The Chinese University of HongKong

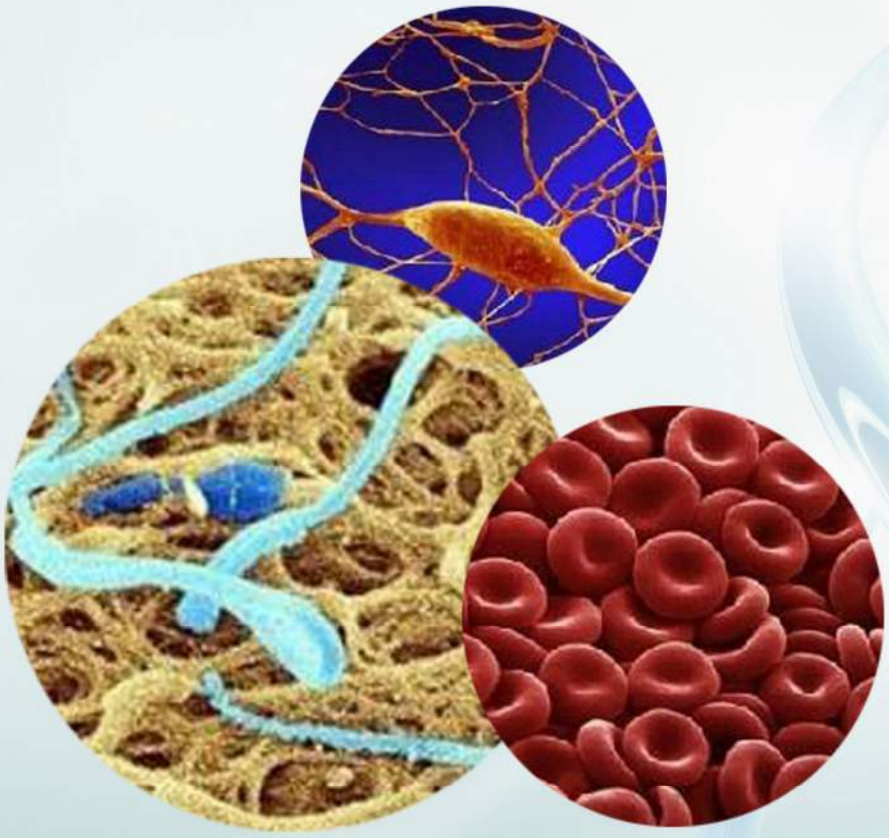
Falculty of Medcine

School of Biomedical Sciences

SCHOOL OF BIOMEDICAL SCIENCES

POSTGRADUATE RESEARCH DAY 2014

11th-12th DECEMBER 2014 Hong Kong Science & Technology Parks



THE CHINESE UNIVERSITY OF HONGKONG

School of Biomedical Sciences
Postgraduate Research Day 2014

香港中文大學 生物醫學學院

CUSBS

11th - 12th December 2014

Faculty of Medicine
The Chinese University of Hong Kong

Welcome Message from the Director of School of Biomedical Sciences

I am most delighted to welcome you all to the *SBS Postgraduate Research Day 2014*, the annual flagship event of the School of Biomedical Sciences organized solely by and for our students. Stepping into its fifth year, the *Postgraduate Research Day 2014* continues to be a successful platform for our students to share their achievements with their peers and supervisors. Different from previous years, the *SBS Postgraduate Research Day 2014* will be held together with the Pan-Asian Biomedical Sciences Consortium Conference in the Charles K. Kao Auditorium at the Hong Kong Science and Technology Park. Researchers and students from a number of Southeast Asian universities in the consortium will join the Conference. This is a golden opportunity for our students to showcase their talents and achievements on an international platform. I am sure our guests will be impressed by the achievements of our students.

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

To provide the environment and training to facilitate our students to achieve research and academic excellence is not our only goal. We strive to provide a holistic education to our students such that they can achieve excellence in whatever career they decide to pursue after they leave the School. I believe the annual Postgraduate Research Day offers the best opportunity for our students to sharpen their other attributes such as leadership, devotion, organization and social skills. The value-added international platform of the Pan-Asian Biomedical Sciences Consortium Conference this year is certainly a very valuable opportunity for our students to interact with and learn from peers from overseas.

I am sure everyone who visits the *Postgraduate Research Day 2014* will, like myself, enjoy learning what our students have achieved. It is indeed a feast of the mind that no one should miss. I would like to take this opportunity to thank all individuals involved in planning, organizing, and coordinating this event, particularly members of the Organizing Committee of *SBS Postgraduate Research Day 2014* for their dedication, commitment and time that make the event a reality. I would also like to extend my heartfelt gratitude to the Graduate Education Office for its continued support to our graduate education and students.

On behalf of all staff of the School of Biomedical Sciences, I wish you all a very successful *Postgraduate Research Day 2014.*

Wai-Yee Chan, Ph.D.
Professor of Biomedical Sciences &
Director, School of Biomedical Sciences
November 2014

Members of the Organization Committee:

Mr. YANG Zhiyuan
Ms. ZHANG Xiaoting
Ms. HEI Lei
Mr. SONG Weicong
Ms. LIANG Shuang
Ms. ZHOU Xunian
Mr. MU Mingdao
Mr. NIU Yuze
Ms. QIAN Yan
Ms. SUN Yayi
Mr. SHI Mai
Mr. LIAN Wei
Ms. ZHAO Qiannan
Ms. CAO Dandan
Ms. MENG Chenling
Ms. LI Mingyue
Mr. LUO Xiaomin
Mr. WANG Zhangting
Ms. LI Jin

Advisers:

Professor CHAN Wood Yee Woody

Mr. WANG Yu Bing

Ms. Joresa NG

Special Acknowledgements:

Ms. CHAN Mei Tak Mandy

Ms. NG Sui Ching Nicole

Ms. LAU Liu Yin Yi Carmen

Acknowledgements

The organizing committee would like to thank the following professors for serving as adjudicators for poster and/or oral presentations:

Prof CHAN Man Lok Andrew
Prof CHAN Sun On
Prof CHAN Wai Yee
Prof CHAN Wood Yee
Prof CHEN Yangchao
Prof CHENG Hon Ki Christopher
Prof CHENG Sze Lok Alfred
Prof CHO Yu Pang Eric
Prof FENG Bo
Prof JIANG Xiaohua
Prof KE Ya
Prof LEE Ka Ho Kenneth
Prof LI Gang
Prof LU Gang
Prof NG Tzi Bun
Prof PONOMAREV Eugene D.
Prof RUDD John Anthony
Prof SHUM Sau Wun Alisa
Prof TSUI Kwok Wing Stephen
Prof WAN Chao
Prof XIA Yin
Prof YAO Xiao Qiang
Prof YU Siu Bun Sidney
Prof YUNG Wing Ho
Prof ZHAO Hui

The Program of SBS Postgraduate Research Day 2014

11th December 2014 (Thursday -Day 1)		
	Session 1 (Room A)	**Session 2(Room B)**
08:00-08:30	Registration	
08:30-09:00	Opening	
09:00-09:15	Photo-taking Session	
09:15-10:00	Keynote Lecture (Reproductive session)	
10:20-11:00	**Neuro-degeneration, development and Repair Session (N1-N4)-poster section 1**	**Vascular and Metabolic Biology/ Stem Cell and Regeneration Session (V1-V4)-poster section 2**
11:15-11:35	Tea Break	
11:40-13:15	**Neuro-degeneration, development and Repair Session (N5-N10)-poster section 1**	**Vascular and Metabolic Biology/ Stem Cell and Regeneration Session (S1-S9)-poster section 2**
13:20-14:30	Lunch	
14:35-15:20	Keynote Lecture (Stem cell)	
15:30-16:10	**Reproduction, Development and Endocrinology Session (R1-R6)-poster section 1**	**Cancer and Inflammation Session (C1-C6)-poster section 2**
16:10-16:30	Tea Break	
16:40-18:30	**Reproduction, Development and Endocrinology Session (R7-R19)-poster section 1**	**Cancer and Inflammation Session (C7-C19)-poster section 2**
18:30-19:30	Welcome Reception	
12th December 2014 (Friday -Day 2)		
08:30-13:05	Pan-Asia Biomedical Science Conference (Immunology Session)	
13:10-14:25	Lunch	
14:30-15:30	**Student Oral Presentation 1-4 (final match)**	
15:30-16:00	Tea Break	
16:00-17:30	**Student Oral Presentation 5-10 (final match)**	
17:30-18:00	**SBS Postgraduate Research Day 2014 Best Posters Presentation Ceremony**	

Index of Abstracts

Neuro-Degeneration, Development and Repair Theme

Title of poster presentation	Name	Abstract No.
Volume reconstruction for in vivo whole cell patch-clamp recordings	Danny Cheuk Wing CHAN	N1
Hepcidin ameliorates mitochondrial damage in a rat model of Parkinson's disease	Tuo LIANG	N2
Expression of Estrogen Receptors on Neurons in the Gastrointestinal Tract of the Mouse: a Potential Target For Flavonoids to Reduce Neurodegeneration	Yuen Hang LIU	N3
The Role of Nogo-A in Endotoxin-Induced Uveitis	Ding MA	N4
Platelets regulate neuroinflammation and cerebral hemorrhage during traumatic brain injury	Marina DUKHINOVA	N5
Growth hormone-releasing hormone signaling in inflammation of posterior segments of the eye in adult rats	Jialin REN	N6
The role of PGC-1a in higher-order brain function: synaptic plasticity and memory	Wei SU	N7
Perturbation of retinoic acid levels reduces nephron endowment in offspring of diabetic pregnancy	Tsz Kwan TAM	N8
Spargel rescues α-synuclein-induced disease phenotypes in a Drosophila model of Parkinson's disease	Ka Chun WU	N9
Restoration of motor function through dopamine replacement in rat model of focal ischemia	Leo Yik Chun YAN	N10

N1

Volume reconstruction for in vivo whole cell patch-clamp recordings

Danny Cheuk Wing CHAN, Ho KO, Wing Ho YUNG

Supervisor: Wing Ho YUNG

The in vivo application of the whole cell patch-clamp technique is fundamental to understanding the subthreshold computational dynamics of individual neurons in an ethologically relevant setting. Recordings of deep cortical and subcortical neurons typically employ a blind patch approach, in which spatial resolution is lost due to variability in pipette fabrication and mounting. We developed an automated tracking system that rapidly registers the micromanipulator position of each freshly mounted pipette relative to a fixed reference frame, prior to insertion into brain tissue. Biocytin labelling of recorded neurons and subsequent volume reconstruction enables us to match neuron morphology with recordings in a post-hoc fashion. Accurate localization of the pipette tip also enables us to approach a volume of interest in close proximity (~30μm), and derive local spatial relationships in microcircui t computation.

N2

Expression of estrogen receptors on neurons in the gastrointestinal tract of the mouse: a potential target for flavonoids to reduce neurodegeneration

Julia Yuen Hang LIU, Ge LIN, Ma Rong FANG, John A. RUDD

Supervisor: John A. RUDD

Alzheimer's disease (AD) is characterized by cognitive deficits and the deposition of beta-amyloid (βA) in the brain. The number of patients exhibiting the disease is increasing worldwide. βA contributes heavily to protein aggregates that form plaques and eventual neurodegeneration in brain. Recently, it has been hypothesized that βA could spread in a prion-like manner from the gastrointestinal (GI) tract to the central nervous system (CNS). If this hypothesis is correct, it may be possible to treat, or contain the progression of disease in the periphery, thus reducing neurodegeneration in the CNS and associated memory impairments.

The present investigations are concerned with developing a potential preventive strategy in the GI tract to reduce the development of AD. In this regard, Soy flavonoids, including genistein, have been widely studied for their ability to reduce βA levels and improve cognition in animal studies and post-menopausal women. It has been hypothesized that the potential mechanism is related to an action to reduce oxidative stress. Recently, it has been proposed that flavonoids may act at estrogen receptors (ER) to induce neurotrophic factor expression in the brain to prevent neurodegeneration in AD. It is not known if similar protective mechanisms exist in the GI tract.

In preliminary experiments, we used immunohistochemistry to examine the distribution and location of ER α/β, and the novel G-protein coupled estrogen receptor, GPR30 or GPER, as well as NGF receptors TrkA and p75 in the enteric nervous system of female adult ICR mice (20-25g, aged 2 months). We also profiled the flavonoid composition of a commercially available soy product. Genistin and diadzin were found in relatively high concentrations in the soy product tested. Enteric neurons of the ileum and colon expressed ERα immunoreactivity to a similar level their cytoplasm and nuclei, while ERβ and GPR30 immunoreactivity was found at higher levels in the cytoplasm relative to the nuclei. p75 neurotrophic receptor and tropomyosin receptor kinase A (TrkA) immunoreactivity were found in both enteric neurons and GFAP-expressing glial cells. In which TrkA expression was found constantly high, while p75 expression varied. We conclude that estrogen receptors exist in enteric nerves. The estrogen receptors may be a target for flavonoids to reduce neurodegeneration, although this requires further investigation.

N3

The role of Nogo-A in endotoxin-induced uveitis

Ding MA, Sun On CHAN

Supervisor: Sun On CHAN

Uveitis, an inflammation of the ocular tissues, is responsible for approximately 10%-20% of the blindness. The treatment of uveitis is mainly focus on suppressing inflammation and achieving regression when it occurs. Recent studies indicate that Nogo receptor (NgR) can modulate macrophage responses during inflammation after peripheral nerve injury. The present work aims to investigate whether there is an anti-inflammatory effects of Nogo in endotoxin-induced uveitis (EIU).

EIU was generated in mice by a footpad injection of Lipopolysaccharide (LPS, 10 mg/kg). Both wild type and Nogo-A knock-out mice were used in this study. Twenty four hours after LPS injection, the mice were anesthetized for collections of ocular tissues.

Result showed that there was an up-regulation of pro-inflammatory genes in the retina after LPS injection and an increase of total protein concentration in aqueous humor. However, the total protein concentration and the expression level of pro-inflammatory genes were decreased in Nogo-A knock-out mice when compared with LPS treated wild type mice. The preliminary results suggest that Nogo-A might mediate the anti-inflammatory effect in EIU. Further experiment will be done to investigate the level of pro-inflammatory factors in the serum and the amount and type of infiltrating cells in different treated groups, also the signaling pathway involved in the inflammatory responses induced by LPS.

