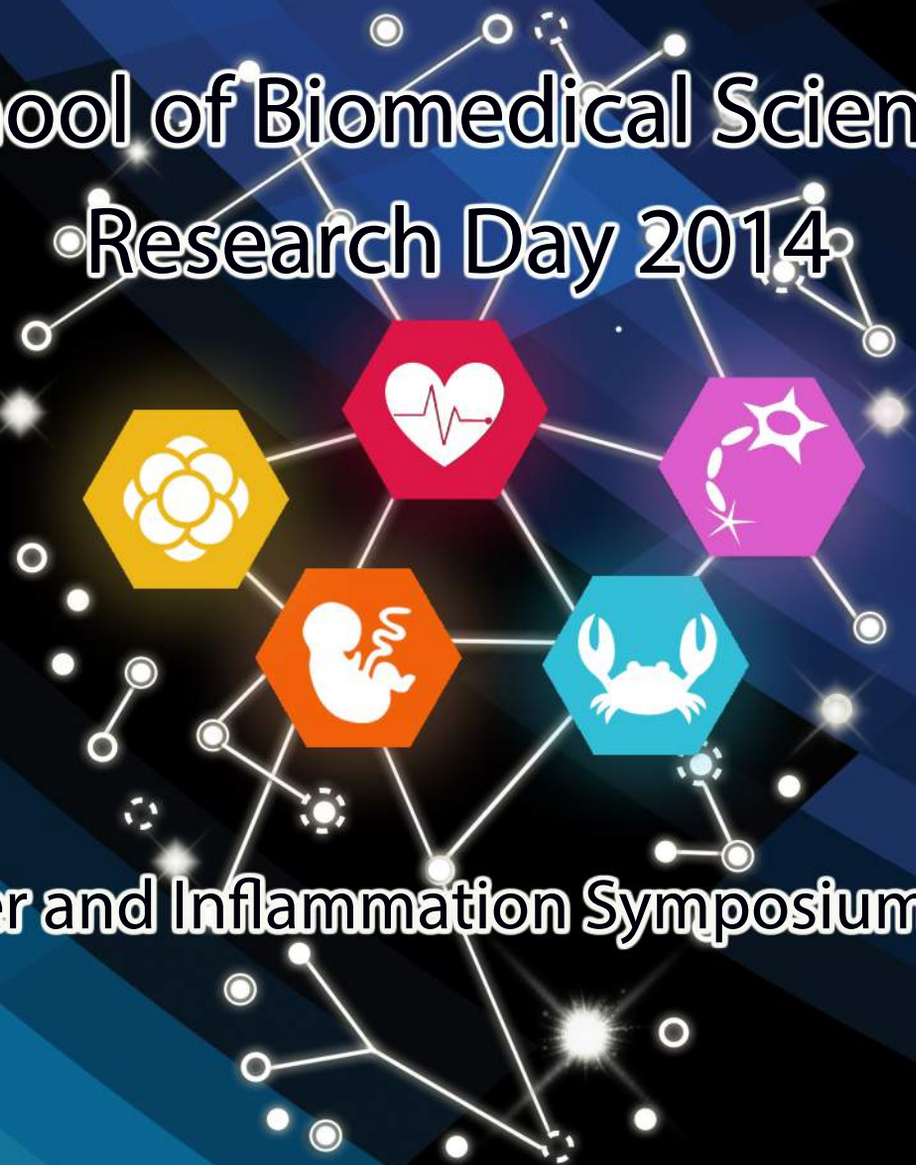
School of Biomedical Sciences Research Day 2014



Cancer and Inflammation Symposium 2014

5 - 6 June 2014

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

香港中文大學
The Chinese University of Hong Kong

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

School of Biomedical Sciences Research Day 2014

Members of the Organizing Committee

Professor Franky L. Chan

Professor Wai Yee Chan

Professor Alfred S.L. Cheng

Professor Wing Tai Cheung

Professor Chi Hin Cho

Professor Xiaohua Jiang

Professor Yiu Wa Kwan

Professor Alaster H.Y. Lau

CONTENTS

Welcome Message from the Dean of Faculty of Medicine

It is with great pleasure to welcome you to the School of Biomedical Sciences Research Day 2014.

Medical researchers worldwide have been trying to understand the intricate interactions between the immune cells, tumor cells and treatment modalities to guide therapeutics to achieve optimal results on the prevention and treatment of cancer, a global health threat. The World Health Organization predicts that by 2035, around 24 million people will have cancer. Developing countries will see the biggest share of preventable new cases due to a shift in lifestyles and other factors. Economic loss from this disease is daunting. The theme of this year "Cancer and Inflammation" is not only a timely response to WHO's warning but also of high relevance to China which a quarter of the world population inhabits.

I would like to congratulate the Organizing Committee on their success in assembling a team of distinguished clinician-researchers and basic medical science researchers to help further our understanding of and to deepen our insights into developing more effective prevention and treatment measures.

Please join me in extending a warm welcome to the three plenary speakers, Professor Rocky Tuan from the University of Pittsburgh, USA; Professor Dongxi Lin from the Chinese Academy of Medical Sciences, The State Key Lab of Molecular Oncology and Professor Phillip Nagley from the Monash University, Australia. We are rejoicing to see 19 delegates from the State Key Lab on Molecular Oncology from Beijing attend the Symposium. Having played leading roles in the battle with cancer in China, they will share their valuable experiences with participants.

I would like to thank the Organizing Committee and the administrative support team for their relentless efforts in attending to every detail to make sure that all participants will be well looked after and feel at home.

The programme is rich and stimulating. I trust that participants will make fruitful and rewarding connections in this cauldron of intellectual ferment, thus opening more doors for mutual support and collaborative work in the years to come.

I am looking forward to meeting you at the opening ceremony.

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

Welcome message from the Director of School of Biomedical Sciences

I am most delighted to welcome you all to the School of Biomedical Sciences (SBS) Research Day 2014 & Cancer and Inflammation Symposium 2014.

This year, it is our honor to have three distinguished guests to give plenary lectures at the Research Day. They are Professor Rocky Tuan from Pittsburgh, Professor Dongxin Lin from Beijing and Professor Phillip Nagley from Monash. I would like to extend the warmest welcome to the Plenary Speakers, Guests and School members for joining this annual flagship event.

A delegation of 19 researchers led by Professor Dongxin Lin from the State Key Laboratory of Molecular Oncology, Chinese Academy of Medical Sciences, Beijing is also joining us this year. Since 2011, the State Key Laboratory of Molecular Oncology and our Cancer & Inflammation Thematic Programme had jointly organized annual symposium in inflammation and cancer held alternately between Beijing and Hong Kong. To solidify our partnership, a formal Memorandum of Understanding (MOU) will be signed immediately after the Cancer and Inflammation Symposium to-morrow.

This is our fifth Research Day. We have gone a long way since the formation of the School. With the increasing scholarly interactions and scientific exchanges amongst investigators both regionally and internationally, we are witnessing continuous growth in our research capacity and strength. The landscape of biomedical research is also changing very fast. Both research areas and research funding modalities take on a new look almost every year. To stay competitive, we have to grow with this evolving scene. It is our hope the Research Day will be able to provide the platform for colleagues to share information of the newest development and discoveries in science and to serve as the conduit to facilitate and enhance collaborations.

I would like to take this opportunity to thank members of the Organizing Committee of the Research Day whose thoughtful planning and unselfish dedication make this event a success. I would also like to thank all staffs who help prepare the meeting site and social events. The Research Day is not possible without the generous support provided by all sponsors.

I sincerely hope you will enjoy and find inspiration in the exciting programmes in the coming two days.

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

Biography of Plenary Speaker

Rocky S. Tuan, PhD

- Director, Center for Cellular and Molecular Engineering
- Arthur J. Rooney, Sr. Professor and Executive Vice Chair, Department of Orthopaedic Surgery
- Associate Director, McGowan Institute for Regenerative Medicine
- Director, Center for Military Medicine Research
- Professor, Departments of Bioengineering and Mechanical Engineering & Materials Science, University of Pittsburgh, Pittsburgh, Pennsylvania

Rocky S. Tuan, PhD, received his PhD in 1977 from the Rockefeller University in New York, under the mentorship of the late Zanvil A. Cohn, MD. His postdoctoral research fellowship was at Harvard Medical School in Boston, first with Melvin J. Glimcher, MD in the Department of Orthopaedic Surgery at the Children's Hospital, and then from 1978 to 1980 with Jerome Gross, MD, in the Developmental Biology Laboratory at the Massachusetts General Hospital. In 1980, Dr. Tuan was appointed as Assistant Professor in the Department of Biology, University of Pennsylvania in Philadelphia, and was promoted to Associate Professor in 1986. In 1988, Dr. Tuan joined Thomas Jefferson University, Philadelphia, to be the Director of Orthopaedic Research and Professor and Vice Chairman in the Department of Orthopaedic Surgery with a joint appointment in the Department of Biochemistry and Molecular Biology. From 1992-1995, Dr. Tuan was the Academic Director of the MD/PhD program at Jefferson, and in 1997, he established the USA's first Cell and Tissue Engineering PhD program at Jefferson, with the mission of training the next generation of "cross-cultural" biomedical scientists committed to regenerative medicine and the development of functional tissue substitutes. In the fall of 2001, Dr. Tuan joined the Intramural Research Program of the National Institute of Arthritis, and Musculoskeletal and Skin Diseases (NIAMS), National Institutes of Health (NIH), as Chief of the newly created Cartilage Biology and Orthopaedics Branch. In 2004, Dr. Tuan received the Marshall Urist Award for Excellence in Tissue Regeneration Research of the Orthopaedic Research Society. In the Fall of 2009, Dr. Tuan was recruited by the University of Pittsburgh School of Medicine to be the Founding Director of the Center for Cellular and Molecular Engineering, and as Arthur J. Rooney, Sr Chair Professor and Executive Vice Chairman of the Department of Orthopaedic Surgery, with a joint appointment as Professor in the Department of Bioengineering. Dr. Tuan is currently Co-Director of the Armed Forces Institute of Regenerative Medicine, a U.S. Department of Defense funded, national, multi-institutional consortium focused on developing regenerative therapies for battlefield injuries. Two recent appointments at Pitt include (1) Associate Director of the McGowan Institute for Regenerative Medicine, and (2) Founding Director of the Center for Military Medicine, both at the University of Pittsburgh. Dr. Tuan has published over 400 research papers, has lectured extensively, and is currently Editor of the developmental biology journal, BDRC: EMBRYO TODAY, and the Founding Editor-in-Chief of STEM CELL RESEARCH AND THERAPY.

