downregulated in pancreatic ductal adenocarcinoma (PDAC), but the underlying mechanism remains elusive as p53 aberration and miR-34a promoter DNA methylation are rare in PDAC cells. We have showed that enhancer of zeste homolog 2 (EZH2), a H3K27 methyltransferase, repressed microRNA in PDAC. Here, we attempted to investigate the role of EZH2 in the silencing of miR-34a.

We showed that miR-34a was aberrantly repressed by EZH2 in PDAC cells. miR-34a was upregulated in EZH2-inhibited PDAC cells, and downregulated in EZH2-overexpressing human pancreatic ductal epithelial (HPDE) cells. EZH2-mediated cell proliferation was attenuated by miR-34a mimics transfection and miR-34a precursor lentivirus transduction. Inhibition of EZH2 increased miR-34a promoter activity in PDAC cells. miR-34a was upregulated upon the inhibition of EZH2 co-silencing partners SUZ12 and HOTAIR but not DNMTs. EZH2 protein and H3K27me3 occupancy at miR-34a promoter was higher in PDAC cells than HPDE cells. Overexpression of EZH2 in HPDE cells induced the enrichment of heterochromatin markers (i.e. H3K9me2, Heterochromatin proteins) at miR-34a promoter, while knockdown of EZH2 in SW1990 reduced the occupancy of these heterochromatin markers. In conclusion, EZH2 suppressed miR-34a that contributed to EZH2-associated cell proliferation in PDAC through trimethylation of H3K27 and heterochromatin formation at miR-34a promoter.

CI-04

Oncogenic cell cycle-related kinase promotes tumor immune escape via modulation of myeloid-derived suppressor cells in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is a sexually dimorphic cancer significantly associated with elevated levels of male sex hormone androgen and pro-inflammatory cytokine interleukin-6 (IL-6). Our previous genome-wide location and functional analysis has pinpointed cell cycle-related kinase (CCRK) as a critical mediator of androgen receptor (AR) oncogenic activity in HCC through a self-reinforcing circuitry involving β-catenin and the Polycomb repressor enhancer of zeste 2 (EZH2). As these key circuit players have also been shown to trigger inflammatory response and aggressiveness of HCC cells, we aimed to investigate whether CCRK modulates tumor microenvironment during hepatocarcinogenesis. Expression profiling of 16 pro-inflammatory cytokines revealed that ectopic expression of wild-type but not kinase-defective CCRK significantly induced the production of IL-6, IL-1β, tumor necrosis factor-α and cyclooxygenase 2 in immortal hepatic and HCC cells. Rescue experiments showed that CCRK-induced IL-6 production was dependent on EZH2 up-regulation and subsequent nuclear factor-κB activation. Based on the causal link between IL-6 and expansion of functional myeloid-derived suppressor cells (MDSCs), we next examined the relationship between CCRK and MDSCs *in vitro* and *in vivo*. By co-culturing CCRK-expressed or silenced hepatic and HCC cell lines with peripheral blood mononuclear cells (PBMCs) isolated from healthy donors, we found that CCRK stimulated the expansion of $Lin^-CD33^+CD11b^+HLA^-DR^-$MDSCs from PBMCs by flow cytometry analysis. The induced MDSCs also showed higher suppressive activity against $CD3^+$ T cell proliferation. In a high-fat diet-fed mouse model exposed to low-dose carcinogen diethylnitrosamine, administration of lentivirus expressing short-hairpin (sh)RNA against *Ccrk* significantly reduced >70% obesity-promoted tumor multiplicity and size compared to mice treated with control shRNA ($p<0.01$). Notably, *Ccrk* down-regulation also significantly reduced the population of circulating MDSCs in the mice ($p<0.05$). In summary, our findings demonstrate that the oncogenic CCRK in hepatic cells can promote MDSC expansion and suppressive functions possibly via IL-6 production. Such tumor-stromal cell interaction provides survival advantage of cancer cells via immune escape. In conclusion, CCRK not only functions as a signaling hub but also an immunoregulator, thus representing a new cancer immunotherapy target.