N4

Platelets regulate neuroinflammation and cerebral hemorrhage during traumatic brain injury

Marina Dukhinova, A.YUNG, T. Veremeyko, Eugene D.Ponomarev

Supervisor: Eugene D.Ponomarev

Inflammation in the central nervous system (CNS) accompanies many neurological disorders such as traumatic brain injury (TBI), stroke or Alzheimer's disease. Although, there are no effective strategies for prevention and treatment of neuroinflammation as well as cerebral hemorrhage, both of which are common complications after TBI or stroke. Platelets are first responders that come on the scene of vascular injury but their role in regulation of inflammation in the CNS is not well understood. We have previously found that sialated gangliosides within neuronal and astroglial lipid rafts were specifically recognized by platelets and this recognition resulted in platelet activation and degranulation. In the CNS, interaction of platelets with lipid rafts occurs after blood-brain barrier disruption initiated by TBI. In our study we compared inflammatory response in the CNS of wild type (WT) vs. St3 gal V deficient (ST3-/-) mice that lack of major brain-specific sialated gangliosides. We found that after TBI, ST3-/- exhibited substantially lower level of CNS inflammation as determined by microglia activation and leukocyte infiltration (16±2% vs. 5±1% of macrophages and 7±1% vs. 2 ±1% of lymphocytes for WT vs. ST3 -/- mice on d2 after TBI). At the same time, we found that ST3-/ mice had enlarged hemorrhagic lesion at the site of TBI, suggesting that interaction of platelets with brain lipid rafts contributed to neuroinflammation and prevented excessive bleeding. Thus our study demonstrated that interaction of platelets with brain-specific ganagliosides increased inflammation and reduced hemorrhage. This indicates that platelet-mediated inflammatory response may have beneficial effect to prevent excessive bleeding and restrict area of damage in the CNS after injury.

N5

Growth hormone-releasing hormone signaling in inflammation of posterior segments of the eye in adult rats

Jialin REN, Sun On CHAN

Supervisor: Sun On CHAN

Growth hormone-releasing hormone (GHRH) signaling is important in regulation of cell growth and proliferation. This signaling pathway involved production of growth hormone (GH) and insulin-like growth factor 1 (IGF-1). Recently, GHRH has been shown to decrease the expression of inflammatory markers in both Alzheimer's disease and chronic prostatic inflammation. Previous data in our lab also showed that GHRH antagonist is a potent anti-inflammatory agent in endotoxin-induced anterior uveitis. However, whether blocking GHRH signaling is effective in alleviating inflammation in the retina and vitreous remains undetermined. Our preliminary findings have shown that inflammation in posterior segments of the eye could be induced by lipopolysaccharide (LPS), with adherence of recruited leukocytes to the retinal vascular endothelium and breakdown of the blood-retinal barrier. In the current study, retinal inflammation was induced in adult SD rats by a footpad injection of LPS. We hypothesize that blocking GHRH signaling with potent antagonist can inhibit the inflammatory responses in the retina and the vitreous. The number of infiltrated inflammatory cells and accumulation of secreted protein in the vitreous will be examined 48 hours after injection. To assess inflammation in the retina, we will examine the number and distribution of activated microglia in the retina after LPS insult with or without GHRH antagonist treatment. Expression of inflammatory markers such as TNF-α, IL-1β and IL-6 will be detected by real time-PCR. The expression of GHRH and its signaling molecules will also be explored with RT-PCR and Western blotting. These findings will demonstrate whether GHRH-GH-IGF1 signaling is involved in retinal inflammation and GHRH antagonist is a potential therapeutic agent for treatment of retinal inflammation.

N6

The role of PGC-1a in higher-order brain function: synaptic plasticity and memory

Wei SU, Linhao XU, Na LU, Danny CHAN, Ya KE, Wing Ho YUNG

Supervisor: Wing Ho YUNG

PGC-1α (peroxisome proliferator-activated receptor gamma coactivator 1-alpha) was firstly identified as transcriptional coactivator of mitochondrial biogenesis and oxidative metabolism in adipose tissue. Subsequent researches demonstrated its complex role in cardiac function angiogenesis, muscle atrophy. Recently, PGC-1a has been proved to have important functions in brain. Loss of PGC-1α in brain causes neurodegeneration in striatum. GABAergic neurons dysfunction and deficiency in parvalbumin is associated with knockout of PGC-1α. Long-time treadmill running would increase the PGC-1α expression in other brain areas. BDNF is induced in hippocampus via PGC-1α/FNDC5 pathway by exercise. In addition, PGC-1α is involved in spine formation and maintenance as well.

Synaptic plasticity kind of modifies synapse and emphasizes its importance in memory and learning process. LTP (long term potentiation) is a form of plasticity in brain and multiple factors participate in its induction. Previous works showed that BDNF signaling and dendritic spines stability was reported in LTP expression. Based on published results, the involvement of PGC-1α remains unknown. Our research purpose is to interpret novel function of PGC-1α in cognition. Given the absence of PGC-1a-specific inhibitor or genetic animal model that provides hippocampus-specific manipulation of PGC-1a, we will develop an AAV shRNA system to manipulate PGC-1a spatiotemporally in hippocampal CA1 region which regulates memory. These findings may suggest the potential role of PGC-1a as a therapeutic target for neurodegenerative diseases.

N7

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

Selina Tsz Kwan TAM, Leo MY LEE, Alisa Sau Wun SHUM

Supervisor: Alisa Sau Wun SHUM

It is well documented that 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 maternal circulation. In humans and in streptozotocin-induced mouse model with diabetes, there is a significant reduction in plasma vitamin A levels. We therefore hypothesize that in maternal diabetes, there is a reduction in RA synthesis and thus a lowering of RA concentrations in the developing kidney of the embryo, which perturbs the expression of developmentally important genes and adversely affects nephrogenesis, thereby reducing nephron mass in the kidney and predisposes the offspring to chronic kidney disease and hypertension after birth.

To investigate our hypothesis, first, we examined the expression of Raldh2, which encodes the key RA synthesizing enzyme, by real-time quantitative RT-PCR. We found that the mRNA level of Raldh2 was significantly reduced in the developing kidney of embryos of diabetic mice in comparison to that of the non-diabetic control. Concomitantly, there was a significant decrease in the concentration of RA, measured using a highly sensitive RA-reporter cell line, in the developing kidney of the diabetic group. This was accompanied by prominent down-regulation of RA-responsive genes Gdnf and c-Ret, which control nephron formation. At birth, the total number of nephrons per kidney in the neonates of diabetic mice was significantly less than that of the control. To conclude, our current findings are in agreement with our hypothesis. Further study will be conducted to determine if there is any causal relationship between a reduction of RA levels in the developing kidney of embryos being exposed to diabetes in utero and a deficit in nephron endowment.

N8

Spargel rescues α-synuclein-induced disease phenotypes in a Drosophila model of Parkinson's disease

Ka Chun WU, Wing Ho YUNG, Ya KE

Supervisor: Ya KE

Parkinson's disease (PD), which is caused by loss of dopaminergic neurons in the substantia nigra, is one of the most common neurodegenerative diseases affecting millions of people worldwide. Abnormal α-synuclein aggregation is the central hallmark of PD and many studies have already demonstrated the deleterious effects of oligomeric and aggregated forms of α-synuclein to dopaminergic neurons. Overexpression of peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), the master regulator of mitochondrial biogenesis and cellular energy metabolism downregulated in PD, has resulted in contradictory outcomes in neurotoxin-induced PD models. In this study, we overexpressed spargel, the PGC-1α fly homolog, in the well-established A53T α-synuclein fly model to examine how spargel modulates α-synuclein toxicity and the underlying mechanisms. Our data indicated that spargel overexpression did not affect the reduced survival rate of A53T α-synuclein flies but improved motor deficits 30 days post-eclosion. Whole-mount immunohistochemistry results indicated that dopaminergic neurons in PPL1 and PPM3 cluster were rescued in 30 day-old A53T α-synuclein flies. To investigate the protective mechanism of spargel against α-synuclein, we first checked whether spargel overexpression could modify α-synuclein level and solubility. Our western blot results showed that spargel overexpression did not significantly affect SDS-soluble α-synuclein level when compared with age-matched A53T α-synuclein flies, but reduced urea-soluble forms of α-synuclein, which may represent the toxic insoluble forms of α-synuclein aggregates. To conclude, our findings support that manipulation of endogenous PGC-1α in dopaminergic neurons, which is possible via a number of FDA-approved drugs, can be a promising and readily translatable treatment strategy against α-synuclein-induced toxicity in PD.

N9

Restoration of motor function through dopamine replacement in rat model of focal ischemia

Leo Yik Chun YAN, Q. Li, Wing Ho YUNG, Ya KE

Supervisor: Ya KE

Background and purpose: Stroke is a major cause of disability, yet there is no pharmacological therapy available to promote recovery after stroke. Dopamine (DA) is a principal neurotransmitter that mediates brain plasticity and motor learning. However understanding is lacking regarding its status after stroke. Here we investigate the impact of ischemia stroke on the dopaminergic system and the effects of therapeutic replacement on motor recovery.

Materials and Methods:Two-months old SD rats received vehicle or L-DOPA 15 mg/kg daily (i.p.) for 3 weeks following focal ischemic stroke in the cortex. The rats underwent motor behavior evaluation by means of open field test, rotarod performance test, horizontal ladder test and limb use asymmetry assessment. Cresyl violet, triphenyltetrazolium chloride (TTC) and immunohistological staining of tyrosine-hydroxylase (TH) were performed to compare the difference in stroke volume and the integrity of dopaminergic projection in the motor cortex pre- and post-ischemic injury.

Results: Stroke volume analysis demonstrates infarct area was confined to the cortex and infarct core was centered on M1 in our model. TH-positive terminals were markedly reduced in the affected hemisphere. When compared with control, L-DOPA-treated animals displayed fewer errors in horizontal ladder test throughout all testing sessions post-stroke. The L-DOPA-treated animals also exhibited improved performance in rotarod test, indicating an improved limb placing ability, balance and inter-limb coordination. In addition, stroke animals exhibited significant asymmetric limb use which persisted throughout the testing period post-stroke. However, L-DOPA-treated animals showed partial restoration of symmetric limb-use.

Conclusions: In summary, an early 3-week daily treatment of L-DOPA can substantially improve motor performance in rat model of cortical stroke, suggesting that dopamine replacement can enhance functional recovery by restoring cortical DA level.

N10

Hepcidin ameliorates mitochondrial damage in a rat model of Parkinson's disease

Tuo LIANG, Zhong-Ming QIAN, Wing-Ho YUNG, Ya KE

Supervisor: Ya KE

Mitochondrial dysfunction is believed to be involved in the pathogenesis of Parkinson's disease (PD). Iron accumulation, a common feature in neurodegenerative disease, may underlie mitochondrial dysfunction in PD. Hepcidin, the main iron regulatory peptide, is widely expressed in the brain. Here we propose that over-expression of hepcidin is beneficial in ameliorating mitochondrial dysfunction in PD. To address this question, we made use of the rotenone injection model in rat, which is a well-established chronic model of PD that is known to affect mitochondrial functions. Adult SD rats received daily intraperitoneal injection of rotenone for 45 days while control animals received vehicle injection. At day 5, rotenone-treated rats received intracerebroventricular injection of either an adenovirus carrying the hepcidin gene (Ad-hepcidin), a blank adenovirus (Ad-blank) or saline. At day 46, the rats were sacrificed for assay of mitochondrial functions. The morphology of the mitochondria was observed by transmission electron microscopy (TEM) and the mitochondria iron content was measured by graphite furnace atomic absorption. We found that rotenone suppressed complex I activity in the mitochondria isolated from the brain, which could be rescued by Ad-hepcidin treatment. Ad-hepcidin also down-regulated the iron content in the isolated mitochondria. Within the substantia nigra (SN), TEM revealed abnormal morphology of the mitochondria, which were characterized by the loss of the cristae. Rotenone also reduced the activity of complex I activity, ATP production and depleted GSH in SN, leading to the rise of reactive oxygen species. All these effects were rectified by Ad-hepcidin but not AD-blank treatment. These results suggest that overexpression of hepcidin can ameliorate mitochondrial dysfunction typically found in Parkinsonism. The protective mechanism of hepcidin and its potential in the treatment of PD warrant further exploration.

Vascular and Metabolic Biology Theme

Title of poster presentation	Name	Abstract No.
Inhibition of miRNA92a prevents endothelial dysfunction induced by high glucose	Lingshan GOU	V1
Molecular Mechanism for Bone Morphogenic Protein-4-induced Up-regulation of Platelet-Derived Growth Factor in Endothelial Cells	Weining HU	V2
Differential mechanisms for insulin-induced relaxations in mouse posterior tibial arteries and main mesenteric arteries	Dan QU	V3
Enhanced autophagic flux improves endothelial function in diabetic mice	Lei ZHAO	V4

V1

Inhibition of miRNA92a prevents endothelial dysfunction induced by high glucose

Lingshan GOU

Supervisor: Yu HUANG

Upregulation of miRNA-92a expression impairs endothelialization and contributes to the neotintima formation after vascular injury. miRNA-92a also decreases the expression of SIRT1, which is involved in the regulation of vascular function. SIRT1 stimulates endothelial nitric oxide synthase (eNOS) activity through deacetylating eNOS, thereby increases endothelial nitric oxide (NO). Moreover, high glucose exposure reduces SIRT1 activity and thereby contributes to the development of diabetic vasculopathy. My preliminary study shows that the miRNA-92a level was up-regulated in the aortas of diabetic db/db mice. miRNA-92a antagomir down-regulates the expression of miRNA-92a in human umbilical vein endothelial cells (HUVECs) in which the mRNA expression of both SIRT1 and eNOS was elevated by miRNA92a antagomir. Therefore, I propose that inhibition of miRNA-92a might be able to improve endothelial function that is impaired under hyperglycemic conditions through up-regulating the expression of SIRT1 and eNOS. I will study how whether miRNA-92a antagomir may protect endothelial cells against high glucose insult to preserve endothelial function in diabetes in coming months.

V2

Molecular mechanism for bone morphogenic protein-4-induced up-regulation of platelet-derived growth factor in endothelial cells

Weining HU, Yu HUANG

Supervisor: Yu HUANG

Bone morphogenetic protein 4 (BMP4) stimulates superoxide anion production and exerts pro-inflammatory effects in blood vessels. However, the underlying mechanisms by which BMP4 impairs endothelial dysfunction remain partly understood. The platelet-derived growth factors (PDGFs) and their receptors (PDGFRs) are major angiogenic regulators and are involved in the development of arteriosclerosis, hypertension and diabetes, although their relations to BMP4 signaling are unclear. The present results show that BMP4 and PDGFAA are elevated in the serum of both diabetic patients and diabetic db/db mice as compared with non-diabetic subjects and db/m+ mice. In vitro treatment with BMP4 (10-40 ng/mL) up-regulates the expression of PDGFAA and PDGFR in human umbilical vein endothelial cells (HUVECs). Both effects are reversed by the BMP4 antagonist noggin, ROS scavengers, tiron plus DETCA, and c-Jun N-terminal kinesis (JNK) inhibitor SP600125. Inhibition of ROS and JNK reduces BMP4-induced increase of SMAD 1/5 phosphorylation while ROS enhance JNK phosphorylation. Furthermore, treatment with PDGFAA attenuates acetylcholine-induced endothelium-dependent relaxations (EDR) in aortas and flow-mediated vasodilatation (FMD) in the second order of mouse resistance mesentery arteries of C57BL/6 mice. PDGFAA neutralizing antibody can improve EDR in BMP4 treated or db/db mouse aortas. The present study provides novel information regarding mechanisms underlying BMP4-mediated harmful effects in blood vessels. Since BMP4 is an important mediator of endothelial dysfunction in diabetes and PDGFAA neutralizing antibody can improve BMP4 induced endothelial dysfunction, reducing PDGFAA could be another useful strategy for the treatment of diabetic vasculopathy.