Dr. Tuan directs a multidisciplinary research program, which focuses on orthopaedic research as a study of the biological activities that are important for the development, growth, function, and health of musculoskeletal tissues, and the utilization of this knowledge to develop technologies that will regenerate and/or restore function to diseased and damaged skeletal tissues. Ongoing research projects are directed towards multiple aspects of skeletal and related biology, including skeletal development, stem cells, growth factor signaling, bone-biomaterial interaction, extracellular matrix and cell-matrix interaction, nanotechnology, biomaterials, 3D printing, mechanobiology, regenerative medicine, and tissue engineering, utilizing an integrated experimental approach combining contemporary technologies of biochemistry, cell and molecular biology, embryology and development, cellular imaging, and engineering.

Biographies of Plenary Speakers

Dr. Dongxin Lin is a professor and the director of Department of Etiology & Carcinogenesis, Cancer Institute and Hospital, Chinese Academy of Medical Sciences (CAMS) & Peking Union Medical College (PUMC), and one of the senior principal investigators of the State Key Laboratory of Molecular Oncology. He is an Academician of Chinese Academy of Engineering.

He graduated from the Graduate School of Beijing Medical University in 1986 and became an assistant professor at Fujian Medical University in 1987. He was a visiting scientist at WHO International Agency for Research on Cancer (IARC) between 1988 and 1990 and at FDA National Center for Toxicological Research (NCTR) between 1990 and 1994. He returned to China in 1995 and became a professor and the department director in 1997 at Cancer Institute and Hospital, CAMS/PUMC.

His major research interest is molecular genetics and molecular epidemiology of human cancer, focusing on the identification of genetic susceptibility loci associated with the development and progression of human cancers including esophageal cancer, pancreatic cancer, gastric cancer, liver cancer as well as lung cancer using candidate gene approaches and genome-wide association studies. His contributions to cancer genetics and molecular cancer epidemiology can be easily identified from his more than 210 publications including 13 papers published in *Nature Genetics* and 17 papers published in *Cancer Research*.

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Professor Phillip Nagley is an adjunct with the Department of Biochemistry and Molecular Biology at Monash University, where he holds the title of Professor Emeritus (having retired at the end of 2012). His research career spans more than 45 years, mostly at Monash University, in Melbourne, Australia. He is also Honorary Professorial Fellow at Florey Institute of Neuroscience and Mental Health, University of Melbourne.

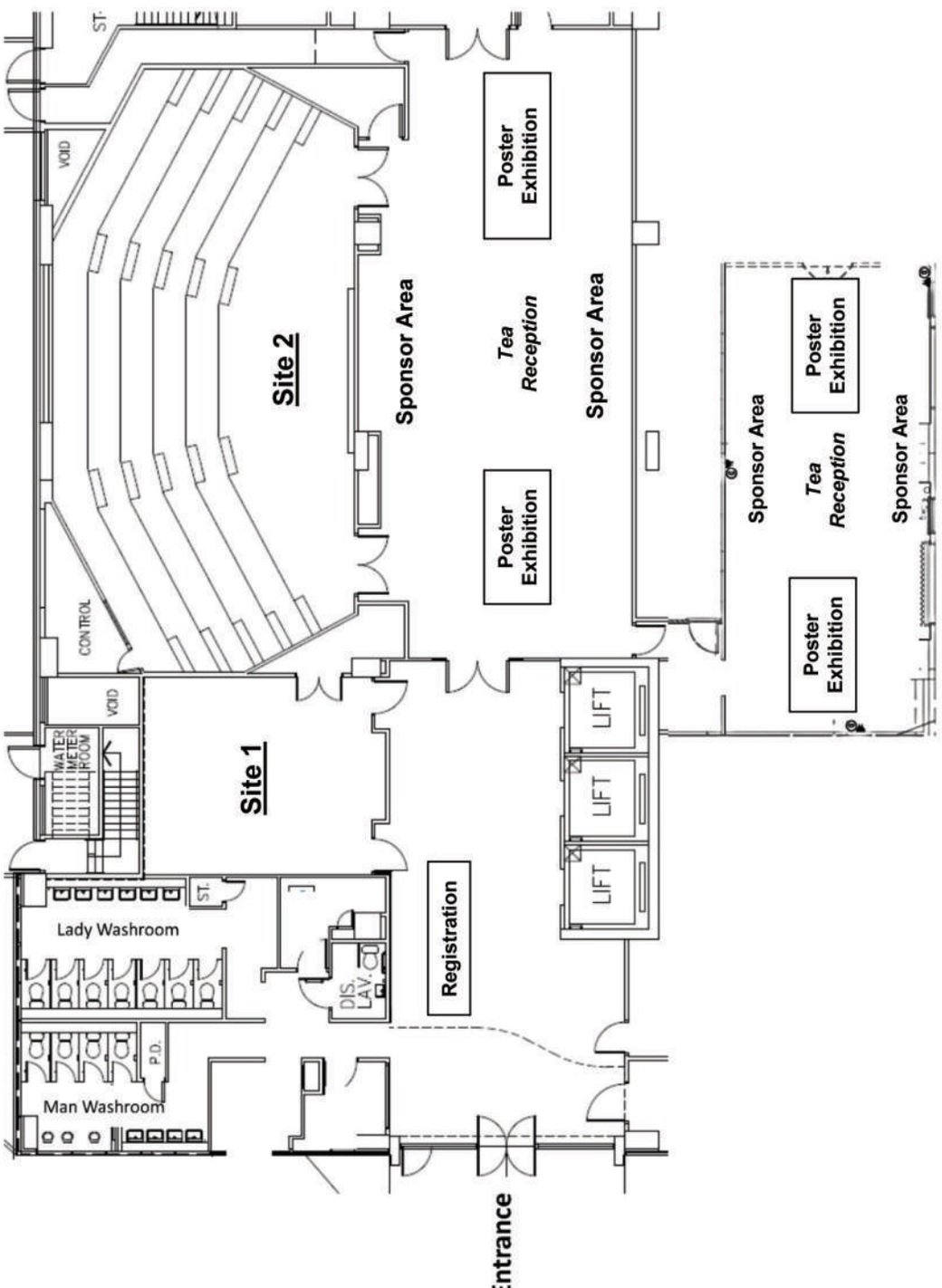
He obtained his BSc (Hons) and MSc degrees from the University of Sydney in the 1960s and graduated PhD from Monash University in 1972. He was awarded DSc in 1986 at Monash University.

Professor Nagley is best known for his work on mitochondria, in both yeast and mammalian cells, where he has studied many topics relating to the formation of mitochondria as well as the role of mitochondria in cell death. His contributions to neuroscience over the past 10 years have been involved with studies on how neurons respond to stress. He has characterised the different sorts of cell death that neurons undergo, using various cellular models of neuronal development and neurodegenerative diseases (including those relevant to stroke and motor neurone disease).

He is a former President of the Australian Society for Biochemistry and Molecular Biology. He is currently Secretary General of FAOBMB.

Map of the Meeting Venue

G/F Lo Kwee-Seong Integrated Biomedical Sciences Building



Programme Summary

SBS Research Day 2014
5 June 2014 (Thursday)

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

09:15-09:30 Photo taking

09:30-10:15 Plenary Lecture by Prof. Rocky S. Tuan, Room G02, Lo Kwee-Seong Integrated Biomedical Sciences Building

Time	*Site 1 (Room G01)*	*Site 2 (Room G02)*
	Cancer & Inflammation (I)	***Reproduction, Development & Endocrinology (I)***
10:15-10:40	Prof. Kwok Pui Fung	Prof. Sidney S.B. Yu
10:40-11:05	Prof. Tzi Bun Ng	Prof. Christopher H.K. Cheng

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

	Cancer & Inflammation (II)	***Reproduction, Development & Endocrinology (II)***
11:30-11:55	Prof. Jun Yu	Prof. Tin Lap Lee
11:55-12:20	Prof. Wing Tai Cheung	Prof. Yin Xia

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

13:20-14:20 ***Poster Presentation Session***

	Cancer & Inflammation (III)	***Reproduction, Development & Endocrinology (III)***
14:20-14:45	Prof. Alaster H.Y. Lau	Prof. Hsiao Chang Chan
14:45-15:10	Prof. Ge Lin	Prof. Wing Hung Ko

	PI Sharing Session: Room G02
15:15-15:50	Professors Andrew M. Chan, Alfred S.L. Cheng, Bo Feng, Xiaohua Jiang, Tin Lap Lee, Eugene Ponomarev & Yin Xia

15:50-16:15 ***Tea Break & Poster Viewing***

	Cancer & Inflammation (IV)	***Reproduction, Development & Endocrinology (IV)***
16:15-16:40	Prof. Stephen K.W. Tsui	Prof. Po Sing Leung
16:40-17:05	Prof. Chi Fai Ng	Prof. Wood Yee Chan
17:05-17:30	Prof. Qian Tao	Prof. Hui Zhao

17:30-18:20 SBS Tour - Assemble outside Room G02

19:00 Conference Dinner (by invitation)

Programme (Site 1)

Room G01, Lo Kwee-Seong Integrated Biomedical Sciences Building

Time	*Title of Presentation*	*Name of Speaker*	*Abstract No.*
	Cancer & Inflammation (I) ***Chairpersons: Prof. Andrew M. Chan and Prof. Jun Yu***		
10:15-10:40	Photodynamic therapy of pheophorbide a on human breast tumour and Methicillin-Resistant Staphylococcus Aureaus (MRSA) infection	Prof. Kwok Pui Fung	S1-01
10:40-11:05	The ribosome inactivating protein trichosanthin promotes apoptosis of breast cancer cells *in vitro* and in nude mice	Prof. Tzi Bun Ng	S1-02
11:05-11:30	***Tea Break & Poster Viewing***		
	Cancer & Inflammation (II) ***Chairpersons: Prof. Alfred S.L. Cheng and Prof. Nathalie Wong***		
11:30-11:55	Characterization of genomic and epigenomic aberrations in experimental obesity-associated hepatocellular carcinoma	Prof. Jun Yu	S1-03
11:55-12:20	G protein-independent suppressor activity of GPCR MAS	Prof. Wing Tai Cheung	S1-04
12:20-13:20	***Lunch Break***		
13:20-14:20	***Poster Presentation Session***		
	Cancer & Inflammation (III) ***Chairpersons: Prof. Yangchao Chen and Prof. Kenneth K.W. To***		
14:20-14:45	Differential effects of toll-like receptor 2 agonists on substance P induced mast cell activation	Prof. Alaster H.Y. Lau	S1-05
14:45-15:10	Translational science: Development of the mechanism-based bio-monitoring marker for clinical diagnosis of pyrrolizidine alkaloid intoxication	Prof. Ge Lin	S1-06
	PI Sharing Session (Please proceed to Room G02) ***Chairperson: Prof. Chi Hin Cho***		
15:15-15:50	Professors Andrew M. Chan, Alfred S.L. Cheng, Bo Feng, Xiaohua Jiang, Tin Lap Lee, Eugene Ponomarev & Yin Xia		
15:50-16:15	***Tea Break & Poster Viewing***		
	Cancer & Inflammation (IV) ***Chairpersons: Prof. Franky L. Chan and Prof. Kwok Wai Lo***		
16:15-16:40	Transcriptional regulation of the tumor suppressor FHL2 by p53 in human kidney and liver cells	Prof. Stephen K.W. Tsui	S1-07
16:40-17:05	Application of new biomarkers for the diagnosis and management of prostate cancer in Chinese population	Prof. Chi Fai Ng	S1-08
17:05-17:30	Epigenetic identification of receptor tyrosine kinase-like orphan receptor 2 as a functional tumor suppressor inhibiting β-catenin and AKT signaling but frequently methylated in common carcinomas	Prof. Qian Tao	S1-09
17:30-18:20	SBS Tour – Assemble outside Room G02		
19:00	Conference Dinner (by invitation)		