CI-05

TCP-1 as a diagnostic agent and drug delivery tool for colorectal cancer in humans

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Colorectal cancer (CRC) remains one of the most prevalent forms of cancer with an increasing trend in Asia, including Mainland China and Hong Kong. Early diagnosis of CRC is the most effective way to improve the life expectancy and quality in cancer patients. Our previous study had demonstrated a novel tumor-blood-vessel-targeting peptide, named as TCP-1 (CTPSPFSHC). This was isolated and identified using a phage-display technology platform. It was found that TCP-1 could selectively bind to the tumor blood vasculature using a mouse orthotopic colon cancer model. In order to extrapolate this animal finding to a more clinical application, we had screened more than 70 CRC patients for the binding capacity for TCP-1 in these human colon cancer samples. We also tried to identify the possible binding site for the peptide in these clinical samples. Results demonstrated that more than 50% of CRC patients showed positive binding with TCP-1 at the tumor blood vessels in different pathological cancer stages and locations in the colon. In addition, there was about another 10% of these patients with positive binding in their tumor tissues other than at the blood vessels. In those positive binding tissues, TCP-1 was found to be co-localized with Target-1, mostly at the tumor blood vessels. With this finding, it is likely that Taget-1 could be a biomarker for CRC and also the target site for chemotherapy. In summary, there is about 62% of CRC patients have positive binding with TCP-1. This peptide can be developed as a diagnostic agent when conjugated with imaging contrast or used as a delivery system when combined with anti-cancer drug for targeted therapy of CRC in humans.

CI-06

Epigenetic loss of cadherin-1 (CDH1) correlates with multidrug resistance in human hepatocellular carcinoma cells

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Promoter-cytosine-phosphate-guanine- (CpG) hypermethylation of tumor suppressor genes is an essential step in cancer progression but little is known about its effect on cancer multidrug resistance. In this study, we showed that CDH1 promoter was hypermethylated in drug resistance of a doxorubicin-induced multidrug resistant hepatocellular carcinoma cell line R-HepG2. Transfection of CDH1 cDNA into R-HepG2 cells led to increased amount of doxorubicin uptake, decreased cell viability, decreased P-glycoprotein expression and increased apoptotic population of cells exposed to doxorubicin. Proto-oncogene tyrosine-protein kinase FYN was over-expressed in R-HepG2 cells which displayed a negative correlation with the expression of CDH1. FYN was knocked down in R-HepG2 cells, leading to less drug resistance by increased cell viability, increased doxorubicin uptake and attenuated P-glycoprotein expression. Our findings identified epigenetic silencing of CDH1 in cancer cells might be a new molecular event of multidrug resistance.

Reference:
Jiang L, Chan JYW and Fung KP (2012) Biochem. Biophys. Res. Commun. 422: 739-744.

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CI-07

Suppression of human mast cell activation by matrix fixed osteopontin

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Mast cells are key multifunctional effector immune cells which release and synthesize myriads of chemical messengers important in the pathogenesis of inflammation. Osteopontin (OPN) is an extracellular matrix-associated glycoprotein which acts as a cytokine in the soluble form (sOPN) or as an adhesion molecule in the matrix fixed form (mOPN) and serves as an important regulator of various immune responses. Both mast cells and OPN have separately been implicated in the mediation of acute and chronic inflammatory reactions but the effects of OPN on mast cells have not been investigated in human. We thus studied this topic by employing human mast cells (HMC) cultured from $CD34^+$ progenitors and OPN isolated from human milk. Mast cell activation was induced by specific antibody against human IgE (anti-IgE). Both sOPN and mOPN did not modulate anti-IgE induced degranulation as determined by histamine release. In contrast, mOPN induced adhesion of HMC and suppressed anti-IgE induced synthesis of cytokines such as IL-5, IL-8 and TNF-α while sOPN was ineffective. Further studies employing blocking agents revealed that the interaction between HMC and mOPN was mediated between the αVβ3 integrin but not CD44 on HMC and the RGD (Arg-Gly-Asp) domain of OPN. In conclusion, we have demonstrated for the first time that OPN in the matrix fixed conformation suppressed mast cell activation in human.