V3

Differential mechanisms for insulin-induced relaxations in mouse posterior tibial arteries and main mesenteric arteries

Dan QU, Jian LIU, Chi Wai LAU, Yu HUANG

Supervisor: Yu HUANG

The characteristics of endothelium-dependent relaxations in response to insulin and acetylcholine (ACh) in the mouse posterior tibial artery (PTA) were studied on wire myograph, and compared to those in the mouse main mesenteric artery (MMA). Insulin-induced relaxation in PTA was reversed by PI3K and Akt inhibitors, LY294002 and triciribine, but not by nitric oxide synthase inhibitor, NG-nitro-L-arginine methyl ester (L-NAME) or guanylate cyclase inhibitor, ODQ. The relaxation in PTA was also inhibited by apamin (small-conductance Ca2+-activated K+ channel blocker) plus charybdotoxin (intermediate-conductance Ca2+-activated K+ channel blocker), elevated KCl or ouabaine (Na+-K+ ATPase inhibitor) plus BaCl2 [inwardly rectifying K+ (KIR) channel inhibitor]; whereas L-NAME but not triciribine inhibited ACh-induced relaxation in PTA. On the other hand, nitric oxide and endothelium-derived hyperpolarizing factor albeit to a less extent mediated both insulin- and ACh-induced relaxations in MMA. The present study is for the first time dissecting out the components of endothelium-dependent relaxation in mouse PTA and suggesting differential responses to different agonists in distinctive blood vessels.

V4

Enhanced autophagic flux improves endothelial function in diabetic mice

Lei ZHAO, Liu Jian, Wang Li, Luo Jiangyun, Lau Chiwai, Yu HUANG

Supervisor: Yu HUANG

Autophagy, also called cellular self-digestion, is a lysosomal catabolic response leading to degradation and recycling of intracellular macromolecules and organelles in mammalian cells. Autophagy plays a critical role in removing protein aggregates, damaged organelles, adapting to stress, and maintaining intracellular homeostasis. In endothelial cells, impaired autophagy leads to oxidant stress and decreased nitric oxide bioavailability. The present study aims to investigate the role of autophagy in endothelial dysfunction in diabetic mice. The levels of both P62 and conversion of LC3-I to LC3-II are elevated in aortas from diabetic db/db mice as compared with those from non-diabetic db/m+ mice. The LC3 turnover assay also shows that the autophagic flux is impaired in db/db mouse aortas. Treatment of db/m+ mouse aortas with autophagy inhibitor 3-MA, results in attenuated acetylcholine-induced endothelium-dependent relaxations (EDRs). MitoSOX and dihydroethidium staining show that autophagy inhibitors chloroquine and bafilomycin A1 increase the production of mitochondrial reactive oxygen species (ROS) in human umbilical vein endothelial cells, indicating that impaired autophagy may contribute to endothelial dysfunction in the aortas of db/db mice through elevating mitochondrial ROS. On the other hand, treatment with autophagy activator rapamycin improves EDRs and reduced ROS generation in the aortas of db/db mice and this vaso-protective effect is reversed by co-treatment with chloroquine. The present results suggest that impairment of autophagy participates in endothelial dysfunction of diabetic mice. Increasing autophagic flux by rapamycin reduces mitochondria ROS generation and thus improves endothelial function, suggesting that the restoration of autophagy may provide a novel therapeutic strategy for treating diabetic vascular vasculopathy.

Stem Cell and Regeneration Theme

Title of poster presentation	Name	Abstract No.
Yap1 plays a negative role in endochondral bone formation by inhibiting chondrocytes maturation	Yujie DENG	S1
Integrative analysis of transcriptomic expression profiles and pathways in postnatal mouse heart	Jingyi GAN	S2
Introduction of Reporter Genes by CRISPR/Cas induced Homologous Recombination in human cells	Xiangjun HE	S3
Revealing Interactors of Nuclear Factor NR5A2	Kai Chuen LEE	S4
Dual functions of Epimedium extract in osteoblastogenesis and osteoclastogenesis	Li LU	S5
Functions of BRE gene in fibroblasts	Wenting SHI	S6
Bioengineered scaffold applied small molecule HIF-α activator enhances articular cartilage regeneration	Yinglan SHU	S7
Hedgehog signaling is required for tendon development and remodeling	Yi WANG	S8
BRE gene facilitates skeletal muscle regeneration by promoting satellite cell motility and fusion	Lihai XIAO	S9

S1

Yap1 plays a negative role in endochondral bone formation by inhibiting chondrocytes maturation

Yujie DENG, Kingston King Lun Mak

Supervisor: Kingston King Lun Mak

Hippo pathway is an evolutionally conserved signaling pathway involved in regulating cell proliferation and organ size control. Here we show the involvement of Yes-associated protein 1 (Yap1), which is the key factor of Hippo pathway, in chondrocyte differentiation. Yap1 was strongly expressed in chondro-progenitor cells but its expression was greatly reduced with chondrocytes differentiation and maturation. Overexpression of Yap1 in limb bud-derived mesenchymal like chondro-progenitor cells attenuated chondrocyte differentiation and maturation by inhibiting the expression of Col2a1, Aggrecan and Col10a1. Interestingly, in collagen type Ⅱ alpha 1 promoter controlled Yap1 (Col2a1-Yap1) transgenic mice, Yap1 overexpression inhibited chondrocyte hypertrophy and calcification in growth plate through downregulating Col10a1 and MMP13 expression. Furthermore, Yap1 transgenic mice exhibited delayed bone formation after birth, which was related with impaired chondrocyte terminal differentiation in vivo. Besides, we demonstrate that Yap1 governs the initiation of fracture repair by regulating cartilage maturation. Yap1 transgenic mice showed severely impaired cartilaginous callus formation after fracture injury. Mechanistically, we proved that Yap1 inhibits chondrocyte maturation and impairs endochondral ossification through directly targeting Col10a1. Taken together, our results suggest that Yap1 acts as a negative regulator of chondrocytes maturation in vitro and in vivo. The inhibitory effects of Yap1 on chondrocytes maturation and endochondral bone formation may be useful for developing a therapeutic strategy for cartilage diseases.

S2

Integrative analysis of transcriptomic expression profiles and pathways in postnatal mouse heart

Jingyi GAN, Kenneth Ka Ho Lee

Supervisor: Kenneth Ka Ho Lee

Background: Cardiomyocytes rapidly proliferate during fetal stage and first days of life but vast majority of them exit cell cycle soon after birth in mammals. However, the detailed molecular mechanism remains to be fully elucidated. To achieve this goal, integrative analysis were used in present study to compare the transcriptomic profile of 13-day-old heart with 2 day-old heart.

Results: The transcriptomic profilling results in 44,171 transcripts were differentially expressed in 13-day-old mice heart versus 2-day-old mice heart. PCA mapping shows a clear separation of heart samples into the two groups. Of these transcripts, 3273 (7.41%) differentially expressed genes (DEGs) had significant change with a fold change cutoff of 1.5 and FDR<0.05. Of these, 1,768 were up-regulated and 1,505 were down-regulated. Functional annotation and gene ontology enrichment analysis shows cell cycle are the most significant downregulated term among the top ten downregulated gene ontology categories, immune response are the most significant upregulated term among the top ten upregulated gene ontology categories. The top 1 KEGG pathways with most representations by the DEGs were cell cycle ($p = 9.31 \times 10^{-9}$). The top 1 associated networks identified were Cardiac Hypertrophy, Cardiovascular Disease and Developmental Disorder. IPA analysis revealed four central node, MYH7, NPPA, NPPB and ACTA1, two core gene GATA4 and IGF1R. Transcriptional regulation of these DEGs was validated by quantitative real-time PCR.

Conclusion: The integrative analysis illustrates that, apart from immune response, down-regulation of cell cycle pathways at the transcriptional level is most important molecular basis underlying the decreased proliferation capacity from 2-day-old to 13-day-old mice heart. Moreover, the four central node, Myh7, Nppa, Nppb and Acta1, the two core gene Gata4 and IGF1R in associated interaction network were identified. This study provides further insights to understand molecular mechanisms behind cell-cycle arrest and cardiac progenitors differentiation in the late stage of postnatal heart. The presumptive targets in our study may be an important component of cardiomyocyte proliferation- or reprogramming- based therapy.

S3

Introduction of reporter genes by CRISPR/Cas induced homologous recombination in human cells

Xiangjun HE, Bo FENG

Supervisor: Bo FENG

Targeted genome editing of embryonic stem cells (ESCs) is one of the prerequisite for exploiting their potential. Such manipulation could be achieved through Cas9 system, a new genome editing tool derived from prokaryotic immune system. However, large fragment recombination mediated by this system for construction of fluorescence-labelled ESC reporter lines, although highly desired, is not achieved. Here, we show that this Cas9 system could be successfully used for establishing HEK293T cell lines with eGFP precisely tagged to endogenous GAPDH and OCT4 for indicating their expression. With this system, we intend to construct stable human ESC lines with fluorescence-labelled lineage markers and pluripotent genes. These modified cells lines will be valuable for understanding of human ESCs and their differentiation as well as optimizing the lineage-specific differentiation protocols.

S4

Revealing interactors of nuclear factor NR5A2

Kai Chuen LEE, Bo FENG

Supervisor: Bo FENG

Nr5a2 (nuclear receptor subfamily 5, group A, member 2/liver receptor homologue-1, Lrh-1) exhibits broad expression during the morula and epiblast stages of development. Genetic ablation of Nr5a2 resulted in embryonic lethality around E6.5–E7. Nr5a2 can promote efficiency of somatic cell reprogramming and replace the role of Oct4 during reprogramming. A later study finds that Nr5a2 can also drive epiblast stem cells (EpiSCs) to ground state pluripotency similar to mouse ESC. These findings showed that Nr5a2 has important role in pluripotency. It has been known that Nr5a2 regulates Oct4 by binding to its proximal promoter and upstream proximal enhancer. ChIP analysis has found that Nr5a2 also regulates Nanog as it binds to the Nanog enhancer and regulates its expression. However, how the Nr5a2 works in protein level is less clear. Being a nuclear receptor, NR5A2 is expected to have protein coactivators. Here, we constructed the expression vector of Flag tagged NR5A2 and transfect it into HEK293T cell line, then we applied the affinity-purification coupled to mass spectrometry techniques to identify interactors of NR5A2. Several candidate interactors have been identified, and the interaction of NR5A2 and PROTEIN L has been confirmed by co-Immunoprecipitation.

S5

Dual functions of Epimedium extract in osteoblastogenesis and osteoclastogenesis

Li LU, Fengjie ZHANG, Wingpui TSANG, Qingnan LI, Ziyin SHEN, Chao WAN

Supervisor: Chao WAN

Epimedium extract (EPE) is a flavone component extracted from Epimedium, a commonly prescribed herbal medicine for the treatment of osteoporosis, while the underling mechanisms of its pharmacological effects remain unclear. Here, we examined the roles of EPE in regulation of osteoblastic and osteoclastic differentiation and functions. Primary osteoblasts and osteoclasts were cultured from bone chips and bone marrow mononuclear cells of 4-week old C57bl mice respectively. The effects of EPE on osteoblastic and osteoclastic differentiation were examined. We found that EPE promotes osteoblast differentiation and bone formation activity indexed by increased alkaline phosphatase (ALP) activity, mineralized nodule formation of the extracellular matrix and upregulated osteogenic maker genes expression including ALP, osteopontin, osteocalcin, and osteoprotegerin. EPE enhanced bone acquisition in the calvaria organ culture as indicated by increased thickness and osteoblast numbers of the calvariae compared with the controls. Signaling study showed that EPE elevated the phosphorylation states of Smad1/5/8 in osteoblast, while this effect was suppressed by Noggin, the BMP antagonist. This suggests that EPE may act through the activation of BMP/Smad signaling to promote osteoblast differentiation. In the osteoclast differentiation model, EPE was shown to inhibit osteoclast differentiation and maturation manifested by significantly decreased numbers of tartrate-resistant acid phosphatase positive multi-nucleated cells, disruption of actin-ring, and eliminated resorptive activity compared with the controls. These effects were not associated with activation of Smad1/5/8, indicating a differential mode of signaling mechanism to that observed in osteoblast. Our results suggest that EPE promotes osteoblast differentiation and bone formation, at least partially, mediated by the BMP/Smad1 signaling, while inhibits osteoclast differentiation and function in a differential mechanism. EPE may serve as a potential therapeutic agent for the treatment of metabolic bone diseases through dual roles in regulating osteoblastogenesis and osteoclatogenesis that awaits further investigations.

S6

Functions of BRE gene in fibroblasts

Wenting SHI, Mei Kuen Tang, Kenneth Ka Ho Lee

Supervisor: Kenneth Ka Ho Lee

The BRE gene, also known as BRCC45 and TNFRSF1A modulator, produces a 44KD protein that is normally distributed in both cytoplasm and nucleus. In the nucleus, BRE has been identified as a member of the BRCA1-A complex which is involved in DNA repair. It functions as an adaptor in the complex and facilities the recruitment of components of the BRCA1-A complex to the site of DNA damage. In the cytoplasm, BRE acts as a death receptor associated anti-apoptotic protein, by binding with the cytoplasmic domains of receptors, TNF-R1 and FAS.

In this study, we use adult fibroblasts isolated from wild-type and BRE-/- mutant mice as a cell model to further investigate the functions of BRE – since the mutant did not produce any phenotype. Compare with wild-type fibroblasts at the same cell passage, BRE-/- cells displayed a premature senescence phenotype which included cell cycle arrest, a flatten cell morphology, positive senescence-associated β-galactosidase staining and an elevated spontaneous production of γ-H2AX foci. This suggests that the absence of BRE will accelerate cellular senescence. As DNA damage is a major reason for cells to commit to cellular senescence, we postulated that the phenomenon is attributed to increased DNA damage and accumulation which could not be effectively repaired during as cells are propagated and gets older. In order to verify our hypothesis that DNA repair is deficient without BRE, wild-type and BRE-/- fibroblasts were exposed to Gamma-irradiation to induce DNA damage. We examined the presence of γ-H2AX, which is the phosphorylated form of H2AX, and it is a marker for DNA damage. Our results demonstrated that IR-induced γ-H2AX foci persisted significantly longer in BRE-/- fibroblasts than compared with wild-type fibroblasts. This suggests that BRE plays an important role of BRE in DNA repair. In addition, the expression of DNA damage responsive BRCA1-RAP80 complex was also significantly down-regulated in the BRE-/- fibroblasts, which suggests that the BRCA1 complex cannot efficiently form without the involvement of the BRE adaptor protein.