Programme (Site 2)

Room G02, Lo Kwee-Seong Integrated Biomedical Sciences Building

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

09:15-09:30 Photo taking

09:30-10:15 Plenary Lecture by Prof. Rocky S. Tuan (Abstract No. PL-01)
"Principles and Applications of Adult Stem Cell Based Tissue Engineering and Regeneration"
Chairperson: Prof. Wai Yee Chan

Time	*Title of Presentation*	*Name of Speaker*	*Abstract No.*
	Reproduction, Development & Endocrinology (I) ***Chairpersons: Prof. Po Sing Leung and Prof. Juliana Chan***		
10:15-10:40	HCV NS5A induces ER-mitochondria tethering	Prof. Sidney S.B. Yu	S2-01
10:40-11:05	Insulin-like growth factors serve as mediators of luteinizing hormone action on oocyte maturation in zebrafish	Prof. Christopher H.K. Cheng	S2-02

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

	Reproduction, Development & Endocrinology (II) ***Chairpersons: Prof. Christopher H.K. Cheng and Prof. Pak Cheung Ng***		
11:30-11:55	Epigenetic memory in spermatogoinal stem cell development	Prof. Tin Lap Lee	S2-03
11:55-12:20	Dragon (Repulsive Guidance Molecule RGMb) induces apoptosis in renal tubular epithelial cells	Prof. Yin Xia	S2-04

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

13:20-14:20 ***Poster Presentation Session***

	Reproduction, Development & Endocrinology (III) ***Chairpersons: Prof. Wood Yee Chan and Prof. Ting Fan Leung***		
14:20-14:45	Multi-signaling pathways underlie tumor-suppressing effect of CFTR	Prof. Hsiao Chang Chan	S2-05
14:45-15:10	G protein-coupled estrogen receptor (GPER/GPR30) activation inhibits P2Y receptor-mediated Ca^{2+} signalling and cytokine secretion in human bronchial epithelia	Prof. Wing Hung Ko	S2-06

	PI Sharing Session ***Chairperson: Prof. Chi Hin Cho***
15:15-15:50	Professors Andrew M. Chan, Alfred S.L. Cheng, Bo Feng, Xiaohua Jiang, Tin Lap Lee, Eugene Ponomarev & Yin Xia

15:50-16:15 ***Tea Break & Poster Viewing***

	Reproduction, Development & Endocrinology (IV) ***Chairpersons: Prof. Wing Hung Ko and Prof. Nelson Tang***		
16:15-16:40	Modulatory actions of vitamin D on the pancreatic renin-angiotensin system	Prof. Po Sing Leung	S2-07
16:40-17:05	Migration of sacral neural crest cells in *Dominant Megacolon*, a mouse model for human Hirschsprung's disease	Prof. Wood Yee Chan	S2-08
17:05-17:30	The proto-oncogene transcription factor ets1 regulates neural crest development through mediating output of BMP signaling with histone deacetylase 1 (HDAC1)	Prof. Hui Zhao	S2-09

17:30-18:20 SBS Tour – Assemble outside Room G02

19:00 Conference Dinner (by invitation)

PL-01

Principles and applications of adult stem cell based tissue engineering and regeneration

Rocky S. Tuan

Director, Center for Cellular and Molecular Engineering, and Professor and Executive Vice Chairman, Department of Orthopaedic Surgery, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA.

The intrinsically low capacity of cartilage for tissue repair and regeneration is a clinical challenge to effective treatment of degenerative joint diseases, such as osteoarthritis, the main cause of physical disability. Tissue engineering and regenerative medicine represent a potentially promising approach. The principal requirements are cells, scaffolds, and biological signals. Adult stem cells, such as mesenchymal stem cells (MSCs), may be harvested from autologous tissues sources, including bone marrow and adipose. MSCs have the ability to undergo multi-lineage differentiation, including chondrogenesis, and are actively being investigated as a candidate cell type for cartilage repair. Critical to successful cell-based tissue engineering is the use of a biocompatible biomaterial scaffold that ideally also enhances proliferation and differentiation of the seeded cells. Biomimetic scaffolds that simulate the structure of native extracellular matrix, e.g., the nanoscalar fibrous nature of collagen, have shown promise in skeletal tissue engineering using MSCs both in vitro and in vivo. Recent work on the use of custom-designed, photo-crosslinked hydrogel scaffolds, which allow cell encapsulation during fabrication, demonstrates high fidelity reproduction of internal structure and excellent cell retention, viability, and differentiation. We are currently applying a 3D printing approach and a custom-designed microbioreactor to construct a microphysiological tissue analogue of the osteochondral junction, based entirely on MSC-derived components, to model the pathogenesis of osteoarthritis. Taken together with their differentiation potential and recently discovered trophic activities, adult stem cells thus present a powerful platform for regenerative, therapeutic, and disease modeling applications in biomedicine.

S1-01

Photodynamic therapy of pheophorbide a on human breast tumour and Methicillin-Resistant *Staphylococcus Aureaus* (MRSA) infection

B.Y. Wang[1], V.K.M. Lau[2], S.W.H. Hoi[1], J.Y.W. Chan[2], B.C.L. Chan[2], J.C.M. Koon[2], G.G.L. Yue[2], J.L. Jiang[1], D.W.S. Cheung[1], S.L. Lui[2,3], C.K. Wong[2,4], M. Ip[3], K.P. Fung[1,2]

[1]School of Biomedical Sciences, [2]Institute of Chinese Medicine, [3]Department of Microbiology and [4]Department of Chemical Pathology, The Chinese University of Hong Kong, Hong Kong SAR, P.R. China.

Using bioassay guided method, our group has identified a photosensitizer, Pheophorbide a (Pa), a chlorophyll derivative, from Traditional Chinese Medicine *Scutellaria barbata* and investigated its photodynamic therapy (PDT) effect on human breast cancer and MRSA infection. The IC_{50} of Pa on human breast tumour MCF-7 cells was 0.5 μM after 24 h incubation post Pa-PDT treatment. Mechanistic studies demonstrated that Pa was localized in the mitochondria and reactive oxygen species (ROS) were generated in MCF-7 cells after PDT treatment. Induction of apoptosis was found to be the major mechanism of tumour cell death. Mitochondrial membrane depolarization and cytochrome *c* release indicated the critical role of mitochondria in Pa-PDT induced apoptosis. Increased expression of tumour suppressor protein p53, cleavage of caspase-9, caspase-7 and Poly (ADP-ribose) polymerase indicated the involvement of caspase-dependent apoptosis pathway. On the other hand, release of apoptosis-inducing factor demonstrated the mediation of caspase-independent apoptosis pathway. Pa-PDT could also exhibit anti-angiogenesis, at 0.1 to 0.5 μM of Pa, Pa-PDT could suppress the cell growth, migration and tube formation of HMEC-1 cells in a dose-dependent manner. In the MCF-7 nude mice xenograft model, short drug-light interval, namely 15 minutes light exposure with Pa at 2.5 mg/kg could cause vascular damages and tumor growth inhibition and apoptosis. In contrast, long drug-light interval, namely 3 h light exposure with same Pa dosage, enhanced neovascularization that favors tumor growth. We also found that Pa showed immunostimulating effect on a murine macrophage cell line RAW 267.4 without irradiation. Pa could significantly stimulate the growth of RAW 264.7 cells with a maximum effect at 1.0 μM after 24, 48 and 72 h incubation post treatment. Intracellular mitogen activated protein kinases (MAPK) such as extracellular signal-regulated kinase (ERK) and p38 MAPK were activated by Pa treatment in a dose-dependent manner, these activations might related to the Pa-induced production of intracellular ROS. Furthermore, Pa could significantly induce the release of interleukin-6 and tumour necrosis factor-α, and promote the phagocytosis of RAW264.7 cells.

In the aspect of anti-MRSA infection, we examined the effect of Pa-PDT on different ATCC MRSA strains and the most prevalent clinical MRSA strains, including hospital acquired MRSA (HA-MRSA) which always come up with multidrug resistance, as well as community acquired MRSA (CA-MRSA) which are characterized by high susceptibility to most antibiotics except methicillin. Preliminary results showed that Pa-PDT could exhibit significant growth inhibition on MRSA strains in a dose-dependent manner. Further studies for the probable wound healing activity of Pa-PDT in a murine model of MRSA-infected wound and the probable immunomodulatory activity of Pa alone on the infected host will be carried out. These studies were supported by grants from GRF (project code: 464507) and HMRF (project code 13120302) of Hong Kong Government.

S1-02

The ribosome inactivating protein trichosanthin promotes apoptosis of breast cancer cells *in vitro* and in nude mice

T.B. Ng, E.F. Fang, J.H. Wong, X.L. Dan, Y.S. Chan, W.L. Pan, C.M. Yin, R.C.F. Cheung

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

Breast cancer is a common malignant disease in females with a rising incidence as well as a high risk of metastasis and relapse. Translational and laboratory-based clinical investigations of new/novel drugs are in progress. Medicinal plants are rich sources of biologically active natural products for drug development. Trichosanthin (TCS) is a ribosome inactivating protein purified from tubers of the Chinese herbal plant *Trichosanthes kirilowii* (common name Tian Hua Fen). In this study, we found that TCS manifested anti-proliferative and apoptosis-inducing activities in both estrogen-dependent human MCF-7 cells and estrogen-independent MDA-MB-231 cells. Flow cytometric analysis disclosed that TCS induced cell cycle arrest. Further studies revealed that TCS-induced apoptosis of the breast cancer cells was attributed to activation of both caspase-8 and caspase-9 regulated pathways. The subsequent events including caspase-3 activation, and increased PARP cleavage. With regard to cell morphology, stereotypical apoptotic features were observed. Moreover, in comparison with control, TCS-treated nude mice bearing MDA-MB-231 xenograft tumors exhibited a significantly reduced tumor volume and tumor weight, due to the potent effect of TCS on tumor cell apoptosis as determined by the increase of caspase-3 activation, PARP cleavage, and DNA fragmentation using immunohistochemistry. Hence TCS is potentially exploitable in the therapy of patients with estrogen-dependent and/or estrogen-independent breast cancers.