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CI-08

Effects of peptides derived from bovine and human lactoferrins on activities of three key HIV-1 enzymes

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The intent of this study was to investigate whether human and bovine lactoferrin fragments comprising lactoferrin (1-11), lactoferricin and lactoferrampin, all of which were devoid of hemolytic activity toward rabbit erythrocytes at 1 mM concentration, exerted inhibitory effects on the activities of HIV-1 reverse transcriptase, protease and integrase. The results indicated that human lactoferricin was the most potent in inhibiting HIV-1 reverse transcriptase (IC50 =2 μM). Bovine lactoferricin (IC50 = 10 μM) and bovine lactoferrampin (IC50 = 150 μM) were less potent. Human lactoferrampin and human and bovine lactoferrin (1-11) at 1 mM concentration did not exhibit any inhibitory effect on HIV-1 reverse transcriptase. All peptides showed only a slight inhibitory effect (from slightly below 2% to 6% inhibition) on HIV-1 protease. Human lactoferrampin and bovine lactoferrampin showed obvious inhibitory effect on HIV-1 integrase at 37 μM and 18.5 μM, respectively. The HIV-1 integrase inhibitory activity of human lactoferrampin and bovine lactoferrampin was dose-dependent. The other peptides were devoid of HIV-1 integrase inhibitory activity. It is concluded that some lactoferrin fragments exert an inhibitory action on HIV-1 reverse transcriptase and HIV-1 integrase.

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CI-09

Potentiating effects of plectasin-derived fragments, cathelicidin and fragments, and lactoferricin with the antibiotic imipenem against drug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*

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Pseudomonas aeruginosa is an opportunistic Gram-negative bacterium that causes a variety of life-threatening infections. It displays high intrinsic drug resistance. The emergence of such resistant isolates is a major obstacle in the treatment and management of infectious diseases. *Acinetobacter baumannii* exhibits a high rate of antibiotic resistance that accounts for nosocomial infections. LL37 is the cationic antimicrobial peptide human cathelicidin. Lactoferricin is derived from lactoferrin. Plectasin is a fungal defensin. In the first experiment, we tested the effects of LL37, its fragments LL 17-32, LL15-37 and LL25-37, and lactoferricin, alone as well as in combination with imipenem, on *P. aeruginosa* strains resistant to imipenem (39 μg/ml). When LL37, lactofericin, or any of the aforementioned LL37fragments, was added to the medium at 156 μg/ml, no bacterial growth was detectable. The peptides alone except LL37 were inactive. The combined effect of lactoferricin, LL37 or fragments of LL37 with imipenem was bactericidal as no growth was detectable when the test medium was re-streaked onto fresh medium. LL37 potentiated bactericidal effects with imipenem as LL37 alone inhibited growth of imipenem-resistant *P. aeruginosa* at 156 μg/ml. In the second experiment, we tested guinea pig, snake and chicken cathelicidins at 100 and 500 ug/ml, without or together with 10 μg/ml imipenem which is below the MIC of the 2 test strains (carbapenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*). Guinea pig cathelicidin, snake cathelicidin and chicken cathelicidin were able to eradicate both test strains hence their MIC must be below 100 μg/ml. Horse cathelicidin eradicated *A. baumannii* but not *P. aeruginosa*. The peptides were also found to potentiate a sub-inhibitory concentration of imipenem so we expect that in the presence of imipenem, the MIC of such peptides can be further reduced. Three plectasin-derived fragments were inactive when tested alone at 500 μg/ml against carbapenem-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* but potentiated a sub-inhibitory concentration of imipenem.

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CI-10

Effects of antimicrobial peptides and fragments derived from them on the pathogens *Helicobacter pylori* and *Candida albicans*

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Helicobacter pylori is a Gram-negative, microaerophilic bacterium present in the stomach and found in patients with chronic gastritis and gastric ulcer. It has been associated with the formation of duodenal ulcers and gastric cancer. *Candida albicans* is a fungus that causes opportunistic oral and genital infections in humans. Systemic fungal infections brought about by *C. albicans* have emerged as a cause of morbidity and mortality in immunocompromised patients. In mammals, cathelicidins and defensins are the two major families of antimicrobial peptides which can be upregulated during infection. Cathelicidins are cationic peptides composed of a conserved N-terminal cathelin-like domain and a variable C-terminal antimicrobial domain. Several cathelicidins have been found in animals, but only a single cathelicidin was identified in humans. Defensins have been reported from fungi, plants and animals. Previously it has been demonstrated that human cathelicidin LL37 was active against *H. pylori*. In this study, guinea pig, snake, chicken, and cow cathelicidins and peptides derived from the fungal defensin plectasin were tested for antibacterial effect against *H. pylori* strain SS1. The data revealed that guinea pig cathelicidin, snake cathelicidin, and chicken cathelicidin suppressed the growth of *H. pylori* cells by 50% at a concentration below 200 μg/ml after exposure of the cells to the cathelicidins for 72 hours. However, cow cathelicidins and peptides derived from the fungal defensin plectasin were inactive toward *H. pylori* cells even at 500 μg/ml.