S7

Bioengineered scaffold applied small molecule HIF-α activator enhances articular cartilage regeneration

Yinglan SHU, Fengjie ZHANG, He Q, Wu Z, Qiu G, Zhou G, Chan WY, Chao WAN

Supervisor: Chao WAN

Articular cartilage, a highly organized avascular connective tissue with substantial durability, has a limited intrinsic repair capacity following trauma or degenerative pathology. Hypoxia is a hallmark for articular cartilage development and regeneration and functions as a stimulus for initiation of gene programs regulating stem cell/progenitor proliferation, differentiation and metabolism. The reparative stem cells or chondrocytes are readily located in a hypoxic microenvironment during cartilage repair. Hypoxia inducible factor-alpha (HIF-α) is the key transcription factor to response to the oxygen fluctuations of the cells during development and repair. However, the role of HIF-α pathway in articular cartilage repair or regeneration remains unclear. In this study, based on a cellular screening assay of hypoxia response element activity, deferoxamine (DFO) was identified as an HIF-α activator. The effects of DFO on the function of chondrocytes and mesenchymal stem cells (MSCs) were systemically examined in vitro and in vivo. Our results showed that DFO increased the proliferation of primary chondrocytes indicated by MTT and BrdU incorporation assays, and increased colony forming efficiency of chondroprogenitor cells. DFO promoted chondrogenic differentiation indexed by upregulation of chondrogenic marker genes expression and increased Alcian Blue staining for proteoglycan synthesis in the chondrogenic micromass cultures. In the three dimension (3D) bioscaffold culture, the expression of Sox 9 and collagen type Ⅱ was upregulated when treated with DFO (50μM) which was comparable to that under hy poxia. This was accompanied by upregulation of mRNA and protein expression of HIF-1α. In a transwell migration assay, DFO increased the total numbers of the migrating cells compared with the controls, while deletion of HIF-1α eliminated the effects of DFO. This was associated with the changes of genes regulating cell adhesion and migration. Next, we generated a 3D bioscaffold incorporated with DFO. The 3D complexes were transplanted in a mouse osteochondral defect model. Histological scoring indicated that the 3D complexes containing DFO significantly increased articular cartilage repair. Quantitation showed increased numbers of proliferating cell nuclear antigen positive cells and Sox9 positive cells in the newly formed cartilage compared with the controls. The tracing of the migration and fate of GFP+ MSCs following transplantation showed that 3D complexes containing DFO recruited more GFP+ Sox9+ MSCs in the defect region at day 14 compared with the controls. The results provide proof of principle that activation of HIF-1α enhances articular cartilage regeneration through coordinating MSCs migration, chondrogenic differentiation and functional engraftment.

S8

Hedgehog signaling is required for tendon development and remodeling

Yi WANG, MAK King Lun, Kingston King Lun MAK

Supervisor: Kingston King Lun MAK

"Tendon surrounding tissues such as tendon sheath and peritenon have a very close functional relationship with tendon fiber. However, their roles in tendon development and remodeling remain largely unclear. Previous reports and our data show that core components of Hh pathway are expressed mainly in the bone-tendon junctions and tissue surrounding tendons in young mice, which suggests a role of this pathway in regulating tendon maturation and remodeling. Here, we investigate its function in vivo by generation of genetically engineered mouse models targeting both Smoothened (Smo) & Patched (Ptch1) receptors respectively, to manipulate Hh signaling in bone-tendon junctions and tendon surrounding tissues.

In the Smo mutant mice where Hh signaling is inhibited, tendons were thickened with significantly increased collagen fibril diameters and collagen fibril number, as well as larger tendon bundles. Stem marker Nanog was downregulated, while Tendon progenitor marker Scx was upregulated in the tendon surrounding tissues. When Ptch1 is knocked out in which Hh signaling is upregulated, the Ptch1 mutant mice showed mirror phenotypes as compared to that of the Smo mutant mice. Consistently, in primary tendon sheath cells with Hh agonists and antagonists revealed similar regulatory pattern for Nanog and Scx expression. In addition, we performed tendon injury experiments and showed that Ptch1 was activated in tendon sheath as revealed by x-gal lineage tracing 7 days after injury. Nanog was downregulated and Scx expression was unregulated which promotes tendon repair.

Taken together, our data suggested that Hh signaling plays important roles in tendon maturation and remodeling. We will further investigate the underlying molecular mechanism how these factors contributed to tendon regeneration and repair."

S9

BRE gene facilitates skeletal muscle regeneration by promoting satellite cell motility and fusion

Lihai XIAO, Mei Kuen Tang, Kenneth Ka Ho Lee

Supervisor: Kenneth Ka Ho Lee

"BRE (Brain and Reproductive organ-Expressed) gene transcribes a highly conserved protein which is expressed in many tissues, including skeletal muscles. In this study, we investigated the potential role of BRE gene on satellite cells mediated skeletal muscle regeneration. The complete knockout of the BRE gene in mice was confirmed at DNA, RNA and protein levels. In vivo study indicated that BRE knockout impaired skeletal muscle regeneration from cardiotoxin induced muscle injury, as proved by less pax7+ cells in the injury site and smaller newly regenerated myofibers in the BRE-KO mice. Satellite cells were isolated from the skeletal muscles of BRE knockout and wild-type mic using the routinely single myofiber culture method. Purity of satellite cells was confirmed by immune-fluorescent staining of pax7 antibody. Scanning Electron Microscope (SEM) and Transmission Electron Microscope (TEM) observations indicated that satellite cells in both groups were rounded morphology with high nuclear to cytoplasmic ration and there were no differences in cells morphology between the BRE-KO and BRE-WT satellite cells.

BrdU staining indicated that there was no difference in cell proliferation between the two groups. Myogenic differentiation results indicated that BRE was necessary for satellite cells fusion as evidence by decline of fusion index and reduced mean length of myofibers during myogenic differentiation in BRE-KO satellite cells compared to BRE-WT group.

Time-lapse was used to track and record the migratory projections of satellite cells. The measurements of satellite cell migration revealed that satellite cells migrated in all directions and there was no difference in directionality between the two types of cells. However there was a significant reduction in velocity of BRE-KO cells. Chemotactic migration assay and immunocytochemistry staining of CXCR4 antibody indicated that BRE promoted the chemotactic migration of satellite cells towards SDF-1α gradient by mediating the express of CXCR4. Ligand induced receptor degradation assay indicated that BRE was important in protecting CXCR4 from degradation. Altogether, our results provided evidence that BRE gene facilitate skeletal muscle regeneration by promoting satellite cell motility and fusion.

Reproduction, Development and Endocrinology Theme

Title of poster presentation	Name	Abstract No.
The AT1R-NHE3-SGLT1 (ANS) axis: a novel player in intestinal glucose absorption and its clinical implications in the treatment of diabetes	Leo Ka Yu CHAN	R1
Glucolipotoxicity Suppresses the Gene Expression of FGF21 and FGF21 Action in the Pancreatic beta Cells	Sam Tsz Wai CHENG	R2
Targeted gene disruption in zebrafish reveals noncanonical functions of Lh signaling in reproduction	Lianhe CHU	R3
Autophagy regulates the early secretory pathway through interaction between ULK1 and Sec23	Wen Jia GAN	R4
A potential molecule regulating the development of the enteric nervous system in mouse embryos	Taida HUANG	R5
Identification of lineage specific miRNA profile in human iPSC differentiation	Lv LI	R6
The role of β-defensins in the process of chemotaxis	Xiaofeng LI	R7
"Identification of Bruton's tyrosine kinase (BTK) as a therapeutic target in Neuroblastoma	Tianfeng LI	R8
Delineating the effect of aging on male germline stem cell development.	Jinyue LIAO	R9
The Role of CFTR in Embryonic Stem Cells Programming and Early Development	Zhenqing LIU	R10
De Novo Identification of Novel Long Non-coding RNAs in Mouse Spermatogenesis Reveals Close Association with Stage-specific Functions	Chunrui LU	R11
The potential role of L3mbtl2 in renal ischemia/reperfusion injury	Huihui HUANG	R12
17β-Estradiol modulates LPS-induced calcium transport and TLR4 expression via inhibition of STIM1 phosphorylation through G Protein-coupled estrogen receptor	Xiao SUN	R13
MicroRNA-29b drives mouse embryonic stem cells mesendoderm differentiation by suppression of Tet1	Jiajie TU	R14
CD147 regulates extrinsic apoptotic pathway in spermatocytes through binding to TRAF2	Chaoqun WANG	R15
Effect of angiotensin II type 2 receptor activation on pancreatic beta-cell regeneration in streptozotocin-treated neonatal rats	Lin WANG	R16
Novel Role of Fatty Acid Receptor GPR120 in Modulating and Protecting Pancreatic Islet Function	Dan ZHANG	R17
A Novel Interaction Between the TRAPP Complex and COPII Vesicle	Shan ZHAO	R18
Functional study of Kindlin-2 during early embryonic development	Xin ZHAO	R19

R1

The AT1R-NHE3-SGLT1 (ANS) axis: a novel player in intestinal glucose absorption and its clinical implications in the treatment of diabetes

Leo Ka Yu CHAN, Tung Po Wong and Po Sing LEUNG

Supervisor: Po Sing LEUNG

The local renin-angiotensin system (RAS) has long been the focus of attention for its crucial roles in various human organ systems. In this regard, we have identified a previously unknown local RAS in the rat intestinal epithelial brush border (BB); angiotensin II (AngII) was found to inhibit the Na+/glucose cotransporter 1 (SGLT1)-dependent intestinal glucose uptake. However, the precise interaction between AngII and SGLT1 still remains elusive. Meanwhile, recent studies have revealed the role of AngII in regulating intestinal epithelial sodium-hydrogen exchanger 3 (NHE3). Interestingly, previous studies have also shown the roles of serum-and-glucocorticoid regulated kinase 1 (SGK1) in phosphorylation of NHE3, and hindrance to the clearance of SGLT1 protein from the intestinal BB; these data suggest a role of SGK1 in regulating NHE3 and SGLT1. In light of these findings, we thus propose a novel homeostatic axis−the AT1R-NHE3-SGLT1(ANS) axis, believed to participate in the regulation of intestinal glucose absorption.

In our current pilot studies, we demonstrated that NHE3 was significantly attenuated at both mRNA and protein levels in the jejunum of db/db mice and Caco2 cells exposed to high-glucose conditions. We also showed that EIPA, an NHE3 blocker, significantly blocked intestinal glucose uptake in the Caco2 cells and murine jejunums. In addition, NHE3-knockdown experiments in Caco2 cells resulted in downregulation of SGLT1 mRNA expression. Consistently, a downregulation of SGK1 transcript levels was observed in db/db mice, high-glucose treated Caco2 cells and NHE3-knockdown Caco2 cells, when compared to their respective controls.

Our preliminary data indicate that the ANS axis is present and functional in regulating glucose transport in the intestine and that this “ANS” might provide answers to a new option for diabetes treatment.

R2

Glucolipotoxicity RegulatestheGene Expression of FGF21 and FGF21 Action in the Pancreatic beta Cells

Sam Tsz Wai CHENG, Stephen Yu Ting Li, and Po Sing LEUNG

Supervisor: Po Sing LEUNG

Hyperglycemia and hyperlipidemia are the two major pathological characteristics of type 2 diabetes mellitus (T2DM). The endocrine fibroblast growth factor 21 (FGF21), mainly secreted from liver,is associated with T2DM as it acts as an important metabolic regulator, mediating lipid and carbohydrate metabolism. In this regard,FGF21 knockout mice have been shown to have decreases in adiposity and glucose homeostasis.[1,2]Besides,therapeutic administration of FGF21 restores plasma triglycerides and glucoseto near normal levels in both obese and diabeticmice.[3]It is also noted that high glucose conditions impair endocrine fibroblast growth factor21 action in mouse pancreatic islets by suppressing the expression of FGF21's cofactor, beta-klotho (also known as KLB)[4]. In addition, administration of exogenous FGF21 to pancreaticislets and beta cell line(INS-1E), which mimics the action of endocrine FGF21, partially protects beta cell against glucolipotoxicity and cytokine-inducedapoptosis.[5]Interestingly, FGF21 is also expressed in pancreatic beta cells; however, its protective role in glucotoxicity remains to be elucidated. Therefore, we hypothesize that pancreatic FGF21 mediates these protective actionin pancreatic beta cells in an autocrine/paracrine manner. Our results showed that gene expression of FGF21, KLB and FGF receptor 1(FGFR1) was regulated by high concentrations of glucose and/or palmitic acid. In addition, administration of exogenous FGF21 could rescue the suppression of pancreatic FGF21 expression induced by high glucose conditions. These data indicate that there may be an interaction between the pancreatic FGF21 and hepatic FGF21, and that pancreatic FGF21 may have a novel role in T2DM pathogenesis.

R3

Targeted gene disruption in zebrafish reveals noncanonical functions of Lh signaling in reproduction

Lianhe CHU, Jianzhen Li, Yun Liu, Wei Hu, Christopher Hon Ki CHENG

Supervisor: Christopher Hon Ki CHENG

The pivotal role of gonadotropin signaling in regulating gonadal development and functions has attracted much research attention in the past two decades. However, the precise physiological role of gonadotropin signaling is still largely unknown in fish. In this study, we have established both luteinizing hormone β-subunit (lhb) and luteinizing hormone receptor (lhr) knockout zebrafish lines by transcription activator-like effector nucleases. Intriguingly, both homozygous lhb and lhr mutant male fish are fertile. The fertilization rate, sperm motility and histological structure of the testis was not affected in both lhb and lhr mutant males. On the contrary, homozygous lhb mutant females are infertile while homozygous lhr mutant females are fertile. Folliculogenesis was not affected in both lhb and lhr mutants. But oocyte maturation and ovulation was disrupted in lhb mutant while only ovulation was affected in lhr mutant. Differential expression of genes in the ovary involved in steroidogenesis, oocyte maturation and ovulation was found between the lhb and lhr mutants. These data demonstrate the essential role of Lh signaling in oocyte maturation and ovulation, and support the notion that Lh acts through Fshr in the absence of Lhr. Moreover, the defects of lhb mutant could be partially restored by administration of hCG. This in vivo evidence in the present study demonstrates, for the first time in any vertebrate species, that Lh signaling is indispensable in female reproduction but not in male reproduction. Lh signaling is demonstrated to control oocyte maturation and ovulation in the ovary.