S1-03

Characterization of genomic and epigenomic aberrations in experimental obesity-associated hepatocellular carcinoma

Jun Yu

Institute of Digestive Disease and Department of Medicine and Therapeutics, State Key Laboratory of Digestive Disease, Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong SAR, P.R. China.

Non-alcoholic fatty liver disease (NAFLD) represents one of the most common liver disorders in developed societies, including Hong Kong. The high prevalence of NAFLD is related to the rising trends of obesity and metabolic syndrome. The population prevalence of NAFLD in Hong Kong Chinese is 27.3%. It is strongly associated with obesity, insulin resistance and dyslipidemia, and is thus regarded as the liver manifestation of metabolic syndrome. Epidemiological studies have shown that obesity plays an important role in the carcinogenesis of hepatocellular carcinoma (HCC). However, the underlying genetic mechanism how obesity promotes HCC development is still unclear. In order to identify the genomic alterations specifically in NAFLD-related HCC, we established two obese animal models: genetic obese mouse model (db/db) and dietary mouse model (C57/BL6 mice fed with high fat diet). Mice were treated with diethylnitrosamine at age of 13-15 days and were harvested after 7 months. As expected, obese mice developed significantly more HCC than control lean mice (100% in db/db *vs* 23% in wt mice, $P< 0.001$; 100% in HFD *vs* 40% in control mice, $P< 0.001$). We therefore performed the integrative genome study to delineate the pathogenic mechanisms of NAFLD-related HCC. By comparing the exome, gene expression array and epigenome data of different NAFLD-HCC and lean HCC, we pinpointed specific novel genetic alternations and signaling pathways associated to NAFLD-HCC development. In conclusion, we delineated molecular components and signaling cascades driving the development of NAFLD-HCC. This leads to discover the clinically-relevant druggable targets to improve the management of NAFLD.

S1-04

G protein-independent suppressor activity of GPCR MAS

J.X. Sun[1], H.K. Yeung[1], M.K. Teng[1], Y.M. Chan[1], L. Zhang[1], S.S.T. Lee[2], Y.Y. Ho[3], N.M. Zhou[4], W.H. Ko[1], W.T. Cheung[1]

[1]School of Biomedical Sciences, [2]School of Life Sciences, The Chinese University of Hong Kong, Shatin, Hong Kong SAR. [3]Genetics of Complex Disorder Program, Departments of Biostatistics and Psychiatry, Columbia University, New York, USA. [4]College of Life Sciences, Zhejiang University, Hangzhou, P.R. China.

Mas oncogene is originally identified from a human epidermoid carcinoma, and is predicted to encode a G protein-coupled receptor. Overexpression and/or somatic mutations of *mas* gene have been noted in human lung, ovarian, and colon cancers. Pharmacological studies suggest MAS can be activated by angiotensin peptides and RFamide neuropeptides. In addition, we further demonstrated that GPCR MAS can be activated by a surrogate peptide named as MBP7. Bioinformatics analysis indicates that a MBP7-like motif is present in purinergic P2Y receptor and glucose transporter. Recently, our studies have indicated that overexpression of MAS in CHO cells suppressed P2Y receptor-mediated calcium mobilization. However, expression of P2Y2 receptors was not significantly altered by overexpression of MAS as evidenced by Western and RT-PCR. Of interest, GPCR MAS overexpression also suppressed the glucose uptake activity of glucose transporter GLUT1. Co-immunoprecipitation study indicated that MAS and GLUT1 were physically in contact. These results suggest that GPCR MAS modulates the P2Y and GLUT1 activity via interacting with a MBP7-like motif, bypassing the heterotrimeric G protein-mediated signalling pathways.

S1-05

Differential effects of the toll-like receptor 2 agonists on substance P induced mast cell activation

H.Y.A. Lau, Y.Y. Yu

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

Mast cells play important roles in innate immunity through their activation via toll-like receptors (TLRs) but also contribute to neuroimmunological responses and inflammation through their activation by the neuropeptide substance P (SP) via Gαi/o proteins. We aim to compare the effects of the TLR2 agonists peptidoglycan (PGN) and tripalmitoyl-S-glycero-Cys-(Lys)4 (Pam3CSK4) on SP induced human mast cell activation. The human mast cell line LAD2 was employed and mast cell activation was determined by assays of β-hexosaminidase, IL-8 and intracellular calcium. TLR2 agonists did not cause degranulation, but induced the release of IL-8. Pretreatment of PGN and Pam3CSK4 inhibited SP induced degranulation but only Pam3CSK4 blocked SP induced calcium mobilization. SP induced IL-8 release was synergistically enhanced by PGN but abolished by Pam3CSK4. Studies with inhibitors of key enzymes implicated in mast cell signaling revealed that synergistic release of IL-8 induced by PGN and SP involved calcineurin, ERK, NF-κB and PI3K signaling cascades whereas Pam3CSK4 inhibited SP induced mast cell activation by interfering with the interaction between SP and Gαi/o proteins. These findings suggest that activation of human mast cells can be differentially modified by TLR2 agonists via distinct signaling pathways through facilitating formation of different TLR2 heterodimers with other TLRs.

S1-06

Translational science: Development of the mechanism-based bio-monitoring marker for clinical diagnosis of pyrrolizidine alkaloid intoxication

L. Zhu, J.Q. Ruan, J.Y. Xue, G. Lin

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

Nowadays, safety of natural products becomes a significant issue with a public consensus in that nature is not always 'good, clean, and healthy'. In the present study, using pyrrolizidine alkaloid (PA)-induced hepatotoxicity as an example, our methodical translational studies, from the identification of clinical problems, to laboratory basic science for finding biomarkers, and then back to clinical assessment, will be presented. PAs widely distribute in about 3% of flowering plants worldwide. Intake of PA-containing/contaminated natural products causes numerous cases of liver injury, especially hepatic sinusoidal obstruction syndrome (HSOS) worldwide. Our recent study revealed that about 75% of retail honey purchased in Hong Kong contained PAs, indicating a potential high risk for the daily exposure to PAs. PAs are pro-toxins and induce hepatotoxicity via metabolic activation to generate toxic pyrrolic metabolites, which are chemically reactive and react rapidly with cellular macromolecules to form pyrrole-protein and pyrrole-DNA adducts leading to hepatotoxicity and tumorigenicity. The delineation of the toxic mechanism of PAs and development of the mechanism-based bio-monitoring marker for clinical diagnosis and assessment of the hepatotoxicity caused by the intake of PA-containing herbal medicines and PA-contaminated foodstuffs will be illustrated.

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S1-07

Transcriptional regulation of the tumor suppressor FHL2 by p53 in human kidney and liver cells

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Four and a Half LIM protein 2 (FHL2) is a LIM domain only protein that is able to form various protein complexes and regulate gene transcription. Recent findings showed that FHL2 is a potential tumor suppressor gene that was down-regulated in hepatocellular carcinoma (HCC). Moreover, FHL2 can bind to and activate the TP53 promoter in hepatic cells. In this study, the activity of the two promoters of FHL2, 1a and 1b, were determined in the human embryonic kidney cell line HEK293 and the activation of these two promoters by p53 was investigated. Our results showed that the 1b promoter has a higher activity than the 1a promoter in HEK 293 cells but the 1a promoter is more responsive to the activation by p53 when compared with the 1b promoter. The same differential action could be also observed in liver Hep3B cells. Combining promoter activity results of truncated mutants and predictions by bioinformatics tools, a putative p53 binding site was found in the exon 1a of FHL2 from +213 to +232. Furthermore, the expression of FHL2 and TP53 were down-regulated in majority of HCC tumour samples (n=41) and significantly correlated (*P* = 0.026). Finally, we found that the somatic mutation 747 (G→T), a hot spot mutation of the TP53 gene, is potentially associated with a higher expression of FHL2 in HCC tumour samples. Taken together, this is the first in-depth study about the transcriptional regulation of FHL2 by p53.

S1-08

Application of new biomarkers for the diagnosis and management of prostate cancer in Chinese population

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Introduction

Prostate cancer (PCa) has becoming an increasing important health care problem in Asian population in the past decades. Currently, it is ranked number three in the incidence of male cancer in Hong Kong. The use of serum prostate specific antigen (PSA) has greatly improved the diagnosis of early PCa and about 2/3 of our prostate cancer patients was now diagnosed by PSA-based care. However, the low specificity of PSA for PCa diagnosis inevitably resulted in many unnecessary stress and morbidities to patients during the workup of patients with elevated serum PSA level. Because of the diversity in the natural history of early PCa, some patients with low risk cancer may not necessary need to have invasive treatment. Currently there is a long list of biomarkers developed to attempt to improve the diagnosis and also stratification of PCa patients. Therefore, we would like to share the experience of using two tests, urine prostate cancer antigen-3 (PCA-3) and pro-PSA / Prostate Health Index (Phi) for the diagnosis and risk stratification of local Chinese patients.

Methodology

Chinese male patient suspected to have PCa and pending for prostatic biopsy would be recruited for these studies. Before they underwent biopsy, serum and post-prostatic massage urine (PPMU) would be collected. A group of patients with clinical benign prostate hyperplasia with no evidence of PCa were also recruited as control for PCA-3 test. The result of PCA3 and Phi was then used to correlate with the diagnosis of PCa and also the pathological staging. A separate group of patients with confirmed PCa underwent radical prostatectomy would also be recruited. Their preoperative paremeters, including Phi, would be used to correlate with the final pathology results.

Results

We established the best cut-off for the PCA3 ratio (defined as the ratio of the Ct value of PCA3/PSA mRNA) was 1.127. Applying this cut-off to the 47 patients with clinically suspected PCa, the sensitivity and specificity of PCA3 for diagnosing PCa were 71% and 92%, respectively. Another 230 consecutive patients, with 21 (9.13 %) diagnosed with PCa, were recruited. The areas under the curve of the receiver operating characteristic curve for total PSA, PSA density, and phi were 0.547, 0.634 and 0.781, respectively. At a sensitivity of 90 %, the use of the phi could have avoided unnecessary biopsies in 104 (45.2 %) patients. Both PCA3 and Phi was shown to correlate with the aggressiveness of tumour.