Human cathelicidin LL37 reduced the viability of *C. albicans* by 80% at 2 μM and by 100% at 10 μM. LL13-37, a fragment of LL37, was also active against *C. albicans* by permeabilizing the fungal membrane and adversely affecting the fungal mitochondria.

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CI-11

The draft genome, transcriptome and microbiome of Dermatophagoides farinae reveal a broad spectrum of dust mite allergens

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Background: A sequenced house dust mite (HDM) genome would advance our understanding of HDM allergens, a common cause of human allergies.

Objective: To produce an annotated *Dermatophagoides farinae* draft genome and develop a combined genomic-transcriptomic-proteomic approach for elucidation of HDM allergens.

Methods: A *D. farinae* draft genome and transcriptome were assembled with high-throughput sequencing, accommodating microbiome sequences. The allergen gene structures were validated by Sanger sequencing. The mite's microbiome composition was determined and the predominant genus was validated immunohistochemically. The allergenicity of a ubiquinol-cytochrome c reductase binding protein (UQCRB) homologue was evaluated with immunoblotting, immunosorbent assays, and skin prick tests.

Results: The full gene structures of 20 canonical allergens and 7 non-canonical allergen homologues were produced. A novel major allergen, UQCRB-like protein, was found and designated Der f 24. All 40 sera samples from mite-allergic patients had IgE antibodies against rDer f 24. Of 10 patients tested, five had positive skin reactions. The predominant bacterial genus among 100 identified species was *Enterobacter* (63.4%). An intron was found in the 13.8-kDa *D. farinae* bacteriolytic enzyme gene, indicating that it is of HDM origin. KEGG pathway analysis revealed a phototransduction pathway in *D. farinae* as well as thiamine and amino acid synthesis pathways suggestive of an endosymbiotic relationship between *D. farinae* and its microbiome.

Conclusion: An HDM genome draft produced from genomic, transcriptomic, and proteomic experiments revealed allergen genes and a diverse endosymbiotic microbiome, providing a tool for further identification and characterization of HDM allergens and development of diagnostics and immunotherapeutic vaccines.

RDE-01

Role of CFTR in insulin secretion: implications in diabetes

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Diabetes is a chronic metabolic disease with over 300 million people suffering worldwide. Insulin insufficiency is found in type 1 diabetes and, combined with insulin resistance, in type 2 diabetes. Although the cause of insulin insufficiency is generally considered to be a result of β-cell damage by autoimmunity, a high percentage of diabetic patients with insulin insufficiency show negative of those autoantibodies. Up to 50% adult patients with cystic fibrosis (CF), a disease caused by mutations in the gene encoding the CF transmembrane conductance regulator (CFTR), develop CF-related diabetes (CFRD) with most patients exhibiting insulin insufficiency. Our recent studies have explored the possible involvement of CFTR in regulating the electrophysiological activities of pancreatic β cells required for insulin secretion. The results show that the glucose-elicited whole-cell currents, membrane depolarization, electrical bursts or action potentials, Ca^{2+} oscillations and insulin secretion are abolished or reduced by inhibitors or knockdown of CFTR in primary mouse β-cells or RINm5F β-cell line, or significantly attenuated in CFTR mutant (DF508) mice compared with wild-type mice. VX-809, a newly discovered corrector of DF508 mutation, successfully rescues the defects in DF508 β-cells. Our results reveal a role of CFTR in glucose-induced electrical activities and insulin secretion in β-cells, and shed light on the pathogenesis of CFRD and possibly other idiopathic diabetes.