R4

Autophagy regulates the early secretory pathway through interaction between ULK1 and Sec23

Wenjia GAN, Hsaio Chang Chan and Sidney Siu Bun YU

Supervisor: Sidney Siu Bun YU

Autophagy is an autodigestive process which maintains the intracellular homeostasis under starvation condition. Although its mechanism has been well studied, its effect on the early secretory pathway has not been documented. In this study, we found that autophagy changes the morphology of ER exit sites and decreases VSVG exportation from ER exit sites. The ER exit sites are specialized regions of the ER dedicated for the budding of COPII coated vesicles, the formation of which requires coat subunits such as Sec23. The serine/threonine kinase ULK1 is a main effector in autophagy. We found that the morphological and functional changes of ER exit sites are mediated by the phosphorylation of Sec23 by ULK1. This event weakens the interaction between Sec23 and Sec31 but strengthens the binding of Sec23 to Sar1. These results uncover a coordination between the autophagic pathway and the exocytic pathway, implicating that ULK1 decreases traffic of protein vesicles at the stage of ER exit and such regulation helps save energy for the cells when they are under starvation condition.

R5

A potential molecule regulating the development of the enteric nervous system in mouse embryos

Taida HUANG, Hou Yonghui, Wang Cuifang, Wood Yee CHAN

Supervisor: Wood Yee CHAN

The enteric nervous system (ENS) is derived from vagal and sacral neural crest cells (NCCs) that migrate into and along the developing gut. Sox10, which is a transcription factor and a marker of NCCs, regulates NCC proliferation, migration and differentiation. Inactivation or mutation of Sox10 may lead to a loss of neurons and glial cells in the gut. In the Dominant megacolon mouse (Dom), a frameshift mutation results in the synthesis of a truncated Sox10 protein which is functionally inactive. Our results on genome wide profiling of RNA expression showed that a neurotrophin-related protein k6 was down-regulated dramatically in the Dom mutant mouse. k6 has been known to be a member of a family of proteins that are involved in the regulation of neurite outgrowth and synaptic development, but its functions in the enteric nervous system development are still unknown. In the present study, we demonstrated that k6 was expressed in normal migrating NCCs both in vitro in a neural tube explant culture and in situ within embryonic gut tube, and its expression was down-regulated in Dom mutant NCCs. In addition, results of luciferase assays indicated Sox10 could directly activate k6 expression by binding to its promoter sequence. Hence, our findings clearly indicated that k6 is a potential target of Sox10 during ENS development in the mouse.

R6

Identification of lineage specific miRNA profile in human iPSC differentiation

Lv LI, Shen Gu, Yick-Keung Suen, Bi-Feng Chen, Hoi-Hung Cheung, Wai-Yee CHAN

Supervisor: Wai Yee CHAN

Human induced pluripotent stem cells (hiPSC) are human embryonic stem cell (hESC)-like cells, with potential of differentiating into all somatic cell types. Because application of hiPSC can avoid immune rejection and ethical issues, it has become the most promising source of cells for therapy. However, until now, the application of hiPSC is restricted by the absence of low-cost, highly efficient and reliable differentiation methods. microRNA (miRNA) is a kind of small RNA that can regulate gene expression post-transcriptionally. miRNAs have shown importance in human stem cell maintenance, germ layer development and lineage specification. According to previous studies, miRNA can induce de-differentiation (from somatic cells to hiPSC) and trans-differentiation (transition between different somatic cell types). These results suggest that the possibility of manipulating hiPSC differentiation with miRNAs is high. So far, we have already established complete hiPSC differentiation system for three germ layers, that is hepatocyte for endoderm, nephron progenitor for mesoderm, and neural progenitors for ectoderm. Furthermore, we perform miRNA microarray with RNA samples collected from different timepoints during hepatocyte and nephron progenitor differentiation. The expression pattern of miRNAs in these two lineages were analyzed and compared. After finishing miRNA microarray analysis in nephron progenitor differentiation, a completely vertical and horizontal comparison will be performed among three representative lineages for three germ layers. In brief, a combination of target gene prediction and gene ontology enrichment analysis will be used to select candidate miRNAs. We hope to reveal the miRNA(s) that is located in the center of the network of hiPSC fate decision and lineage specification.

R7

The role of β-defensins in the process of chemotaxis

Xiaofeng LI, Zhenqing Liu, Chun Yuan, Yechun Ruan, Xiaohua JIANG, Hsiao Chang CHAN

Supervisor: Xiaohua JIANG

In mammals, only a few spermatozoa arrive at the fertilization site. During the step in the journey to the egg, apart from their self propulsion, spermatozoa may be assisted by oviduct movement and/or a guidance mechanism, the so-called chemotaxis. But the molecular mechanisms involved in the chemoattraction of specific sperm subsets prior to fertilization remain elusive. Interestingly, defensins have been proposed to play versatile role in sperm function. Here, we examined the expression of several beta-defensins in the female reproductive tract and follicular Fluid. RT-PCR results showed that beta-defensin 19 is differentially expressed along the female reproductive tract as well as in the follicular Fluid. The results of the sperm ascending chemoattractant gradient assay showed that mouse sperm could be attracted by beta-defensin 19 in a concentration-dependent manner. Further experiment indicated that this attractive force could be blocked by CCR6 antibody or catsper inhibitor. Sperm intracellular calcium measurements showed that beta-defensin 19 may induce calcium influx. Moreover, Co-IP experiment verified that beta-defensin 19 can interact with CCR6. Our data indicate that beta-defensin 19 may play an important role in sperm chemotaxis which may be an important mechanism to maximize the chance of fertilizing an egg.

R8

Identification of Bruton's tyrosine kinase (BTK) as a therapeutic target in Neuroblastoma

Tianfeng LI, Hui ZHAO

Supervisor: Hui ZHAO

Neuroblastoma (NB) is a common and lethal childhood cancer after leukemia and brain cancers, and accounts for 8% to 10% of all childhood cancers. It is one of the leading causes (approximate 15%) of childhood death by cancer. Despite of considerable progress on its diagnosis and therapy over the last few decades, it remains one of the leading causes of childhood cancer deaths, and the long-term cure rate in children with advanced NB is less than 40%, which is far from satisfactory. Even the children who survive from NB will still be suffered with long-term side effects. Therefore it is still under the urgent need to identify the crucial molecules that are involved in the pathogenesis of neuroblastoma. Anaplastic lymphoma kinase (ALK) (OMIM: 105590) is a receptor tyrosine kinase (RTK) and exhibits the classical structure of RTKs which consist of the extra-cellular domain, trans-membrane domain and intra-cellular domain. ALK is recognized as a major predisposition gene in both sporadic and familiar neuroblastomas. Among the ALK mutations, ALK F1174L has constitutive active kinase activity, and has been identified in sporadic and familial in neuroblastoma tumors. In an attempt to identify ALK interaction partners, we found that ALK physically interacted with Bruton's tyrosine kinase. The BTK belongs to a TEC family kinase (TFK), which is the second largest non-receptor kinase family in humans, and has been implicated in a number of B cell leukemia. Kaplan-Meier analysis indicated that high-level BTK expression in neuroblastoma tumors correlated both with poor overall survival probability and poor relapse-free survival, which is in line with the effects of ALK. The high expression of ALK reduced the overall survival probability and the event free patient survival. A BTK inhibitor, ibrutinib (PCI-32765), inhibited the neuroblastoma cell proliferation. Taken together, our studies suggested an oncogenic role of BTK in neuroblastoma pathogenesis.

R9

Delineating the effect of aging on male germline stem cell development.

Jinyue LIAO

Supervisor: Tin-Lap Lee

"Evidences from animal aging models revealed that significant alterations occur in germ cells and mature sperms as males enter advanced age, and such changes have negative consequences on their progeny. Despite advances in molecular biology and genomics, previous attempts to delineate the developmental programs in male germline stem cell had limited successes. This is largely because the focus was limited to coding RNAs from protein-coding genes, which represent less than 10% of RNA transcripts. The function of lncRNAs in maintenance of male germline stem cell and its regulatory role in advanced paternal age are unknown. To better understand the self-renewal mechanism of germline stem cells and impact of aging at the cellular and molecular level, we aim to study the high-resolution transcriptional profiling of both known coding genes and lncRNAs.

In the present study, we isolated male germline stem cells using fluorescence-activated cell sorting and defined distinctive gene expression signatures between self-renewing and differentiating states by in-depth RNA-seq analysis. We firstly identified 427 known coding genes and 63 lncRNAs with expression selectively in undifferentiated germline stem cell. Around 60% of these potential self-renewal associated transcripts were found be differentially expressed in aged mice compared to their young counterparts. Pathway analysis revealed that the top networks that were altered were associated with gonadotropin releasing hormone receptor pathway and Insulin/IGF pathway which are both important for spermatogenesis, suggesting that this difference may account for the deleterious effects of aging on male germline."

R10

The role of CFTR in embryonic stem cells programming and early development

Zhenqing LIU, Xiaohua Jiang, Jieting Zhang, Jinghui Guo, Hsiao Chang CHAN

Supervisor: Hsiao Chang CHAN

"Embryonic stem cells (ESC) are unique cell types characterized by their capacity of self-renewal and their developmental potential to differentiate into any type of cells in response to environmental cues. The balance between self-renewal and differentiation in ESC is tightly controlled by a complex interplay between signaling from the extracellular environment and the intrinsic transcription factor networks. However, it remains largely unknown how environmental signals are transduced into alterations of transcriptional networks that lead to cell-fate conversion.

CFTR is a cAMP-regulated anion channel capable of conducting both Cl- and HCO3-. Mutations in the gene encoding region of CFTR cause cystic fibrosis (CF), a common autosomal recessive disease in Caucasian populations. While CFTR was first thought to be predominantly expressed in a wide range of epithelial tissues, subsequent studies have found that CFTR is also expressed in tissues and cells of non-epithelial origin. Recently, we have found that CFTR is functionally expressed in ESC. Our RT-PCR, sequencing, patch clamp and immunofluorescence staining results have revealed that CFTR is expressed in both human and mouse in ESC. To further understand the role of CFTR in ESC pluripotency and differentiation, we derived CFTR knockout(KO) ESC with C57BL6 background. We found that KO ESC proliferated faster than wild type cells, and had increased expression of self-renewal markers such as Oct4, Nanog and Sox2 in ES basal culture condition. However, when cultured in 2i condition, which indicates a naïve state of ES, CFTR KO ESC tended to be less pluripotent. Wnt/β-catenin and MAPK/ERK pathways are important pathways that control mouse ESC pluripotency. Intriguingly, our Western Blot analysis revealed that Wnt/β-catenin pathway was significantly downregulated while MAPK/ERK pathway was significantly upregulated in KO ESC upon different extracellular stimuli. More interestingly, CFTR expression increased along with embryoid body (EBs) formation day by day, indicating the critical role of CFTR in differentiation. Furthermore, we found that neuronal differentiation was significantly impaired in KO ESC.

Taken together, our results suggest CFTR may sense environmental signals and transduce them into activation/inactivation of key transcription factors governing stem cell self-renewal/differentiation through Wnt/β-catenin and MAPK/ERK pathways.

R11

De novo identification of novel long non-coding RNAs in mouse spermatogenesis reveals close association with stage-specific functions

Chun Shui LUK, H. GAO, J. LIAO, Tin-Lap LEE

Supervisor: Tin-Lap LEE

The unique cellular dynamics in germ cell development provides essential elements for understanding the fundamentals of developmental biology. Long noncoding RNAs (lncRNAs) recently emerged as key regulators in normal and disease development, but their roles in mouse spermatogenesis were not fully elucidated. Furthermore, most of current lncRNA annotations are developed from non-germ cell tissues or cell lines, which may miss germ cell-specific lncRNAs.

To reveal novel lncRNAs in spermatogenic cells, we assembled male germ cell transcriptome datasets from our previously published tiling arrays and Serial Analysis of Gene Expression (SAGE), and RNA-Seq data in recent studies by a novel bioinformatics pipeline known as Hybrid Transcriptome Assembly (HTA). Intriguingly, over 2000 novel intergenic lncRNAs were discovered in three key stages of spermatogenesis. Selected lncRNA candidates were validated for stage-specific expression by real-time PCR and size confirmation by Northern Blot. Functional association analysis revealed some stage-specific novel lncRNAs were associated with spermatogenesis-related genes, including Lin28, Trp53, and Yy1 in spermatogonia, Mov10l1 and Nr2c2 in pachytene spermatocytes, and Fndc3a, Ccin, and Catsperb in round spermatids. Moreover, our Reduced Representation Hydroxymethylation Profiling (RRHP) results revealed a significant rise in 5-hydroxymethylatedcytosine (5hmC) content during kit- to kit+ spermatogonia transition within promoter and exon regions of a novel spermatogonia-specific lncRNA which is predicted to be associated with Trp53 in trans, suggesting a possible regulation on spermatogonial apoptosis by lncRNA and 5hmC through Trp53 pathway. In conclusion, our work provided insights to a previously hidden network of molecular regulations on male germ cell development, and revealed a close relationship between lncRNAs and genes with stage-specific functions.

R12

The potential role of L3mbtl2 in renal ischemia/reperfusion injury

Huihui HUANG, Yin XIA

Supervisor: Yin XIA

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 project, 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.

R13

17β-Estradiol modulates LPS-induced calcium transport and TLR4 expression via inhibition of STIM1 phosphorylation through G Protein-coupled estrogen receptor

Xiao SUN, W. H. Ko, Christopher Hon Ki CHENG

Supervisor: Christopher Hon Ki CHENG

Sex plays a significant role in the development of bacterial infections and inflammation diseases. Nowadays, there is increasing evidence that estrogen, the female sex hormone, exerts anti-inflammatory and anti-atherogenic effects in various tissues. Vaginal epithelial cell (VEC) is characterized as stratified squamous nonkeratinizing epithelia which acts as a powerful physical barrier to pretect women from bacterial and fungal infections, where estrogen presents as an priority regulator on its functional modification. However, the underlying molecular mechanism(s) leading to estrogen attenuates TLR4 ligand Lipopolysaccharide (LPS)-stimulated proinflammatory cytokines overproduction and intracellular Ca2+ overload remain elusive. To determine whether estrogen-drived Ca2+ signaling of human vaginal epithelial cells (hVEC) prevents epiethlial inflammation and which estrogen receptors is involved in this process. We investigated the action of 17β-Estradiol (E2) in depleting intracellular Ca2+ and suppressing the store-operated Ca2+ entry (SOCE) by inhibiting ER Ca2+ sensor stromal interaction molecule 1 (STIM1) phosphorylation. Here our results describe a novel regulatory role of estrogen in modulating the LPS-mediated IL-8 secretion, Ca2+ trafficking and TLR4 expression through G protein-coupled receptor 30 (GPR30). LPS-induced SOCE and ER Ca2+ release were significantly abrogated by E2 and G1 pretreatment. Moreover, we also find that TLR4 expression is decreased when intracellular Ca2+ signaling is blocked by Ca2+ chelator BAPTA-AM or PLCγ2-IP3-Ca2+ cascade inhibitor U73122. These results indicate that estrogen-driven Ca2+ signaling plays an essential role in epithelium innate immune function and may provide crucial therapeutic targets to limit bacterial infections in female reproductive tract.