Conclusion

The application of new biomarkers, PCA3 and Phi, helped to avoid unnecessary biopsy in patients suspected to have PCa. They could also help to stratify the risk of individual patients and guided the treatment planning for individual patients.

S1-09

Epigenetic identification of receptor tyrosine kinase-like orphan receptor 2 as a functional tumor suppressor inhibiting β-catenin and AKT signaling but frequently methylated in common carcinomas

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Through subtraction of tumor-specific CpG methylation, we identified receptor tyrosine kinase-like orphan receptor 2 (ROR2) as a candidate tumor suppressor gene (TSG). ROR2 is a specific receptor or co-receptor for WNT5A, involved in canonical and non-canonical WNT signaling, with its role in tumorigenesis controversial. We characterized its functions and related cell signaling in common carcinomas. *ROR2* was frequently silenced by promoter CpG methylation in multiple carcinomas including nasopharyngeal, esophageal, gastric, colorectal, hepatocellular, lung, and breast cancers, while no direct correlation of *ROR2* and *WNT5A* expression was observed. Ectopic expression of *ROR2* resulted in tumor suppression independent of WNT5A status, through inhibiting tumor cell growth and inducing cell cycle arrest and apoptosis. ROR2 further suppressed epithelial-mesenchymal transition and tumor cell stemness through repressing β-catenin and AKT signaling, leading to further inhibition of tumor cell migration/invasion and increased chemo-sensitivity. Thus ROR2, as an epigenetically inactivated TSG, antagonizes both β-catenin and AKT signaling in multiple tumorigenesis. Its epigenetic silencing could be a potential tumor biomarker and therapeutic target for carcinomas.

S2-01

HCV NS5A induces ER-mitochondria tethering

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The hepatitis C virus is a positive strand RNA virus affecting more than 170 million people worldwide. Its compact viral genome produces approximately 10 viral proteins needed for the complete life cycle of the virus. The non-structural protein 5A (NS5A) is essential for viral replication in the host cells. One of its functions is re-arrangement of internal membranes to form membranous web, in which viral replication takes place. We have recently discovered that NS5A has a novel ability to tether mitochondria and endoplasmic reticulum(ER) and fragment mitochondria. This effect is coordinately regulated by a number of host proteins that are known to interact with NS5A. These results indicate a previously ignored functional role of the mitochondria in HCV pathogenesis.

S2-02

Insulin-like growth factors serve as mediators of luteinizing hormone action on oocyte maturation in zebrafish

J.Z. Li, L.H. Chu, X. Sun, C.H.K. Cheng

The surge of luteinizing hormone (Lh) triggers oocyte maturation in vertebrates. However, this action is thought to be indirect and requires certain factors to transmit its signal. Herein we report for the first time in vertebrates that insulin-like growth factors (Igfs) serve as mediators of Lh action on oocyte maturation in zebrafish. The dynamic expression profiles of Igfs including *igf1*, *igf2a*, *igf2b* and *igf3* were demonstrated during follicullogenesis in the ovary. Regulation of the ovarian *igfs* by Lh was investigated in intact follicles as well as in primary culture of follicular cells. Upregulation of *igf2b* and *igf3* expression but downregulation of *igf2a* expression in the follicular cells by Lh through a cAMP pathway was demonstrated, but no effects on *igf1* was found. Recombinant zebrafish Igf2a, Igf2b and Igf3 proteins significantly enhanced oocyte maturation *in vitro* and *in vivo*, such actions being completely blocked by the Igf type 1 receptors (Igf1rs) inhibitors NVP-ADW742 and NVP-AEW541. Western blot studies demonstrated that phosphorylation of Igf1rs in the follicles could be activated in naturally matured follicles or treatment by Igfs or hCG. Interestingly, hCG activation of oocyte maturation could be significantly blocked by Igf1r inhibitors *in vitro* and *in vivo*. Furthermore, using *lhb* (luteinizing hormone beta chain) knockout zebrafish, the differential regulation of *igfs* in ovarian follicles was confirmed and the defects of the *lhb* mutant in oocyte maturation could be rescued by Igf3 *in vivo*. Thus, all above results clearly demonstrated that Igf3 is the predominate mediator that propagates the action of Lh on oocyte maturation in zebrafish while other Igfs play less important roles.

S2-03

Epigenetic memory in spermatogoinal stem cell development

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Spermatogonial Stem Cells (SSCs) are group of pluripotent cell populations in the mammalian testes. SSCs are critical for male fertility; any developmental defects in SSCs could pass to the offspring. In fact, spermatogenic failure accounts for at least 40% of infertility cases. SSCs is not only important in reproductive biology, but also in regenerative medicine as an alternative pluripotent stem cell source. DNA methylation is an epigenetic mark critical in normal and disease development. Despite epigenetic modifications by 5-methylcytosine (5mC) have been well studied, the regulation of demethylation pathways remains largely elusive. Not until recently that active DNA demethylation through novel modifications of cytosine was found to be essential for mouse embryonic stem cell development. Here we hypothesized that the array of chemical modifications on cytosine served as molecular switches governing the cellular fate of SSCs, and this epigenetic memory is required to restrict SSC development. We showed the global 5hmC level correlated with undifferentiated SSC and involved in transcriptional regulation of SSC related genes and lncRNAs we identified previously. With these intriguing findings, we aim to map and delineate their functional roles in SSC culture and animal models.

S2-04

Dragon (Repulsive Guidance Molecule RGMb) induces apoptosis in renal tubular epithelial cells

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Dragon is one of the three members of the repulsive guidance molecule (RGM) family, i.e. RGMa, RGMb (Dragon), and RGMc (hemojuvelin). We previously identified the RGM members as bone morphogenetic protein (BMP) co-receptors that enhance BMP signaling. Our previous studies found that Dragon is highly expressed in the tubular epithelial cells of mouse kidneys. However, the roles of Dragon in renal epithelial cells are yet to be defined. We now show that overexpression of Dragon increased cell death induced by hypoxia in association with increased cleaved poly(ADP-ribose) polymerase and cleaved caspase-3 levels in mouse inner medullary collecting duct (IMCD3) cells. Previous studies suggest that the three RGM members can function as ligands for the receptor neogenin. Interestingly, our present study demonstrates that the Dragon actions on apoptosis in IMCD3 cells were mediated by the neogenin receptor but not through the BMP pathway. Dragon expression in the kidney was up-regulated by unilateral ureteral obstruction in mice. Compared with wild-type mice, heterozygous Dragon knock-out mice exhibited 45–66% reduction in Dragon mRNA expression and decreased epithelial apoptosis, and had attenuated tubular injury after unilateral ureteral obstruction. Our results suggest that Dragon induce tubular epithelial cell apoptosis both *in vitro* and *in vivo*.

S2-05

Multi-signaling pathways underlie tumor-suppressing effect of CFTR

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CFTR is a cAMP-activated anion channel expressed in epithelial cells of various tissue origins. A recent study has reported an elevated risk of digestive tract cancer in patients with CFTR mutations. More interestingly, the CFTR gene itself has been reported to be frequently hypermethylated in various cancer cell lines and tumor samples, indicating possible involvement of CFTR in the pathogenesis of cancer. However, the underlying mechanisms remain elusive. Our recent studies have demonstrated down-regulation of CFTR in prostate, breast and colon cancers, which is strongly correlated with poor prognosis of the patients, suggesting a tumor-suppressing role of CFTR. Knockdown or inhibition of CFTR is found to promote epithelial-mesenchymal-transition (EMT), migration and invasion of all cancer cell types examined. However, the CFTR-dependent signaling appears to be different in different types of cancer. While NF-kB and uPA are activated by down-regulation of CFTR in both prostate and breast cancers, additional activation of uPA via CFTR-dependent regulation of miR-193b is found in prostate cancer but not other cancer types. Interestingly, directly protein-protein interaction of CFTR with a tight junction complex protein AF-6/afadin is found to activate ERK pathway leading to enhanced invasive phenotype in colon cancer. Thus, CFTR appears to mediate multi-signaling pathways in the development of different types of cancer.

S2-06

G protein-coupled estrogen receptor (GPER/GPR30) activation inhibits P2Y receptor-mediated Ca^{2+} signalling and cytokine secretion in human bronchial epithelia

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P2Y receptors are expressed in airway epithelia and play a significant role in regulating transepithelial ion transport. In addition, recent data support the role of P2Y receptors in releasing inflammatory cytokines in the bronchial epithelium and other immune cells. In addition to the classical nuclear hormone receptors ERα and ERβ, a novel estrogen (E2) receptor, G protein-coupled estrogen receptor (GPER/GPR30), was recently identified. The action of GPR30 is unclear, but it has been implicated in mediating anti-inflammatory responses. Our study aimed to investigate the inhibitory effect of GPR30 or E2 receptor activation on P2Y receptor-mediated Ca^{2+} signalling pathway and cytokine production in human bronchial epithelia.

Data in our study demonstrate that both primary normal human bronchial epithelial cells and the 16HBE14o- cell line express GPR30 at the mRNA and protein levels, as demonstrated by real-time PCR and western blotting, respectively. Stimulation of epithelial cells with E2 or with the specific agonist of GPR30, G1, rapidly attenuated a UDP- or UTP-evoked increase in $[Ca^{2+}]_i$, while this effect was reversed by GPR30 specific antagonist, G15. E2 or G1 also inhibited the secretion of two pro-inflammatory cytokines, interleukin (IL) - 6 or IL-8, in cells stimulated by different nucleotides, including UDP, UTP and ATP S. Taken together, our data suggest that the anti-inflammatory role of GPR30 may be due to its opposing effect on the pro-inflammatory pathway activated by the P2Y receptors in inflamed airway epithelia.

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S2-07

Modulatory actions of vitamin D on the pancreatic renin-angiotensin system

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Vitamin D is necessary for normal insulin secretion and it also suppresses renal renin secretion; thus hypovitaminosis D leads to defective insulin secretion, reduced glucose homeostasis, and increased risk of type 2 diabetes mellitus (T2DM). Meanwhile, increased pancreatic islet renin-angiotensin system (RAS) activity in hyperglycemia impairs islet function and structure. We postulate that vitamin D regulates pancreatic islet function through suppression of the pancreatic islet RAS.

To address this issue, RAS component expression and production were examined in isolated islets treated *ex vivo* with or without active vitamin D (calcitriol) under physiological and high-glucose conditions from normal, hypovitaminosis D and vitamin D receptor-knockout (VDR-KO) mice.

Results showed that the upregulated expression of islet RAS components by high-glucose conditions was both prevented and corrected by calcitriol. Consistently, VDR-KO mice exhibited overactive islet RAS compared with wild-type mice. In addition, mice of hypovitaminosis D treated with RAS inhibitors, without correction of hypovitaminosis D, reduced islet RAS over-activity, ameliorated islet dysfunction and improved glucose tolerance.