RDE-02

MicroRNA in cell fate determination

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Human induced pluripotent stem cells (hiPSC) have the potential to differentiate into all somatic cell types. Because the introduction of these cells does not evoke immune rejection and the use of these cells does not encounter any ethical issues, iPSCs have become the most promising source of cells for therapy as well as for developmental studies. microRNAs (miRNA) are known to regulate gene expression post-transcriptionally. Previous studies have shown that miRNA can induce de-differentiation and trans-differentiation of cells. These observations suggest that the possibility of manipulating hiPSC differentiation with miRNAs is high.

We have established hiPSC differentiation platform for three germ layers, namely, hepatocyte for endoderm, nephron progenitor for mesoderm, and neural progenitors for ectoderm. Profiling of miRNA expression in samples collected from different time points during differentiation with microarrays allowed us vertical and horizontal comparisons among the three representative lineages of the three germ layers. A number of apparently lineage-specific miRNAs were identified. A combination of target gene prediction and gene ontology enrichment analyses were used to select candidate miRNAs, the expression of which was confirmed by qPCR. The roles of candidate miRNAs in lineage specification will be further studied via functional analysis, including detection of downstream targets genes, preformation of gain- and loss-of-function assays, and generation of miRNA-specific expressing transgenic mice. We hope to reveal the miRNA(s) that is located in the center of the network of cell fate decision and lineage specification.

RDE-03

Semaphorin 3A signaling during the entry of sacral neural crest cells into the hindgut of mouse embryos

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Sacral neural crest cells (NCCs) enter the distal hindgut and contribute to enteric neurons and glia in the post-umbilical gut of the mouse and chick embryos. Previous studies have shown that before entering, mouse sacral NCCs stay close to the hindgut for two days from E11.5 to E13.5, but the regulatory mechanism underlying the entry remains unknown. We hypothesized that semaphorin 3A (Sema3A)-neuropilin-1 signaling is involved in the entry by inhibiting the sacral NCC migration. In this study, with immunofluorescence localization, *in vitro* culture and time-lapse live cell imaging, we found that Sema3A was transiently expressed in the mouse distal hindgut epithelium at E12.5 to E13.5, while its receptor, neuropilin-1, was expressed by sacral NCCs. Sacral NCC migration was retarded in a culture medium containing Sema3A, but cell proliferation and apoptosis were not affected. When a hindgut epithelium expressing Sema3A was co-cultured with pelvic ganglia containing sacral NCCs, both the NCC migration and the extension of neuronal processes were suppressed. These findings suggest that the transient expression of Sema3A in the hindgut epithelium regulates the entry of sacral NCCs into the hindgut by suppressing not only the sacral NCC migration but also the extension of neuronal processes into the hindgut.

RDE-04

Modeling Werner syndrome and autism spectrum disorder with iPSC

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We are interested in understanding the molecular and genetic factors leading to syndromic and idiopathic diseases. In particular, we have been working on the premature aging disease Werner syndrome (WS), which is caused by germline mutations on the WRN DNA helicase, and idiopathic autism spectrum disorder (ASD), which is a complex neurodevelopmental disorder. We established human iPSC models for WS and ASD respectively, using patient fibroblasts. Genomic profiling of the transcriptome revealed high similarity between normal and WS or ASD iPSC. However, when differentiating to specific lineages of cells, we observed differential properties relating to aging (for WS) and neurophysiology (for ASD). In WS, mesenchymal progenitor cells entered senescence prematurely, with obvious accelerated shortening of telomeres and loss of sister telomeres at lagging strand. In ASD, mature neurons showed aberrant expressions of genes related to ligand-receptor interaction, ECM-receptor interaction and ion channel function. Taken together, the iPSC-based disease models for WS and ASD provide insights into understanding the pathogenesis of progeroid and neurodevelopmental disorders.