R14

MicroRNA-29b drives mouse embryonic stem cells mesendoderm differentiation by suppression of Tet1

Jiajie TU, Shuk Han Ng, Alfred Chun Shui Luk, Jason Jingyue Liao, Bo Feng, Kingston King Lun Mak, Wai-Yee Chan, Tin-lap Lee

Supervisor: Tin-lap Lee

5-Hydroxymethylcytosine (5hmC) represents a novel epigenetic marker in mammalian epigenetic regulation and involves in maintaining pluripotent state and differentiation potential of embryonic stem cells (ESCs). The transitional process from 5-methylcytosine (5mC) to 5hmC is dynamically regulated by Ten-eleven translocation (Tet) family. Despite it provides new insights on transcription control in demethylation pathway, the regulatory network between Tet family and 5hmC in ESCs remains elusive, especially the upstream regulators of Tet family and the interaction between non-coding RNAs and Tet family are largely unknown. Here we demonstrate microRNA-29b (miR-29b) regulates 5hmC level during mouse embryonic stem cells differentiation, which has impact on ectoderm and mesendoderm differentiations via repression of Tet1. MiR-29b level is increased selectively when mouse ESCs commits differentiation, leading to significant reduction of cellular 5hmC level by direct binding at the 3' un-translated region (3'UTR) of Tet1. The regulation caused aberrant gene expression and phenotype changes associated with pluripotency and ectoderm/mesendoderm differentiation. We also show that the role of miR-29b/Tet1 axis in mESCs is via up-regulation of DKK1, an inhibitor of Wnt pathway. Besides, miR-29b directly targets Tet2 and Tdg and regulates global expression level of other derivatives of 5mC (5fC and 5caC) in mESCs. These findings underscore the contribution of small non-coding RNA mediated 5mC demethylation in ESCs differentiation, and provide new targets and insights for developing strategies in regenerative medicine of stem cells.

R15

CD147 regulates extrinsic apoptotic pathway in spermatocytes through binding to TRAF2

Chaoqun WANG, Hao Chen, Hsiao Chang CHAN

Supervisor: Hsiao Chang CHAN

Cluster of differentiation 147 (CD147), a transmembrane protein, has been recently recognized to play an important role in spermatogenesis, which is regulated by germ cell proliferation and apoptosis. CD147 null mutant male mice are infertile, with increased apoptotic germ cells and arrested spermatogenesis observed. Interfering with CD147 with its antibody induces apoptosis in spermatocytes. However, it is still elusive how CD147 is involved in germ cell fate regulatory network. Apoptosis, a process of programmed cell death, is thought to facilitate the removal of abnormal germ cells. While, canonical NFκB signaling protects germ cell from death; the role of non-canonical NFκB signaling on germ cell apoptosis is still unclear. It has been reported that TRAF2 inhibits extrinsic apoptosis and non-canonical NFκB signaling, but activates canonical NFκB signaling. Interestingly, TRAF2 is also known to bind to CD147. In this study, we aimed to clarify the mechanism underlying CD147-regulated germ cell apoptosis with mouse spermatocyte cell line GC-2 in vitro and mouse testicular germ cells in vivo. We found that blocking of CD147 induced extrinsic apoptosis in GC-2 cells and mouse testicular germ cells, companied by non-canonical NFκB signaling activation and canonical NFκB signaling suppression, suggesting that CD147 might regulate germ cell survival/apoptosis via TRAF2. Further, we confirmed the interaction between CD147 and TRAF2 with co-immuneprecipitation technique. These findings support an important role of CD147 in regulating germ cell apoptosis through its interaction with TRAF2.

R16

Effect of angiotensin II type 2 receptor activation on pancreatic beta-cell regeneration in streptozotocin-treated neonatal rats

Lin WANG, J. Liang, Po Sing LEUNG

Supervisor: Po Sing LEUNG

Our previous studies have shown the presence of the angiotensin II type 2 receptor, AT2R, in pancreatic islets and that the activation of AT2R plays an important role in regulating pancreatic islet development. In the present study, we aimed at investigating into the effects of C21, a non-peptide agonist for AT2R on AT2R-mediated islet regeneration using a regeneration animal model of streptozotocin (STZ)-treated neonatal rats.

Neonatal rats were assigned to 5 groups: normal, STZ vehicle, STZ plus C21 (0.25, 0.5 and 1 mg/kg). C21 was intraperitoneally injected into STZ-treated neonatal rats continuously for 7 days, body weight and blood glucose were routinely monitored every day. On the last day, serum insulin was measured, and the pancreata were extracted to examine islet function and changes in markers of β-cell expression and oxidative stress, as well as potential signalling mechanisms involved.

Our results showed that the body weight, blood glucose and serum insulin level of STZ-treated rats were significantly lower than the normal control rats, as expected. However, C21-treated STZ rats, particularly those with 0.5 and 1mg/kg groups showing significant decreases in blood glucose and increases in serum insulin concentrations. The C21-treated STZ rats also exhibited significantly higher GSIS activity in response to high glucose challenge as compared to STZ vehicles. In addition, C-21 treated rats displayed larger islet mass and up-regulated expression of insulin and Ngn3 mRNA in the pancreas. Furthermore, it was also found that STZ treatment induced higher levels of reactive oxygen species and apoptosis, as evidenced by expression of SOD1 mRNA and caspase-3 and p38 protein, these effects were reduced in C21 treated rats. All of these data suggest that C21 may be involved in the regeneration of β-cells, probably via anti-oxidative and anti-apoptotic effects.

R17

Novel role of fatty acid receptor GPR120 in modulating and protecting pancreatic islet function

Dan ZHANG and Po Sing LEUNG

Supervisor: Po Sing LEUNG

GPR120 belongs to the G protein-coupled receptor (GPCR) family and is thought to be a target for the management of T2DM. However, potential roles of GPR120 in modulating pancreatic islet function remain elusive. Our study was aimed at investigating this issue using the models of pancreatic beta-cell line (INS-1E) and Wistar rat and C57BL/6 mouse islets.

The GPR120 expression was identified in INS-1E, rat and mouse islets, and it was decreased in cells and islets in exposure to high glucose. Consistently, GPR120 expression was also diminished in islets of db/db and high-fat diet/streptozotocin (HFD/STZ)-induced mice. In vitro and ex vivo studies showed that DHA, a GPR120 agonist, potentiated glucose stimulated insulin secretion (GSIS) in INS-1E as well as in rat and mouse islets; this stimulatory effect of DHA on GSIS was suppressed by BSA (known to block interaction between DHA and GPCR). Furthermore, in HFD/STZ-induced T2DM mouse islets, DHA did not enhance GSIS as it did in normal mouse islets. More importantly, the acceerative effect of DHA on GSIS in INS-1E was attenuated by GPR120 knockdown but improved by GPR120 overexpression. Apart from its regulatory action in GSIS, GPR120 was found to be related to insulin synthesis, since insulin expression was reduced in INS-1E transfected with GPR120 siRNA.

On the other hand, DHA exerted inhibitory effect on palmitic acid-induced inflammation through down-regulating expression of pro-inflammatory mediators in INS-1E and mouse islets, of which GPR120 may act as a DHA receptor.

All these data provide evidence suggesting the role for GPR120 in regulation of pancreatic islet function.

R18

A novel interaction between the trapp complex and COPII vesicle

Shan ZHAO, Chunman LI, Xiaomin LUO, Sidney Siu Bun YU

Supervisor: Sidney Siu Bun YU

The transport protein particle (TRAPP) complexes take part in various membrane trafficking pathways, although this complex was initially regarded as a tethering factor for ER-derived COPII vesicle. In yeast and mammalian, three different forms of TRAPP complexes, TRAPPI, II, and III, have been identified. TRAPPC12(trafficking protein particle complex 12，TTC15，CGI-87),which has no homolog in yeast, has already been determined as a subunit of mammalian TRAPP complex. Nonetheless, it is unclear to which form of TRAPP complex TRAPPC12 belongs. In mammalian TRAPP, our preliminary data supported that TRAPPC12 is the component of TRAPPIII.

However, what the function(s) of TRAPPIII in mammalian is still poorly understood. While a role in autophagy has been implicated, we have mass spec. analysis indicating a potential role of TRAPPIII with COPII vesicle. Using TRAPPC12 as a representative subunit of TRAPPIII, we could try to elucidate the functional relationship between the subunit of TRAPPIII and COPII vesicle by genetic and epigenetic manipulations. These results provide a novel perspective on TRAPP biology.

R19

Functional study of Kindlin-2 during early embryonic development

Xin ZHAO and Hui ZHAO

Supervisor: Hui ZHAO

Kindlin-2, a co-activator of integrin, is well known to enhance talin-mediated cell to matrix adhesion and cell spreading. Increasing evidence suggests that kindling-2 can regulate signaling transduction directly. In addition, kindlin-2 is also implicated in tumor cell invasion, tubulointerstitial fibrosis, hemostasis and epithelial mesenchymal transition. Kindlin-2 expresses throughout the development process, but its function in early embryonic stages remains unclear. Here we demonstrate that in Xenopus embryos, Kindlin-2 is expressed in the animal pole by blastula stages. The expression is enriched in the anterodorsal most region during neurula stages and it becomes restricted in the somites and neural crest during tailbud stages. Either loss or gain of Kindlin-2 in Xenopus embryos resulted in abnormal body axis and loss of tail development. Blocking Kindlin-2 translation reduced the expression of brachyury and goosecoid, which are respectively pan mesoderm and dorsal mesoderm marker genes. Overexpression of Kindlin-2 enhanced the expression of brachyury, chordin and sox2, suggesting a dorsalized phenotype. Kindlin-2 expression is not effected by overexpression of eFGF, Wnt3a or BMP4 signaling in animal cap assay. Our data suggest that Kindlin-2 is essential for early embryonic development and promotes the dorsal formation.

Cancer and Inflammation Theme

Title of poster presentation	Name	Abstract No.
Purification, characterization and anti-osteocarcinoma study of a novel hemagglutinin from the Northeast China black beans	Xiuli DAN	C1
Thetherapeutic effect of vitamin D3 inconjunctionwithcathelicidin forgastric cancer	Mingxing LI	C2
A potential inhibitor in treating Alzheimer's disease by docking the PDZ-2 binding pocket	Lisha LI	C3
An in vitro model to screen the pyrrolizidine alkaloid-induced toxicity in hepatocytes	Yao LU	C4
Guttiferone K induces apoptosis in colorectal cancer through endoplasmic reticulum stress aggravation	Kai Kei MIU	C5
Osteopontin differentially regulates human mast cell functions induced by FceRI and TLR2 activation	Chun Wai NG	C6
A potential risk variant in HOMER1 regulatory block for major depression disorder and suicidal behavior	Shitao RAO	C7
Histone deacetylase 8 impairs insulin sensitivity and activates Wnt pathway in NAFLD-associated hepatocellular carcinoma	Yuan TIAN	C8
Elucidating the nuclear functions of PTEN: Post-translational modifications and cell homeostasis	Yubing WANG	C9
Properties of PTEN Mutants Found in Autism	Chi Wai WONG	C10
Pharmacokinetic interaction between paclitaxel and polyoxypregnanes	Xu WU	C11
Investigation of the mechanism of antiplatelet aggregation effects of senkyunolide A, ligustilide, and butylidenephthalide	Di XIE	C12
Identification of novel long non-coding RNAs regulated by Polycomb Repressive Complex 2 in hepatocellular carcinoma	Feiyue XU	C13
Investigating Dominant-acting Effects of Cancer-associated PTEN Mutants	Mingfei YAN	C14
Pyrrolizidine alkaloids-induced hepatotoxicity on hepatic sinusoidal endothelial cells and hepatic parenchymal cells	Mengbi YANG	C15
Reactivation of Growth/Differentiation Factor 1 Contributes to the Chemopreventive Effect of 5-aza-2'deoxycytidine in Gastric Cancer	Weiqin YANG	C16
Investigation of herb-drug interaction between Statins and Danshen-Gegen Decoction on Cardiovascular Disease	Ka Chun YAU	C17
Herbal components, acting as pro-drugs, reverse P-gp-mediated multidrug resistance of anticancer agents	Chun YIN	C18
Identification of Alterations in Host Genome Methylation Driven by the Human Immunodeficiency Virus Type 1	Yinfeng ZHANG	C19

C1

Purification, characterization and anti-osteocarcinoma study of a novel hemagglutinin from the northeast China black beans

Xiuli DAN, Jack Ho WONG, Evandro Fei FANG, Francis Chun Wai CHAN, and Tzi Bun NG*

Supervisor: Tzi Bun NG

In the present study we isolated a novel hemagglutinin from an edible legume and explored the potential of this protein in the treatment of osteocarcinoma and liver cancer. The protein was purified by liquid chromatography techniques which entailed affinity chromatography on Affi-gel blue gel, ion exchange chromatography on Mono Q and gel filtration on Superdex 75 with an FPLC system. The hemagglutinating activity of this hemagglutinin was demonstrated to be ion-dependent and stable over a wide range of temperature and pH values. Anti-proliferative activity was observed in the tumor cell lines MG-63 and HepG2, but not in the normal cell line WRL 68. Osteocarcinoma cells treated with the hemagglutinin underwent obvious cell shrinkage, chromatin condensation, mitochondrial membrane depolarization and apoptosis. The mRNA expression level of interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-1 beta (IL-1β), interferon- gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α) were found to be up-regulated to different extents after the treatment of this hemagglutinin.

C2

The therapeutic effect of vitamin D3 inconjunctionwithcathelicidin forgastric cancer

Ming Xing LI, William K.K. WU, Lin ZHANG, J. SHEN, Ruby L.Y. CHAN, Xiao Min LUO, Franky L. CHAN, Chi Hin CHO

Supervisor: Chi Hin CHO

1α,25(OH)2 vitamin D3 (1α,25(OH)2D3), the active form of vitamin D3, could significantly inhibit the proliferation and induce cell cycle arrest through stimulating the expression of p21 in gastric cancer cells (TMK1) but not in the normal gastric epithelial cells (HFE145). This vitamin analogue also successfully induced the expression of cathelicidin and provoked autophagy in TMK1. Knockdown of cathelicidin by siRNA abolished the anti-proliferative effect of 1α,25(OH)2D3. To confirm this anti-cancer action in vitro, we established an animal model of orthotopic gastric tumor with the same gastric cancer cell line in nude mice. In this tumor model we found that daily oral administration with vitamin D3 significantly inhibited tumor growth in the stomach. Such treatment also increased mucosal cathelicidin and p21 expressions and induced LCB3 II level in the gastric tumor tissues. In conclusion, our results suggest that vitamin D3 inhibits gastric cancer in a cathelicidin-dependent manner. As this vitamin is currently used as a dietary supplement mainly for bone diseases in humans, it could also function as a novel therapeutic agent or prophylactic treatment for gastric cancer in the future.