These data indicate that suppression of the RAS over-activity in hyperglycemia and hypovitaminosis D protects pancreatic islet and preserves insulin secretion, thus improving glucose tolerance. These findings also suggest the possibility of synergism between correction of hypovitaminosis D and treatment with RAS inhibitors for T2DM.

S2-08

Migration of sacral neural crest cells in *Dominant Megacolon*, a mouse model for human Hirschsprung's disease

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Hirschsprung's disease (HSCR) is a common gastrointestinal motility disorder which is generally considered as a developmental disorder related to the defective migration of neural crest cells (NCCs). During embryonic development, NCCs migrate along defined pathways from the neural tube (developing central nervous system) to the gut to form enteric neurons. Abnormal migration of NCCs along the gut tube leads to a regional reduction or absence of enteric ganglia in the distal colon, thus resulting in abnormal gut motility. Recently, we found that sacral NCCs (NCCs at the sacral level) also contribute to the formation of enteric ganglia and possess distinct migratory characteristics in the mouse. However, it is unclear about the role of sacral NCCs in the pathogenesis of HSCR. In the present study, we aimed to identify anomalies in the migration of sacral NCCs in *Dominant megacolon*, a mouse mutant model for HSCR, by using a combination of techniques including whole embryo culture, *in situ* cell labelling, gut explant culture, immunofluorescence staining and cell transplantation. Our results suggested that although mutant sacral NCCs were able to migrate along the normal migration pathway, fewer NCCs managed to enter the gut and their migration along the gut tube was also retarded.

S2-09

The proto-oncogene transcription factor ets1 regulates neural crest development through mediating output of BMP signaling with histone deacetylase 1 (HDAC1)

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The neural crest (NC) is a transient, migratory cell population that differentiates into a large variety of tissues including craniofacial cartilage, melanocytes, and peripheral nervous system. NC is initially induced at the border of neural plate and non-neural ectoderm by balanced regulation of multiple signaling pathways, among which an intermediate BMP signaling is essential for NC formation. *Ets1*, a proto-oncogene playing important roles in tumor invasion, has also been implicated in delamination of NC cells. In this study, we found that the expression of *Xenopus ets1* in NC was regulated by Lrig3 and FGF signal. Overexpression of *ets1* repressed NC formation through down-regulation of BMP signaling. Moreover, *ets1* repressed the BMP-responsive gene *id3* that is essential for NC formation. Conversely, overexpression of *id3* can partially rescue the phenotype of NC inhibition induced by ectopic *ets1*. Ets1 can bind to *id3* promoter as well as Histone Deacetylase 1 (HDAC1). Mechanistically, this was attributed to multivalent interactions that permit Ets1, a transcription factor, to physically interact with HDAC1, and bind to *id3* promoter, resulting in recruitment of HDAC1 to the promoter of *id3*, thereby inducing histone deacetylation of the *id3* promoter. Thus, our studies indicate that *ets1* regulates NC formation through attenuating BMP signaling epigenetically.

Poster Presentation Session

5 June 2014 (Thursday) 13:20 – 14:20

*****Presenting Author please be available besides your poster for answering questions*****

Title of Presentation	*Abstract No.*
Therapeutic targeting of an oncogenic fibroblast growth factor-FGF19, which promotes cell proliferation and induces EMT of carcinoma cells through activating ERK and AKT signaling L. Zhong, L.L. Li, A. Chan, Q. Tao	P-01
CUDC-101, a hybrid molecular targeted agent, reverses resistance to Pt anticancer drugs via multiple mechanisms K.K.W. To, D.C. Poon, Y.M. Wei, F. Wang, G. Lin, L.W. Fu	P-02
Green tea extract is a potent anti-inflammatory agent in a rat model of endotoxin-induced uveitis Y.J. Qin, J.L. Ren, C.P. Pang, S.O. Chan	P-03
Remote ischemic postconditioning promotes retinal ganglion cell survival after optic nerve injury X. Liu, O. Sha, A.W.S. Cheung, E.Y.P. Cho	P-04
Hepcidin ameliorates mitochondrial damage in a rat model of Parkinson's disease T. Liang, C. Chu, W.H. Yung, Y. Ke	P-05
Regulation of inflammation in the central nervous system: Platelets as sensors of neuronal damage T. Veremeyko, M. Duhinova, A. Yung, E.D. Ponomarev	P-06
The GLP-1 receptor antagonist, exendin (9-39) antagonizes acute but not delayed cisplatin-induced emesis in the ferret Z. Lu, C.K. Yeung, G. Lin, D.T.W. Yew, P.L.R. Andrews, J.A. Rudd	P-07
Dysregulation of retinoid metabolism underlies diabetic embryopathy L.M.Y. Lee, R.C.C. Wang, A.S.W. Shum	P-08
Acetylshikonin, a novel AChE inhibitor, inhibits apoptosis via upregulation of heme oxygenase-1 expression in SH-SY5Y cells Y. Wang, W.L. Pan, W.C. Liang, W.K. Law, D.T.M. Ip, T.B. Ng, M.M.Y. Waye, D.C.C. Wan	P-09
Beneficial effect of phosphatidylcholine supplementation in alleviation of hypomania and insomnia in a bipolar disorder boy: a case report and its possible explanation at the genetic level S.S. Rao, M.H.B. Lam, Y.K. Wing, L.C.L. Yim, W.C.W. Chu, V.S.Y. Yeung, M.M.Y. Waye	P-10
Neuron-glial interactions regulating Toll-like receptor-4 (TLR4) activity in sensory ganglia K.H. Tse, K.B.S. Chow, H. Wise	P-11

Title of Presentation	*Abstract No.*
Impact of chronic intermittent hypoxia on hippocampal neuronal function: an *in vivo* recording study L.H. Xu, Q. Li, Z.H. Shi, Y. Ke, W.H. Yung	P-12
Maternal transfer, distribution and reproductive toxicity of low-dose melamine in fetuses and infants C.Y. Chu, K.P. Fung, C.C. Wang	P-13
Molecular evaluation of *in vitro* embryonic stem cell test to assess embryotoxicity of Chinese herbal medicines B. Liang, L.Y. Tang, C.C. Wang	P-14
Macrophages play an important role in the early angiogenesis and development of endometriosis C.W. Man, C.C. Wang	P-15
Evaluations of *in vitro* embryotoxicity tests for Chinese herbal medicines L.Y. Tang, C.C. Wang	P-16
Immunosuppression of myeloid derived suppressor cells during early development of endometriosis T. Zhang, C.C. Wang	P-17
Technical and biological variations in RNA-seq data N. Tang, H.M. Yeung, F.Y Hu, X.M. Zhang	P-18
Direct activation of human and mous Oct4 genes using engineered TALE and Cas9 transcription factors J. Hu, Y. Lei, W.K. Wong, S. Liu, K.C. Lee, X. He, W. You, R. Zhou , J.T. Guo, X. Chen, X. Peng, H. Sun, H. Huang, H. Zhao, B. Feng	P-19
Human embryonic stem cells as tools to study the oncogenic basis of neuroblastoma J.T. Zhang, L.L. Tsang, X.H. Jiang	P-20
Acid bath does not induce Oct4 expression in mouse splenocytes T.L.M. Lo, F.M.K. Tang, W.T. Shi, Y. Yao, K.K.H. Lee	P-21
The role of CFTR on tenogenic differentiation and tendinopathy Y. Liu, X.H. Jiang, G. Li	P-22
Systematic or local administration of allogeneic mesenchymal stem cells promoted fracture healing S. Huang, G. Li	P-23
GLP1R agonist on hippocampus of aging brain J.Y. Zhou, G. Xu, W.S. Poon, G. Lu	P-24
Circulating microRNAs in aneurysmal subarachnoid hemorrhage (SAH) patients G. Lu, X.W. Su, J.Y. Zhou, Q. Liu, W.S. Poon, G.K.C. Wong	P-25
Functional roles of Hedgehog signaling in tendon remodeling Y. Wang, K.K. Mak	P-26

Title of Presentation	*Abstract No.*
Role of lysosomal enzyme cathepsin D in regulation of bone formation F.J. Zhang, Q. He, L. Schneider, W.P. Tsang, J. Zhang, C. Wan	P-27
PPARδ involves in the vascular benefits of metformin in obese mice W.S. Cheang, X.Y. Tian, W.T. Wong, Y. Huang	P-28
Bone anabolic effects, *ex vivo*, of alendronate and Herba Epimedii extract in osteoblasts of ovariectomized rats W.Q. Lv, C.C.W. Poon, J.H.K. Yeung, L. Qin, P.P.Y. Lui, G.P.H. Leung, S.M. Y Lee, M.P.M. Hoi, S.W Chan, S.K. Kong, H.P. Ho, Y.W. Kwan	P-29
The anti-arthritis effects of Aconitum vilmorinianum, a folk herbal medicine in Southwestern China M. Li, J. He, L.L Jiang, E.S.K. Ng, H. Wang, F.F.Y. Lam, Y.M. Zhang, N.H. Tan, P.C. Shaw	P-30
Potential role of TM9SF4 in starvation-induced autophagy L. Sun, Z. Meng, L. Jiang, X.Q. Yao	P-31
HOTAIR mediates tumorigenicity by suppressing glucose-regulated protein 78 (GRP78) in nasopharyngeal carcinoma J.F. Zhang, Y.F. Lu, W.M. Wang, H. Wang, H.F. Kung	P-32
RRP15, a nucleolar protein, controls ribosomal biogenesis and cell proliferation in mammalian cells Z.X. Dong, C.J. Zhu, W. Jiang	P-33
Genomic rearrangements and chromosomal aneuploidies for the detection and prognostic prediction of esophageal squamous cell carcinoma J.J. Hao, Z.Z. Shi, H.Q. Yao, G.Y. Dai, W. Kang, T. Gong, C.L. Wang, X.M. Jia, X. Xu, Y. Cai, G.Q. Wang, M.R. Wang	P-34
Identification of serum peptide biomarkers for esophageal squamous cell carcinoma by MALDI-TOF Mass spectrometry K. Jia,Y.L. Sun, F. Wang, X.F. Liu, Y.Y. Qiao, Q.W. Ma, X.H. Zhao	P-35
Detection of circulating tumor cells from patients with hepatocellular carcinoma H. Mu, K.X. Lin, H. Zhao, C. Li, Y.L. Sun, J.Q. Cai, X.H. Zhao	P-36
A Mir-182/snail feedback loop regulates epithelial-mesenchymal transition Y. Zhan, Z.H. Liu	P-37
YM155 induced caspase-independent cell death in esophageal squamous cell carcinoma N. Zhao, L.P. Zhou, F. Liu, Y. Xu, X.H. Zhao	P-38
Cdk1 phosphorylated chromokinesin Kif4A is critical for chromosome condensation and alignment in early mitosis C.J. Zhu, Z.X. Dong, Q.M. Zhan, W. Jiang	P-39