RDE-05

Molecular mechanisms underlying the anti-inflammatory effect of carbon monoxide in human bronchial epithelial cells

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Extracellular nucleotide release and the subsequent activation of P2Y receptors have been implicated in the pathogenesis of several inflammatory lung disorders, such as asthma (Mortaz et al., 2010). During airway inflammation, damage to the surface epithelium is due to the secretion of eosinophil derived, highly toxic cationic proteins, such as the major basic protein (MBP). Our recent study demonstrates that, when the human bronchial surface epithelia are chemically damaged by poly-L-arginine as a surrogate of MBP (Chow et al., 2010), nucleotides such as ATP and UDP are released into the extracellular medium. The extracellular nucleotides then activate cell surface P2Y receptors to release two pro-inflammatory cytokines, interleukin (IL)-6 and IL-8, via a Ca^{2+}-dependent process (Hao et al., 2014). The aim of this project is to investigate the anti-inflammatory role of carbon monoxide (CO) in a human bronchial epithelial cell line, 16HBE14o-.

Data demonstrate that the 16HBE14o- cell line expressed heme oxygenase (HO) enzymes, HO-1 and HO-2, at the mRNA and protein levels. Poly-L-arginine stimulated the mRNA expression of HO-1 and HO-2 in 16HBE14o- cells. CO-releasing molecules (CORMs) inhibited extracellular nucleotide- or poly-L-arginine- induced IL-6 and IL-8 release in 16HBE14o- cells. In the presence of CORM-A1, CORM2 or CORM3, a significant reduction in the level of $[Ca^{2+}]i$ was witnessed in cells stimulated with different extracellular nucleotides, compared with untreated control epithelia. Our data suggest that CO could be produced endogenously by HO-1 or HO-2 in 16HBE14o- cells. CO possessed anti-inflammatory effect, which may be partly mediated by its inhibitory action on the P2Y receptor-mediated $[Ca^{2+}]i$ increase.

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RDE-06

Epigenetic and transcriptional dynamics in neonatal spermatogonial stem cell development

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Spermatogonial stem cells (SSCs) are male germline stem cell that either self-renew or differentiate to produce progenitor spermatogonia to initiate spermatogenesis. Similar to other stem cells, it is believed that these processes are tightly regulated through precise epigenetic and transcriptional dynamics, and its aberrant alterations could lead to significant health impact to the offspring. Despite recent studies have suggested that epigenetic profiles in SSCs shift dramatically when committing differentiation, and is concordant with gene expression of established differentiation markers like c-Kit. The interplay between epigenetic and transcriptional regulations in mammalian SSCs remain largely unknown. To this end, we examined the dynamics of DNA epigenetic modifications and gene expression in neonatal undifferentiated and differentiating SSCs. Epigenetic modifications on DNA methylation and demethylation in form of 5-methylcytosine (5mC) and 5-Hydroxymethylcytosine (5hmC) were revealed by whole genome 5mC/5hmC profiling at single-base resolution, whereas the transcriptomes were profiled by RNA-seq. The high-resolution DNA methylome maps revealed significant differential gene methylation, which were enriched in various cellular developmental processes. In particular, we observed methylation changes associated with OCT4 and GDNF, which are both important for undifferentiated spermatogonia maintenance. 5hmC profiling suggested 5hmC marks were enriched specifically in proximal upstream and downstream regions relative to TSS and their presence correlated positively with gene expression. Strikingly, we also observed that genes enriched in undifferentiated SSCs displayed higher promoter 5hmC levels, indicating 5hmC might be associated with stemness-related genes and stem cell capacity. Taken together, the results provides comprehensive information of the transcriptional and epigenetic landscapes in neonatal SSC development, and highlight the active involvement of DNA methylation and demethylation on various transcripts, which will allow identification of novel molecular regulations.

RDE-07

Fibroblast growth factor 21: a potent metabolic regulator in the protection against pancreatic islet dysfunction and type 2 diabetes mellitus

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Type 2 diabetes mellitus (T2DM) results when insulin resistance develops in association with pancreatic islet failure. Despite being a critical regulator for glucose and lipid homeostasis in major metabolic targets, the exact actions of fibroblast growth factor 21 (FGF21) in pancreatic islets remain ambiguous.