C3

A potential inhibitor in treating Alzheimer's disease by docking the PDZ-2 binding pocket

Lisha LI, Penelope OR,Mingfei YAN,Wong Chi WAI,Yubing WANG, Jing LI,Cheung Ka WING, Andrew Man Lok CHAN*

Supervisor: Andrew Man Lok CHAN

Phosphatase and tensin homolog (PTEN), a critical regulator on neuronal morphology and migration, has been linked to autism and ischemic neuronal injuries. PTEN has been implicated in NMDAR-mediated long-term depression (LTD). The carboxyl-terminal Postsynaptic density-95/Dlg1/ZO1 binding domain (PDZ-BD) of PTEN is required for LTD, and this is mediated through the PSD-95 scaffold protein. In Alzheimer's Disease (AD), pathogenicβamyloid peptides are known to induce LTD in hippocampal neurons. Thus the hypothesis is that Aβamyloid induces synaptic malfunction in Alzheimer's disease(AD) can be relieved by blocking PTEN-mediated long-term depression of synapse. Specifically, inhibiting the interaction between PTEN and PSD-95 is a potential approach in treating AD. For this, we identified the second PDZ domain (PDZ-2) of PSD95 to interact with the PDZ-BD of PTEN. To discover inhibitors of this protein-protein interaction, we perform an "in silico" screening in FDA approved drug database for compounds that can dock the PDZ-2 binding pocket, a series of compounds have been selected. For in vitro affinity evaluation we developed a fluorescence polarization (FP) assay to screen the possible inhibitor for this interaction. Then we elected several compounds with high score on screening to test on the FP assay. According to the FP's binding curve, a compound has been identified to show significant inhibition at the nanomolar range. It's ability to overcomeβamyloid toxicity will be evaluated in primary neuronal cultures.

C4

An in vitro model to screen the pyrrolizidine alkaloid-induced toxicity in hepatocytes

Yao LU, Ge LIN

Supervisor: Ge LIN

Pyrrolizidine Alkaloids (PA) are naturally occurring heterocyclic compounds found in a variety of plants. PA-induced hepatotoxicity via hepatic metabolism is mediated by phase I drug metabolizing CYP450 enzymes. Over past decades much progress has been made in our understanding of PA induced liver injury through study of rodent models. Experiments on large-scale animal models are expensive and usually facing ethical issues. Thus, a human-relevant in vitro system is needed for early screening of metabolism-induced hepatotoxicity. In the present study, a liver cell line of human origin, known as HepaRG cells, was used for the development of a screening model. In comparison with the most widely used human liver cell line hepG2, the differentiated HepaRG cells were confirmed to exhibit hepatocyte-like properties with high expression levels of major CYP450 mRNAs (e.g. CYP3A4, CYP2E1 and CYP2B6). Comparable CYP450 activities in differentiated HepaRG cells were also demonstrated by fast turnover rate of major CYP450 substrates. On the other hand, the expression and activities of CYP450 enzymes were extremely low in HepG2 cells. Exposure of HepaRG cells to PA at low concentrations resulted in dramatic cell death. In contrast, no significant cytotoxicity was observed with the same concentrations of PA incubated in HepG2 cells.

Conclusions: The levels of the phase I drug metabolizing enzymes, in particular CYP450 in HepaRG cells were found to be significantly higher than those in HepG2 cells, and thereby HepaRG cells more closely reflect metabolism in human liver. Our preliminary results demonstrated that HepaRG cells could be used for early screening of metabolism-induced hepatotoxicity, including PA-induced hepatotoxicity.

C5

Guttiferone K induces apoptosis in colorectal cancer through endoplasmic reticulum stress aggravation

KaiKei MIU, Kenneth Kin Wah TO, Hong Xi XU, Ge LIN

Supervisor: Ge LIN

Guttiferone K (GutK) is a polycyclic polyprenylated acylphloroglucinol (PPAP) isolated from Garcinia spp. Our group had previously confirmed that GutK can trigger G1/S phase arrest and apoptosis in colorectal cancer cells, however, the mechanism underlying GutK-induced apoptosis is unknown. The present study demonstrated that GutK elicited endoplasmic reticulum (ER) stress in a variety of colorectal cancer cells. Furthermore, prolonged treatment of GutK enhanced CHOP expression that potentially channeled massive population of these cells to apoptosis.

ER stress marker activities were evaluated after the treatment of HCT116 and HT29 cancer cells with GutK through Western blotting and/or qRT-PCR. GutK induced 1) GRP78 and GRP94 both in mRNA and protein level; 2) eIF2α phosphorylation; 3) transient CHOP expression and ATF6 localisation to Golgi apparatus; and 4) XBP-1 mRNA with 26-nt intron cleaved and JNK phosphorylation within 24 hours.

Furthermore, the pro-apoptotic arm of ER stress could be activated when GutK was treated for a prolonged period. Propensity to apoptosis was characterized by probing PARP cleavage as a measure of caspase-3 activation. The cells underwent apoptosis in a GSK3β-dependent manner. CHOP, as an apoptotic inducer in ER stress, was expressed after treatment with GutK. Knockdown and dominant negative approaches were adopted to assay the role of CHOP in the subsequent apoptosis. Both siCHOP and dnCHOP effectively suppressed GutK-induced apoptosis.

In conclusion, the mechanism of GutK-induced apoptosis was partly due to ER stress activation which eventually led to CHOP accumulation, resulting in a GSK3β-dependent apoptosis. These phenomena are linked to the newly characterized process coined “ER stress aggravation”. As recent researches suggested that ER stress-induced apoptosis is cancer selective, such finding facilitates GutK to be exploited as a novel anti-cancer agent through this mechanism.

C6

Osteopontin differentially regulates human mast cell functions induced by FceRI and TLR2 activation

Chun Wai NG, Alaster Hang Yung LAU

Supervisor: Alaster Hang Yung LAU

Mast cells are key multifunctional effector cells of the immune system which release and synthesize myriads of chemical mediators and cytokines. Osteopontin is an extracellular matrix-associated glycoprotein which is recognised to be an important regulator of various immune responses. Although both mast cells and osteopontin have separately been implicated in the mediation of inflammatory reactions that are associated with allergy, innate immune host defence responses, angiogenesis, wound healing and metastasis, there is a lack of studies of their interactions. It is hence the aim of the present study to investigate the regulation of human mast cells (HMC) by osteopontin by employing HMC cultured from CD34+ progenitors. The adhesion and pro-inflammatory cytokine release of HMC following incubation with osteopontin were determined by CyQUANT cell proliferation assay and commercial ELISA kits respectively. Matrix-fixed OPN could induce HMC adhesion after anti-IgE and PGN activation. Following adhesion, only anti-IgE but not PGN induced release of IL-8 and TNF-α from HMC was suppressed. In contrast, soluble OPN did not affect anti-IgE and PGN-mediated IL-8 and TNF-α release. Blocking the RGD domain of OPN with cyclic RGD could reverse the adhesion of HMC to both anti-IgE and PGN-induced adhesion and adhesion-mediated inhibition of IL-8 and TNF-α synthesis. After blocking the integrin αVβ3 of HMC with a specific antibody, anti-IgE-induced adhesion was partially inhibited and only the suppressed TNF-α production could be reversed. These studies demonstrate that OPN regulates different signaling pathway of HMC when they are activated by anti-IgE and PGN, which means that OPN would implicate regulate functions of HMC differently in adaptive and innate immunity.

C7

A potential risk variant in HOMER1 regulatory block for major depression disorder and suicidal behavior

Shitao RAO, Marco HB LAM, Venus SY YEUNG, Cynthia O SIU, YK WING, Stephen Kwok Wing TSUI, Mary Miu Yee WAYE

Supervisor: Stephen Kwok Wing TSUI

Objectives: Animal model and genetic studies suggest that HOMER1 is involved in the etiology of major depression disorder (MDD) and suicidal behavior. However, most of the genetic studies were performed in Caucasians and the results were not consistent (Orsetti et al. , 2008, Rietschel et al. , 2010, Strauss et al. , 2012). This study aimed to investigate the association of HOMER1 polymorphism with MDD and suicidal behaviorin Hong Kong Chineseand assess the change of patients'psychological state. **Methods:** Personality inventory, impulsivity and depression scale were used to assess psychological state in MDD patients and suicide attempters. The association of HOMER1 polymorphism rs7713917 with MDD or suicidal behavior was performed by case-control association study. **Results:** In psychometric measures, both MDD patients and suicide attempters were likely to be less conscientious, less extroverted and more neurotic than healthy subjects. Moreover, the HOMER1 polymorphism rs7713917 was found to be significantly associated with both MDD and suicidal behavior in homozygous model (AA vs. GG, adjusted p-value= 0.035 and 0.013, respectively) and recessive model (AA vs. AG+GG, adjusted p-value= 0.009 and 0.008, respectively). In addition, through bioinformatics tools we have found that rs7713917 is located in the potential binding site of GATA-1, whose increased expression levels had been found in individuals with MDD and could result in a decrease of the expression of synapse-related genes (Kang et al. , 2012). Furthermore, this variant falls in a large linkage disequilibrium (LD) block with HOMER1 promoter region indicating its potentially close relationship with the change of HOMER1 expression. **Conclusions:** Our findings suggest that personality traits are linked to risk for MDD and suicidal behavior. Moreover, the association study implies that HOMER1 AA homozygote is significantly associated with susceptibility to MDD and suicidal behavior in Hong Kong Chinese population.

C8

Histone deacetylase 8 impairs insulin sensitivity and activates Wnt pathway in NAFLD-associated hepatocellular carcinoma

Yuan TIAN, Ka F. TO, Paul B. S. LAI, Yue S. CHEUNG, Jun YU, Vincent W. S. WONG, Henry L. Y. CHAN, Alfred Sze Lok CHENG

Supervisor: Alfred Sze Lok CHENG

Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of metabolic syndrome which elevates the risk of hepatocellular carcinoma (HCC). Epigenetics converts alterations in metabolism into heritable pattern of gene expression. Previously, we identified Hdac8 as the sole commonly induced chromatin regulatorin NAFLD-associated HCC. However, the role of HDAC8and the mechanism by which HDAC8 promotes cell growth remains to be defined. In this study, we found that SREBP-1 could bind to the promoter of HDAC8 for transcriptional up-regulation. Western analysis of AKT phosphorylation and quantitative RT-PCR of lipogenic genes expression suggested that HDAC8 promotes insulin resistancein HCC cells. Down-regulation of HDAC8 significantly inhibited HCC growth *in vivo* as demonstrated by both xenograft and orthotopicmodels. Functional characterization demonstrated the pro-proliferative and anti-apoptotic activities for HDAC8, which were associated with reduction in histone H4 acetylation.HFD-fed mice exposed to low-dose diethylnitrosaminewere used to validate the functional significance of HDAC8. Knockdown of HDAC8 dramatically reduced HFD induced up-regulation of metabolic profiles as well as tumor numbers. Luciferase-based pathway array revealed that Wnt/β-catenin pathway was prominently activated by HDAC8. Mechanistically, HDAC8 was found to physically interact with EZH2 to cooperatively repress Wnt antagonists via concomitant promoter binding and histone methylation and deacetylation. Notably, HDAC8, SREBP-1, EZH2, and active β-catenin were concordantly over-expressed in both obesity-induced HCC models and NAFLD-associated HCC patient samples. Collectively, these findings demonstrate the functional significance of HDAC8 in NAFLD-associated hepatocarcinogenesis and its mechanistic interaction with EZH2 to repress Wnt antagonists, providing a strong impetus for therapeutic intervention.

C9

Elucidating the nuclear functions of PTEN: Post-translational modifications and cell homeostasis

Yubing WANG, Chiwai WONG, Mingfei YAN, Lisha LI, Penelope OR, Andrew Man Lok CHAN

Supervisor: Andrew Man Lok CHAN

PTEN possesses both protein and lipid phosphatase activities, with its tumor-suppressor function mainly depends on its lipid phosphatase activity through inhibiting the PI3K/AKT signaling pathway at the cell membrane. However, increasing evidence have demonstrated that PTEN also localizes to the nucleus where it plays important roles in cell-cycle regulation and genomic integrity. PTEN have several putative nuclear import and exclusion signals, but the mechanism in how PTEN is shuttled between the cytoplasm and nucleus is not entirely clear. Previous studies have shown that PTEN ubiquitination and phosphorylation are involved. More recently, SUMOylation events at K266 and K254 have been shown to promote membrane binding and nuclear retention, respectively. How all these PTEN post-translational events are coordinated is not entirely clear.

For this, both nuclear- (NLS) and cytoplasmic- (NES) targeted PTEN harboring SUMOylation-defective mutations, K254R, K266R, and K254R/K266R, were constructed. In human glioblastoma cell lines, U87MG and U373MG, NLS- and NES-PTEN proteins showed different post-translational modifications as well as different AKT activities when compared to the wild type PTEN.
To further delineate the nuclear functions of PTEN, a glioblastoma cell line, U87MG, stably expressing either wild-type or NLS-tagged PTEN was generated. Gene expression profiling will be performed to explore how nuclear PTEN carry out its function at the gene expressions level.

C10

Properties of PTEN Mutants Found in Autism

Chi Wai WONG, Andrew Man Lok CHAN

Supervisor: Andrew Man Lok CHAN

PTEN (Phosphatase and tensin homolog deleted on chromosome ten) is a tumor suppressor gene, which is frequently mutated or lost in cancer cells. It is a lipid and protein phosphatase, and locates at cell membrane, cytosol and nucleus. Besides working as a tumor suppressor, PTEN is also important in neurons. Germline mutation of PTEN has been implicated to cause autism spectrum disorders (ASD). ASD is a neurodevelopmental disorder with affected individuals harboring social interaction deficits. It is believed that the inactivation of PTEN will hypoeractivate the mTORC signaling pathway causing uncontrolled neurite outgrowth. To determine how PTEN ASD mutations affect its biochemical and biological funcitons, a panel of PTEN ASD mutants was generated. These point mutations are located in either the phosphatase domain or C2 domain of PTEN. Characterization of these mutants revealed that they have different protein stability, phosphatase activity and subcellular localization. Based on these different properties, we are currently trying to find out how PTEN functions in neurons are affected by changes in stability, phosphatase activity and subcellular localization.