P-01

Therapeutic targeting of an oncogenic fibroblast growth factor-FGF19, which promotes cell proliferation and induces EMT of carcinoma cells through activating ERK and AKT signaling

L. Zhong, L.L. Li, A. Chan, Q. Tao

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The endocrine fibroblast growth factor 19 (FGF19) subfamily contains three members-FGF19, FGF21 and FGF23, critically implicated in the pathogenesis of multiple malignancies. Here we report that, although with limited expression in few normal adult tissue types, FGF19 is overexpressed in multiple carcinomas especially of aero- and digestive tissues. We further found that overexpression of FGF19 promoted tumor cell proliferation and colony formation, and also enhanced tumor cell migration and invasion. Consistently, knockdown of endogenous FGF19 suppressed the viability, clonogenicity, and migration and invasion abilities of tumor cells. FGF19 activated ERK, AKT and β-catenin signaling through upregulating the phosphorylation of AKT, β-catenin and ERK1/2. We also found that knock-down of FGF19 inhibited tumor cell epithelial-mesenchymal transition (EMT), accompanied by increased expression of epithelial marker E-cadherin and decreased expression of mesenchymal markers N-cadherin, SLUG and fibronectin. FGF19 regulated F-actin rearrangement through promoting RhoA phosphorylation, further modulating the EMT process. Moreover, FGF19 could induce tumor cell resistance to doxorubicin through ERK signaling pathway. Application of an anti-FGF19 antibody successfully suppressed tumor cell migration and invasion. Thus, FGF19 exerts oncogenic functions in multiple carcinoma cells, and could serve as a potential therapeutic target.

P-02

CUDC-101, a hybrid molecular targeted agent, reverses resistance to Pt anticancer drugs via multiple mechanisms

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CUDC-101 is a potent molecular targeted anticancer agent, rationally designed to simultaneously inhibit HDAC, EGFR and HER2. Platinum (Pt)-based anticancer drugs, exemplified by cisplatin and oxaliplatin, are the mainstay of treatment for most solid cancers. However, resistance to Pt anticancer drugs develops rapidly during the course of chemotherapy, which is caused by overexpression of multidrug resistance (MDR) transporters and activated DNA repair mechanisms.

We investigated the potentiation effect of CUDC-101 on the anticancer activity of conventional cytotoxic drugs in MDR cells with overexpression of various ABC transporters and in Pt-resistant cancer cells. CUDC-101 was found to increase the cytotoxicity and accumulation of substrate anticancer drugs in MRP-1 (doxorubicin) and MRP-2 (methotrexate) overexpressing cells, while no effect was observed in the parental sensitive cells. Mechanistically, CUDC-101 was found to inhibit MRP-1 and MRP-2 efflux function, probably by acting as an uncompetitive inhibitor and interfering with the ATPase activity of the transporters. In Pt-resistant cancer cells, CUDC-101 appears to circumvent the resistance through inhibition of both MRP-2 and DNA repair-mediated mechanisms. The combinations of CUDC-101 with cisplatin or oxaliplatin were found to display synergistic cytotoxic effect in cisplatin- or oxaliplatin-resistant cancer cell lines, respectively. Upon the concomitant administration of CUDC-101, cellular accumulation of Pt drugs and formation of DNA-Pt adducts were found to be increased whereas expression of ERCC1 (DNA repair gene) was inhibited in Pt-resistant cells. CUDC-101 was also evaluated for its potential inhibitory effect on cytochrome P450 enzymes. The minimal inhibition of CYP3A4 and CYP2D6 by CUDC-101 could help avoid undesirable drug-drug interactions in combination therapy. Our result thus advocates further development of CUDC-101 as a novel Pt-drug sensitizer for use in combination cancer chemotherapeutic regimens.

P-03

Green tea extract is a potent anti-inflammatory agent in a rat model of endotoxin-induced uveitis

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Purpose: We have previously shown epigallocatechin-3-gallate (EGCG) is the most biologically active constituent of catechins in green tea extract (GTE). In this study we determine the anti-inflammatory effects of a natural GTE (Theaphenon E), catechin mixtures and EGCG alone on endotoxin-induced uveitis (EIU) in Sprague Dawley (SD) rats.

Methods: EIU in rats was induced by a footpad injection of 1 mg/kg lipopolysaccharide (LPS), followed by 2 times oral administration of GTE (550 mg/kg), catechins mixtures, EGCG (375.2 mg/kg), or Dexamethasone (Dxm, 1 mg/kg) at 2 hours and 9 hours post-injection. Twenty four hours after injection, the eyes were examined by slit-lamp prior to terminating the animals for collection of aqueous humors for cell count and protein assay. The eyeballs were fixed and processed for immunohistochemistry.

Results: LPS caused severe hyperemia and edema in the iris, and accumulation of infiltrated cells and proteins in the aqueous humor. The infiltrated cells in anterior/posterior chambers and iris stroma were immunopositive for CD 43 (surface antigen on leukocytes) and CD68 (cytoplasmic antigen in monocytes/macrophages), with 80% of these cells being leukocytes and 20% monocytes/macrophages. GTE, catechin mixtures and EGCG all significantly reduced the infiltrated cell number and total protein concentration in aqueous humor ($P<0.05$). All animals treated with GTE (n=5 rats) and catechins mixtures (n=5 rats) survived after the treatment, but two of five rats died after EGCG treatment.

Conclusions: Our data showed that GTE and its catechin combination exhibited potent anti-inflammatory action in an experimental model of acute ocular inflammation. Further experimentations are being done to investigate whether similar therapeutic action is obtained at lower dosages and with different combinations of catechins.

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P-04

Remote ischemic postconditioning promotes retinal ganglion cell survival after optic nerve injury

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Background
Application of a mild ischemic stimulus to the heart or brain, either before (preconditioning) or after (postconditioning) its exposure to a lethal ischemic insult, is known to elicit endogenous protective mechanisms. However, most studies of ischemic conditioning in the central nervous system (CNS) have focused on ischemic damage or related conditions like hypoxia, whereas its potential in treating other neural diseases remains unknown. The recent finding that a mild ischemia applied to a region *remote* from the target *after* induction of the lethal ischemic insult could also confer protection - remote ischemic postconditioning (RIPC), further suggests potential application in other types of neural injury. We have studied the benefit of RIPC on retinal ganglion cell (RGC) survival after optic nerve injury.

Methods
RGCs in adult hamsters were injured by cutting the optic nerve. RIPC was performed by inducing temporary ischemia of one hind limb (via a series of alternate compression and release of the femoral artery) at various times post-optic nerve injury. Control animals had the optic nerve cut but with sham RIPC performed. Survival of RGCs was quantified at 7 or 14 days post-injury, and the expression of heat shock protein HSP27 and microglial reaction in the retina were studied.

Results
RGCs exhibited extensive cell death after optic nerve injury. However, application of RIPC at 10 minutes or 6 hours post-optic nerve injury promoted RGC survival at 7 days post-injury, with the 10 minute postconditioning still exerting protection at 14 days post-injury. Enhanced RGC survival was not associated with changes in microglial proliferation. Rather, the number of RGCs that expressed HSP27 was increased by 51% when RIPC was performed at 10 minutes post-injury, as compared to the sham conditioning group.

Conclusion
Our results suggest the potential of employing remote ischemic postconditioning as a non-invasive neuroprotective strategy in different CNS disorders like spinal cord and traumatic brain injury.

P-05

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

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Background
Impairment in brain iron homeostasis is believed to be a major factor involved in the pathogenesis of Parkinson's disease (PD). Iron accumulation may underlie mitochondrial dysfunction, a common pathological finding in PD. Recent findings show that hepcidin, the main iron regulator of the body, is also widely expressed in the brain. Here we propose that over-expression of hepcidin is beneficial in reducing 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.

Methods
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). In addition, the mitochondria iron content was measured by graphite furnace atomic absorption and the expression level of transferrin receptor 2 (Tfr2) was determined by Western analysis.

Results
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 and Tfr2 expression 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 ameliorated by Ad-hepcidin but not AD-blank treatment.

Conclusion
These results suggest that overexpression of hepcidin can ameliorate mitochondrial dysfunction typically found in Parkinsonism. In the SN, hepcidin presumably suppressed iron accumulation in the mitochondria by down-regulating the expression of Tfr2, therefore protecting dopaminergic neurons. The protective mechanism of hepcidin and its potential in the treatment of PD warrant further exploration.

P-06

Regulation of inflammation in the central nervous system: Platelets as sensors of neuronal damage

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Inflammation in the central nervous system (CNS) is a complex process with a high social and economic impact worldwide. Currently there is no effective therapy for prevention and treatment of CNS inflammation that accompany many neurodegenerative diseases such as multiple sclerosis (MS) or Alzheimer's disease. In this talk I will give an example of regulation of inflammation in the CNS by platelets. Platelets respond to a vascular damage, but their role in the neurodegenerative diseases is not well known. We found that administration of brain lipid rafts induced a massive platelet activation and degranulation resulting in anaphylaxis in mice. Platelets reacted with sialated gangliosides integrated in lipid rafts of astrocytes and neurons. The brain-specific gangliosides GT1b and GQ1b were recognized by the platelets and this recognition occurred during disruption of blood brain barrier. During neuroinflammation, platelets accumulated in the CNS and secreted proinflammatory factors such as IL-1 and serotonin (5-HT). Further implications of pathogenic and regulatory roles of platelets and their direct interactions with autoimmune CD4 T cells in MS will be further discussed. Thus the study determines a new role of platelets as "innate immune cells" that directly recognize a neuronal damage and contribute to inflammation in the CNS.

P-07

The GLP-1 receptor antagonist, exendin (9-39) antagonizes acute but not delayed cisplatin-induced emesis in the ferret

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Chemotherapy induced nausea and vomiting are not fully prevented by current therapies. Studies in *Suncus murinus* have shown that glucagon-like peptide-1 (GLP-1) receptor antagonist, exendin (9-39), reduced cisplatin-induced acute emesis. Therefore, we investigated if the ferret, a carnivore with a well characterised emetic response and greater translation value than *Suncus murinus*, could identify the anti-emetic potential of exendin (9-39), and assessed the effect of exendin (9-39) on gastric function, appetite and cardiovascular homeostasis. Continuous infusion of exendin (9-39) (4.2 nmol/h, i.c.v.) antagonized cisplatin (5 mg/kg)-induced acute emesis by 39.5% ($P<0.05$), but failed to antagonize delayed emesis or to reverse cisplatin-induced weight loss, or reductions of food and water intake ($P>0.05$). Exendin (9-39) increased blood pressure ($P<0.05$), and partially reversed the cisplatin-induced hypothermia occurring during delayed phase ($P<0.05$). Exendin (9-39) had no effect on heart rate, heart rate variability, or gastric myoelectric activity in animals that received cisplatin ($P>0.05$). Although blocking GLP-1 receptors may reduce cisplatin-induced acute emesis, the hypertensive effect of exendin (9-39) may preclude its use as an anti-emetic in man.