We firstly investigated the effects of hyperglycemia on islet FGF21 action in diabetic db/db mice. Results showed that high-glucose conditions attenuated islet FGF21's signaling, via decreases in β-klotho (an FGF21 cofactor) and peroxisome proliferator-activated receptor gamma (PPARγ) levels, indicative of its involvement in glucose-mediated islet dysfunction. We secondly elucidated the physiological role of islet FGF21using FGF21-knockout mice. Results showed that FGF21 was important for the maintenance of insulin sensitivity, islet insulin synthesis, β-cell proliferation and islet function, via its inhibitory effect on islet growth hormone's signalling, indicative of an obligatory metabolic regulator of FGF21 in islets. We further explored the role of FGF21 in islet lipid metabolism and its relevance to obesity-induced insulin resistance, islet dysfunction and T2DM. Preliminary results showed that FGF21 was responsible for the regulation of several critical genes involved in the modulation of fatty acid metabolism and in the protection against lipotoxicity-induced islet inflammation.

Taken together, our data will provide new insights into the actions of FGF21 and into a scientific basis for the preservation of islet function/survival against obesity-associated lipotoxicity and resultant T2DM.

RDE-08

The role of L3mbtl2 in renal ischemia/reperfusion injury

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L3mbtl2 has been implicated in early embryonic development. It is expressed in many organs including the kidney. However, the role of L3mbtl2 in adult kidney is still undefined. In this study, we first demonstrated that L3mbtl2 is highly expressed in renal tubular epithelial cells in mice. We then generated kidney epithelial cell specific L3mbtl2 knockout mice (L3mbtl2 cKO) by crossbreeding floxed L3mbtl2 mice with Ksp-Cre mice. These mice were grossly normal with no phenotypes found in the kidney under basal conditions. However, when the kidneys were subjected to ischemia/reperfusion injury (IRI), the kidneys of L3mbtl2 cKO mice were much less injured compared to the kidneys of wild-type (WT) mice, as determined by the decreased tubular necrosis and cast formation. Kidneys of L3mbtl2 cKO mice exhibited decreased numbers of TUNEL-positive cells compared with those of WT mice 12 hours after IRI. In human proximal tubular HK-2 cells cultured in serum-deprived medium, knockdown of L3mbtl2 reduced cleaved caspase3 expression and increased cell viability. Conversely, overexpression of L3mbtl2 increased cleaved caspase3 expression and decreased cell viability. These results suggest that disruption of L3mbtl2 may protect the kidney from apoptosis and tubular injury in injured kidneys. The detailed phenotypes of L3mbtl2 cKO mice and the molecular mechanisms underlying L3mbtl2-induced apoptosis are still under active investigation.

RDE-09

Regulation of lipid homeostasis by mammalian TRAPP complex

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The transport protein particle (TRAPP) complex is a multi-subunit tethering factor that has been identified as (GEF) for the Rab GTPase Rab1/Ypt1p. This complex is present in three forms: TRAPPI complex composed of seven core subunits that is required for ER-Golgi transport; TRAPPII complex, which has additional three subuints,TRAPPC6, TRAPPC9 and TRAPPC10, mediates post-Golgi trafficking and TRAPPIII complex, which includes at least all core subunits and TRAPPC8, involves in autophagosome formation. Here, we demonstrate that the TRAPPII complex is also a Rab18 GEF and regulates lipid homeostasis. When treated with oleic acid, a skin fibroblast cell line derived from patient with TRAPPC9 loss-of-function mutation showed significantly greater size of lipid droplets (LDs) compared to identically treated controls. The similar phenotype was also observed from TRAPPC9 knock down Hela cells, TRAPPC9&C10 knock out HEK293T cells as well as Rab18 knock down Hela cells. In addition, we found that when treated with oleic acid, overexpressed wild type Rab18 accumulated around lipid droplets in 293T cells but still localized to ER in TRAPPC9&C10 knock out 293T cells. Our findings uncover a function for TRAPP complex at LDs and suggest that TRAPPII complex controls the ER-LD shuttling of Rab18.