C11

Pharmacokinetic interaction between paclitaxel and polyoxypregnanes

Xu WU, Chun YIN, Yang YE and Ge LIN

Supervisor: Ge LIN

Xiao-ai-ping, a proprietary herbal product containing extract from a Chinese medical herb, Marsdeniae tenacissimae Caulis., has long been used in China in combination anti-cancer therapy. Our previous study demonstrated that the three most abundant ingredients, polyoxypregnanes (POPs), in the extract can reverse P-glycoprotein-mediated paclitaxel multi-drug resistance. The aim of the present study is to investigate the potential pharmacokinetic (PK) interaction between paclitaxel and POPs in the rats. We successfully established a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the simultaneous detection of paclitaxel and POPs in rat plasma. In the male Sprague Dawley rat PK model, moderate PK interaction was observed between paclitaxel and POPs. POPs did not affect the Cmax of paclitaxel (around 2.7 μM). However, in the presence of POPs, the area under the concentration-time curve (AUC) of paclitaxel was significantly increased by 1.5 folds [AUC(pacltaxel+POPs) of 2030±247 nM·h vs AUC(paclitaxel) of 1343±107 nM·h, $p<0.01$], indicating enhanced plasma exposure. Significantly reduced clearance (CL) of paclitaxel [CL(paclitaxel) of 1.03±0.07 L/h/kg vs CL(paclitaxel+POPs) of 0.65±0.09 L/h/kg, $p< 0.001$) was also observed. These results suggest that the reduced clearance and enhanced systemic exposure of paclitaxel might be due to the involvement of POPs inhibited P-glycoprotein-mediated biliary excretion of paclitaxel. Our study revealed PK interaction between paclitaxel and POPs, and provided the basis for the future pharmacodynamic and toxicological evaluation of this combination therapy.

C12

Investigation of the mechanism of antiplatelet aggregation effects of senkyunolide A, ligustilide, and butylidenephthalide

Di XIE, Andrew Man Lok CHAN, Ge LIN

Supervisor: Ge LIN

Senkyunolide A (B14), ligustilide (B7), and butylidenephthalide (B5) are three major activeingredients in both Ligusticumchuanxiongand Angelica sinensis. It has been illustrated that these three chemically related compounds showed potent antiplatelet aggregation effects. The present study attempted to delineate the mechanism of theirantiplatelet aggregation effects.

Different platelet aggregation inducers, including collagen, TXA2, thrombin, and ADP,were used for functional tests to evaluate the antiplatelet aggregation effects of these three compounds. Data showed that the sensitivities of the three compounds towards different stimulator-induced platelet aggregation varied significantly,B7 inhibited the platelet aggregation induced by all four inducers, while B5 and B14 mainly inhibited collagen-induced platelet aggregation. Among the three compounds, B7 showed the highest potency of antiplatelet aggregation. These results indicated that the mechanisms of antiplatelet aggregation of the three compounds might be different, because individual inducers triggered different receptors and induced distinct pathways of platelet aggregation.

Further mechanism study investigated the role of Rap1,which is a crucial downstream small GTPase. The GTP-bound active form of Rap1 activates the GP IIb/IIIa receptor, which is the final step of platelet aggregation. Our results demonstrated that B7 alleviated the activation of Rap1in both collagen-induced and thrombin-induced platelet aggregation,whileB14 and B5 only inhibited the Rap 1 activation in collagen-induced platelet aggregation. The results suggested that the three compounds might exert their antiplatelet aggregation effects via regulating Rap 1 or it supstream factors associated with different inducers, and the targets of antiplatelet aggregation of the three compounds might be different.

In conclusion, the present study demonstrated the antiplatelet aggregation effects of the three compounds, and provided useful indication for the furtherin-depth study of the mechanism underlying antiplatelet aggregation.

C13

Identification of novel long non-coding RNAs regulated by Polycomb Repressive Complex 2 in hepatocellular carcinoma

Feiyue XU, Lu FENG, Chi Han LI, Zhangang XIAO, Yangchao CHEN

Supervisor: Yangchao CHEN

Long non coding RNAs (lncRNAs) are being increasingly recognized to contribute to many biological processes through diverse mechanisms in cancer. As a transcriptional repressor, enhancer of zeste homolog 2 (EZH2), a subunit of Polycomb repressive complex 2 (PRC2), silences gene expression via its histone methyltransferase activity. Here, we identified two lncRNAs, DN2 and DN5, were regulated by EZH2 in HCC cell lines. We also found that DN2 and DN5 were down regulated in hepatocellular carcinoma (HCC). DN2 and DN5 promoted cell growth when they were knockdown in vitro. In this study, biological function and regulation mechanisms of DN2 and DN5 will be investigated in HCC.

C14

Investigating Dominant-acting Effects of Cancer-associated PTEN Mutants

Mingfei YAN, Andrew Man Lok CHAN

Supervisor: Andrew Man Lok CHAN

PTEN, a potent tumor suppressor, plays an important role in the pathogenesis of a spectrum of cancers. Previous studies proved that PTEN can homodimerize to exert its lipid phosphatase activity. What's more, cancer-associated PTEN mutants can bind wild-type PTEN and dampen its phosphatase activity in a dominant-negative manner. However, in my study, dominant-acting effects are observed in several cancer-associated PTEN mutants. By transfecting or infecting PTEN-null cancer cell lines like U87, U373 and PC3, higher level of PI3K-AKT pathway activation are observed in several PTEN mutants, like R130Q, I135R, G129E, DC, when compared with empty vector control. This result, in contrast to the previously reported dominant-negative activity, indicates that cancer-associated PTEN mutants may actually function in a dominant-acting manner in driving oncogenesis. Further work is needed in order to elucidate the underlying mechanisms and to provide potential therapeutic applications.

C15

Pyrrolizidine alkaloids-induced hepatotoxicity on hepatic sinusoidal endothelial cells and hepatic parenchymal cells

Mengbi YANG, Jianqing RUAN, Ge LIN

Supervisor: Ge LIN

Exposures to pyrrolizidine alkaloids (PAs)-contaminated food and herbal products are one of the major causes for hepatic sinusoidal obstruction syndrome (HSOS). PAs undergo metabolic activation in the liver to generate electrophilic metabolites: dehydropyrrolizidine alkaloid (DHP) and dehydroretronecine (DHR). These reactive metabolites can bind with proteins to form the pyrrole-protein adducts, leading to hepatotoxicity, or be detoxified by glutathione (GSH). Unlike most hepatic disorders, PA-induced HSOS typically presents as sinusoidal damage followed by parenchymal dysfunction. The current study aims to investigate the selective toxicity of the active PA metabolites between the hepatic sinusoidal endothelial cells (HSEC) and parenchymal cells, and to explore the role of GSH level and pyrrole-protein adduct in this susceptibility difference

The cytotoxicity of two reactive metabolites was examinated in two different cell lines: HSEC and HepG2. Dose-dependent cytotoxicity of DHP and DHR was observed in both cell lines. HSEC was found to be more susceptible to these metabolites compared to HepG2. GSH depletion and pyrrole-protein adduct formation were evaluated. Compared to HepG2, HSEC had significantly lower basal GSH level, and demonstrated quicker and more severe depletion of GSH when exposed to the reactive metabolites. Moreover, HSEC generated significantly greater amount of pyrrole-protein adducts compared to HepG2.

In conclusion, we firstly confirmed in an in vitro cell models that PA metabolites are more toxic to HSEC compared to the parenchymal cell line HepG2, which explained the specific early damage in sinusoids in PA-induced HSOS. Profound depletion of GSH and higher formation rate of pyrrole-protein adducts might partially explain this susceptibility of HSEC to PA intoxication.

[Supported by Research Grant Council of Hong Kong (GRF Grants no.471013 and 469712) and CUHK (Direct Grants no.2041744 and 4054047)].

C16

Reactivation of Growth/Differentiation Factor 1 Contributes to the Chemopreventive Effect of 5-aza-2'deoxycytidine in Gastric Cancer

Weiqin YANG, May S. M. LI, Wei KANG, Li Han ZENG, Haitian WANG, Anthony W. CHAN, Enders K. W. NG, Ka F. TO, Francis K. L. CHAN, Jun YU, Michael W.Y. CHAN, Joseph J. Y. SUNG, Alfred Sze Lok CHENG

Supervisor: Alfred Sze Lok CHENG

INTRODUCTION: The limited benefit of Helicobacter pylori (HP) eradication or nutritional intervention in risk reduction and the dismal prognosis of gastric cancer (GC)underlie the urgent need for new preventive strategies.We have recently shown that Hp causes aberrant DNA methylation of tumor suppressor genes to promote gastric carcinogenesis(ref 1). However, the functional and mechanistic relationships between aberrant DNA methylation and GC remain elusive. **AIMS&METHODS:** We investigated the effect of 5-aza-2'-deoxycytidine (5'Aza-dC), a FDA-approved demethylating agent in a murine GC model induced by N-Nitroso-N-methylurea (MNU). Using MethylCap-microarray and quantitative RT-PCR, we identified novel DNA methylation-controlled genes in paired GC tumors/adjacent tissues, 5'Aza-dC-treated and -untreatednormal mucosa samples, followed by pyrosequencing and gene expression validation in human GC samples and cell lines.Gene methylation levels were examined in clinical samples by pyrosequencingand correlated with patient survival. **RESULT:** At 52weeks-post MNUexposure, GC was developed in 8/19 mice. Administration of 5'Aza-dC for 24 weeks significantly reduced GC incidence from 42.1% to 11.1%. Microarray analysis and qRT-PCR identified that only *growth/differentiation factor 1* (*Gdf1*), a ligand for TGF-β signaling,showingsignificant down-regulated expression in tumors compared to both tumor-adjacent and normal tissues. Notably, 5'Aza-dC treatment reactivated *Gdf1* expression to the normal mucosal level. Ectopic GDF1 expression increased the phosphorylation of SMAD2/3and significantly suppressed GC cell proliferationin vitro and in vivo at least partially through G1 phase cell cycle arrest.Furthermore, GC patients with hypermethylation of *GDF1*correlated with poor overall survival rate. **CONCLUSIONS:** Our findings demonstrate a causal relationship between DNA methylation and GC development. Epigenetic silencing of *GDF1*may abrogate the growth-inhibitory effects of TGF-β signaling and render selective growth advantage to gastric epithelial cells during carcinogenesis. This study lends support to demethylating drugs for GCchemopreventiontrial and identifies a potential biomarker for prognosis.

C17

Investigation of herb-drug interaction between Statins and Danshen-Gegen Decoction on Cardiovascular Disease

Ka Chun YAU, David CHEUNG, Chi Man KOON, Kwok Pui FUNG

Supervisor: Kwok Pui FUNG

Introduction

Statins, HMG-CoA reductase inhibitors, is a class of cardiovascular drug having been used to treat hyperlipidemia. Danshen and Gegen (DG), traditional Chinese medicines, have been used for treating cardiovascular disease.Statins and DG have been shown to inhibit the vascular smooth muscle cell (vSMC) proliferation, which is an important event for the occurrence ofatherosclerosis and cardiovascular disease. Therefore, it is worth investigating the combination use of statins and DGon treating cardiovascular disease and their herb-drug interactions.

Methods

For *in vitro* study, the anti-proliferative effect on vSMC (A7r5) of Atorvastatins and DG were studied by BrdU cell proliferation assay. For *in vivo* study, balloon-injury (BI) on carotid arteries of Sprague Dawley ratwas adopted to study the efficacy of Atorvastatin and DG on intima-media thickening of vessel.

Results

For *in vitro* study, Atorvastatin supplemented with DG has been demonstrated to inhibit vSMC proliferation. Our results revealed that the co-treatment of Atorvastatin and DG could inhibit the proliferation of A7r5 more than those groups treated with Atorvastatin or DG alone. For *in vivo* study, Atorvastatin supplemented with DG has been shown to reduce the vascular wall thickening (intima-media ratio) in rat carotid arteries with BI. Our results demonstrated that BI rats fed by 80 mg/kg Atorvastatin with 300 mg/kg DG combination (-27.34% as compared to control) could be significantly ($P<0.05$) reduced the intima-media ratio more than the sum of the effect of those fed by 80 mg/kg Atorvastatin (-1.12% as compared to control) alone and 300 mg/kg DG alone (-9.85% as compared to control).

Conclusion

To sum up, statins supplemented with DG has been shown synergistically to inhibit the proliferationof vSMCin both *in vitro* and *in vivo* study.

C18

Herbal components, acting as pro-drugs, reverse P-gp-mediated multidrug resistance of anticancer agents

Chun YIN, Kenneth K.W. TO, Xu WU, Stella CHAI, Yang YE, Ge LIN

Supervisor: Ge LIN

Three abundant but ineffective components (POP68, 69 and 70) present in a TCM herb Marsdeniatenacissimawere hypothesized to be biotransformed to the corresponding metabolites (POP62, 63 and 66), which were found to inhibit P-glycoprotein (P-gp). This study aimed to demonstrate how the three most abundant components were biotransformed to the corresponding active metabolites to exert their reversal effect on P-gp-mediated MDR of anticancer drugs in vivo.

Pharmacokinetics of a mixture of POP68, 69 and 70 were evaluated in SD rats. After i.v. injection of the mixture, only the intact POPs were detected in the blood. While, after oral administration of the same mixture, the concentrations of effective metabolites were significantly higher than those of the administered POPs in the blood circulation. Furthermore, oral administration of the POPs mixture to rats pretreated with antibiotics to eliminate intestinal microbiota, such biotransformation was significantly inhibited, indicating that the biotransformation was mediated by intestinal microbiota. Furthermore, the mixture of these POPs was orally administered concomitantly with intravenous injection of paclitaxelto the mice bearing sensitive LCC6 or P-gp-overexpressing LCC6/MDR1 xenograft to investigate the reversal effect of the three abundant POPs. The concurrent administration of paclitaxel with the POPs mixture significantly retarded tumor growth in mice bearing the resistant LCC6/MDR1 xenograft, demonstrating the reversal of MDR.

In conclusion, the abundant but ineffective components from Marsdeniatenacissima, acting as pro-drugs, were biotransformed to the corresponding effective metabolites by intestinal microbiota, leading to MDR reversal effects in vivo.

C19

Identification of Alterations in Host Genome Methylation Driven by the Human Immunodeficiency Virus Type 1

Yinfeng ZHANG, Stephen Kwok Wing TSUI

Supervisor: Stephen Kwok Wing TSUI

Nowadays, the knowledge in DNA methylation-mediated gene regulation has brought a step closer to understand the virus-host interplay in the context of genome alteration. HIV has shown the ability to change the DNA methylation pattern by DNA methyltransferases and therefore to affect host genes transcription. In addition, the methylation status may be correlated with the progression of acquired immunodeficiency syndrome (AIDS). However, the detailed DNA methylation pattern caused by HIV is still not clear. To better understand the precise mechanism, it will be necessary to evaluate the pattern alterations of DNA methylation across the genome. To clarify the alteration of the profiles of host genome methylation caused by HIV infection, we conducted a study on HIV-1 associated genome-wide DNA methylation pattern by using the MeDIP - microarray method. In addition, the identified pattern was validated in T cell lines. A pair of monozygotic twins was recruited in mainland China. One of the twins was infected with HIV while the other was not. Based on the data from NimbleGen DNA Methylation 2.1M Promoter Array, 4679 differentially methylated regions in the HIV positive subject with the peak value more than 3.0 were identified and analyzed. Furthermore, the validation result from T cell line confirmed the efficacy of the DNA methylation patterns well. The hyper-methylation pattern identified by this research project could be a very helpful guidance to reveal the interaction between the host and the virus through DNA methylation while further study would contribute to better understanding of the development of HIV/AIDS.