RDE-10

Genetic disruption and molecular study on an atypical small GTPase

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Small GTPases Rab regulate many steps of membrane traffic process. Typical Rab act as molecular switches cycling between an “on” and “off” mode through exchange of "Rab^{GTP} " to "Rab^{GDP} ". Nevertheless, the subcellular location and biological relevance of an atypical small GTPase, Rab40c (which contains a SOCS (suppressor of cytokine signaling) box that recruits a ubiquitin ligase complex.) is still elusive. In this study, we use CRISPR-Cas9 (CRISPR (clustered regularly interspaced palindromic repeats)-Cas (CRISPR associated protein)) system for genomic sequence-specific disruption of Rab40c via non-homologous end joining (NHEJ). Further examination between in single-colonized wide-type and KO ($Rab40c^{+/+}$ vs $Rab40c^{m/m}$) cells revealed abnormal morphology of lipid droplets in the KO cells. We have identified a number of proteins that interacted with Rab40c by mass spectrometry analysis, including proteins functioning in lipid metabolism and others regulating cell proliferation. These data indicate a potential dual function of Rab40c in lipid droplet metabolism and cancer.

RDE-11

Heat shock 70kDa protein 5 (Hspa5) is essential for pronephros formation by mediating retinoic acid signaling

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The heat shock 70kDa protein 5 (Hspa5) also known as binding immunoglobulin protein (Bip) or glucose regulated protein 78 (Grp78), belongs to the heat shock protein 70kDa family. As a multifunctional protein, it participates in protein folding, calcium homeostasis and serves as an essential regulator of the endoplasmic reticulum (ER) stress response. It has also been implicated in signal transduction by acting as a receptor or co-receptor residing at the plasma membrane. Its function during embryonic development, however, remains largely elusive. In this study, we used morpholino antisense oligonucleotides (MO) to knockdown Hspa5 activity in *Xenopus* embryos. In Hspa5 morphants, pronephros formation was strongly inhibited with the reduction of pronephric marker genes *lhx1*, *pax2* and *atp1b1*. Pronephros tissue is induced *in vitro* by treating animal caps with *all-trans* retinoic acid (atRA) and activin. Depletion of Hspa5 in animal caps, however, blocked the induction of pronephros as well as reduced the expression of RA-responsive genes, suggesting that knockdown of Hspa5 attenuated RA signaling. Knockdown of Hspa5 in animal caps resulted in decreased expression of *lhx1*, a transcription factor directly regulated by RA signaling and essential for pronephros specification. Co-injection of Hspa5MO with *lhx1* mRNA partially rescues the phenotype induced by Hspa5MO. These results suggest that the RA-lhx1 signaling cascade is involved in Hspa5MO induced pronephros malformation. This study shows that Hspa5, a key regulator of the unfolded protein response, plays an essential role in pronephros formation, which is mediated in part through RA signaling during early embryonic development.

ACKNOWLEDGEMENTS

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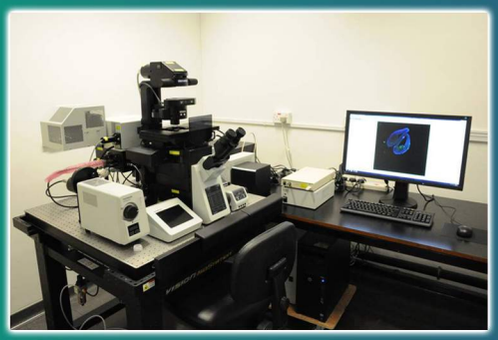

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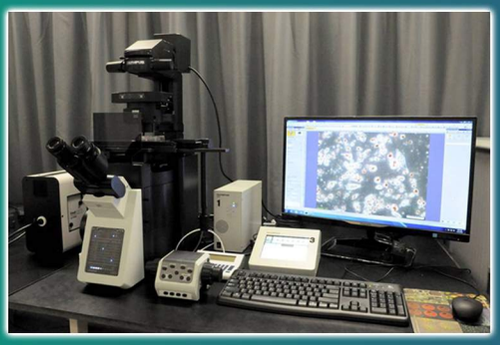

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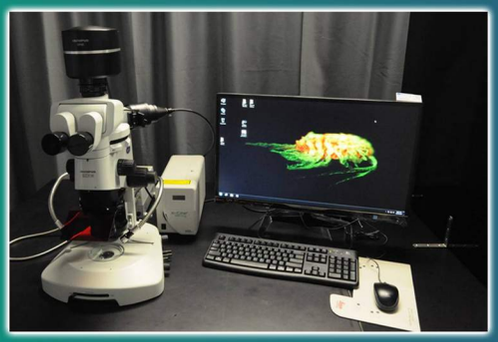

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