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P-08

Dysregulation of retinoid metabolism underlies diabetic embryopathy

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Babies born to women with diabetes mellitus are prone to congenital anomalies. Here, we identify a previously unrecognized mechanism that underlies this increased risk. Normal embryogenesis requires tight regulation of the concentration of retinoic acid (RA), a crucial signaling molecule. We found that the key RA metabolizing enzyme *Cyp26a1*, which is expressed in specific tissues for protection against inappropriate exposure to RA, was significantly down-regulated in embryos of diabetic mice. Concomitantly, these tissues exhibited reduced efficacy of RA inactivation, and were more vulnerable to the deleterious effects of aberrant RA levels. By comparing mutant mouse embryos with haploinsufficiency of CYP26A1 with their wild-type littermates, in diabetic and non-diabetic pregnancies, we found a causal relationship between *Cyp26a1* expression levels, efficacy of RA metabolism and sensitivity to RA teratogenesis in diabetic embryopathy. Notably, normalization of *Cyp26a1* expression and RA metabolism in embryos of diabetic mice completely abolished their increased susceptibility to various birth defects when exposed to a teratogenic RA dose. Collectively, our findings support a mechanism by which dysregulation of RA metabolism, via specific down-regulation of *Cyp26a1*, increases malformation risk in diabetic pregnancy. Unraveling this causal linkage between disrupted RA metabolism and diabetic embryopathy may form the basis for developing new preventive measures and therapeutic interventions to reduce adverse fetal outcome in pregnancies complicated by diabetes.

P-09

Acetylshikonin, a novel AChE inhibitor, inhibits apoptosis via upregulation of heme oxygenase-1 expression in SH-SY5Y cells

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Although beta-amyloid aggregation and fibrillar tau-tangles have been implicated as the major pathogenic markers of Alzheimer's disease (AD) and served as potential targets for drug development, the acetylcholinesterase inhibitors (AChEIs) are still the popular choice in current clinical treatment for AD patients. Therefore, there is a continued need to search for novel AChEIs with good clinical efficacy and less side-effects. Using our in-house natural product database and Autodock vina as tool for docking, we have identified acetylshikonin and its derivatives as candidates of AChEIs that were not previously reported in the literatures. A series of cell-based analysis were conducted for their neuroprotective activities. We found that acetylshikonin and its derivatives prevented apoptotic cell death induced by hydrogen peroxide in human and rat neuronal SH-SY5Y and PC12 cells at 10 μM. We showed that acetylshikonin exhibited the most potent anti-apoptosis activity through inhibition of the generation of reactive oxygen species (ROS) as well as protection of the loss of mitochondria membrane potential. Furthermore, we identified for the first time that upregulation of heme oxygenase 1(HO-1) by acetylshikonin is a key step mediating its anti-apoptotic activity from oxidative stress in SH-SY5Y cells.

P-10

Beneficial effect of phosphatidylcholine supplementation in alleviation of hypomania and insomnia in a bipolar disorder boy: a case report and its possible explanation at the genetic level

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Objects: In this study we aimed to clearly describe a 16 year old boy's symptoms of bipolar disorder (BPD) and his responsiveness after phosphatidylcholine supplementation, and find out whether his responsiveness is consistent with the fact he has a risk allele of DGKH (diacylglycerol kinase eta) for BPD.

Methods: We report on a case of a 16 year old boy with BPD who was initially psychotic, and upon treatment with medication, responded well and stabilized after discharge from hospitalization. However, he still suffered from monthly episodes of insomnia accompanied by hypomania for 4 months despite adherence to medication. It is expected that supplementation of phosphatidylcholine will alleviate the symptoms of hypomania and insomnia for BPD patients, and it might be explained by risk variants located in the susceptibility genes of BPD.

Results: The 16 year old boy's hypomanic symptoms responded well to supplementation with phosphatidylcholine. He has been free of any episodes for approximately one year after initiation of supplementation with phosphatidylcholine. Moreover, genotyping showed that the boy has the risk genotype (G/C, OR= 14.4, 95% CI= 1.6-131.5, P= 0.003) in rs77072822 which is a novel SNP located in intron 1 of DGKH for BPD, whereas his normal sister has the non-risk genotype (G/G). Though this SNP has not been previously reported being a risk allele for BPD, it lies in the same region of DGKH as those alleles that associated with BPD by other previous studies.

Conclusions: Supplementation of phosphatidylcholine might be a useful strategy for those BPD patients who carry the risk genotype in intron 1 of DGKH. However, this finding should be viewed with caution and further studies assessing different populations and larger samples are necessary in support of this finding.

P-11

Neuron-glial interactions regulating Toll-like receptor-4 (TLR4) activity in sensory ganglia

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In the brain and spinal cord TLR4 is expressed by astrocytes and microglial cells, therefore, it was expected that the satellite glial cells, which surround the soma of primary sensory neurons, would also be the cell type expressing TLR4 in sensory ganglia. However, in the adult rat dorsal root ganglia (DRG), it is the neurons and not the glial cells which ordinarily express TLR4. Surprisingly, DRG glial cells in mixed DRG cell cultures are packed full of TLR4-immunoreactivity (TLR4-ir), but this TLR4-ir is best expressed on the cell surface of glial cells only in the absence of neurons (17 ± 2% in mixed DRG cells and 77 ± 10% in pure glial cell cultures; $p < 0.01$). In the absence of neurons, DRG glial cells show increased sensitivity to stimulation by TLR4 agonists such as lipopolysaccharide ($p < 0.05$), while the gene expression profile remains similar. The use of co-culture systems indicated that neuron-glial cell contact was required for DRG neurons to suppress cell surface expression of TLR4-ir on glial cells. In conclusion, DRG neurons negatively regulate TLR4 expression by associated glial cells. We propose that in the presence of damaged neurons, DRG glial cells will become reactive to DAMPs and PAMPs leading to unregulated inflammatory responses.

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P-12

Impact of chronic intermittent hypoxia on hippocampal neuronal function: an *in vivo* recording study

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Chronic intermittent hypoxia (IH) occurs in obstructive sleep apnea (OSA), a very common sleep and breathing disorders that is associated with central nervous system dysfunction, including learning and memory impairment. Despite the obvious importance, the question of exactly what happens to neuronal activities in different brain regions during intermittent hypoxia has never been addressed. We envision that long-term recording of the firing activities of neurons and brain rhythms *in vivo* during and after the IH will provide direct information to address this question and thus considerable insight into the cause of cognitive dysfunctions in OSA. Based on a well-established OSA model in which the rats were subject to daily 8-hr cycling of oxygen between 21% and 10% every 90s, the firing activities of neurons in the CA1 region of the hippocampus were followed for 14 days by chronically implanted multi-electrode arrays. We found that both CA1 principal neurons and interneurons tended to increase their firing activities during the first week of IH treatment, particularly apparent at the late hours (at 8 h) but not early hours (at 5 min and 3 h) of daily IH paradigm. The hyper-excitability, however, was followed by gradual suppression of firing in the second week. At Day 14, the firing rates of the neurons were typically less than 40% of those at Day 1. Partial recovery of the neuronal activities was found after 1-week recovery in normoxia but in principal neurons only. Interestingly, analysis of the field potential revealed that the hippocampal theta rhythm (4-8 Hz) was diminished by chronic IH with no sign of recovery 1 week after the IH treatment. Together, these data suggest that hippocampal neurons respond to short-term hypoxia treatment by boosting their firing rates. However, long term IH impairs the firing activities and suppresses learning and memory related theta rhythm, which is likely contributed by impaired functions of the interneurons rather than pyramidal neurons. These results provide the cellular correlates of the memory deficit observed in OSA subjects.

P-13

Maternal transfer, distribution and reproductive toxicity of low-dose melamine in fetuses and infants

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Deliberate melamine adulteration in pet food and infant formulas caused renal failure in pet as well as nephrolithiasis and death in human infants. Trace amounts of melamine are still contaminating our daily foods and beverages. Knowledge of adverse outcomes of the low-dose melamine in pregnant or lactating mothers and offspring during and after pregnancy are unknown.

Single TDI dose of melamine was gavaged to pregnant, lactating and neonatal rats. Lactational and placental transfer of maternal melamine was demonstrated in breast milk, amniotic fluid and foetuses in late gestation. Distribution of oral bolus of melamine was different between foetal and neonatal rats. Melamine was evenly distributed in foetal serum and organs, namely liver, brain, kidney, lung and heart, in different gestational periods but was accumulated mainly in neonatal serum and kidney, particularly in early infants. Reduced number of somites and litter size, and increased post-implantational, prenatal loss and stillbirths were identified under *in utero* melamine exposure. Size was significantly increased in the neonatal kidneys but number of matured glomeruli was lower in melamine –received rat foetuses. Renal tubular hyperplasia and crystal formation were observed in the maternal kidneys.

This is the first study to show significant maternal transfer, distribution and reproductive toxicity of low-dose melamine in fetal and infant rats.

P-14

Molecular evaluation of *in vitro* embryonic stem cell test to assess embryotoxicity of Chinese herbal medicines

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Background: As one of the widely validated in vitro embryotoxicity test methods, embryonic stem cell test (EST) was evaluated for the embryotoxicity of Chinese herbal medicines (CHMs) . We determined that the in vitro EST was inferior to predict strongly embryotoxic CHMs, suggesting new evaluation parameters or specific prediction modelis required.

Objectives: To determine embryotoxicity of CHMs by cytotoxicity in fibroblasts and inhibition of cardiomyocyte differentiation (cellular EST) could be difficult to standardize. To improve the prediction value, we used molecular endpoints to evaluate the EST (molecular EST) for CHMs.

Methods: CHMs with well-known in vivo reproductive and developmental toxicity were selected. Mouse embryonic stem cell line D3 and mouse fibroblast cell line 3T3 were used. Molecular markers for pluropotency and cardiomyocyte differentiation were quantified. Prediction model was used to classify the embryotoxic potential of each CHM.

Results: Molecular EST could be more sensitive than cellular EST to predict the embryotoxicity of CHM.

Conclusion: in vitro molecular EST could provide a more comprehensive and accurate evaluation of embryotoxic potential for CHMs. We could obtain more detailed dose respond and underlying signaling pathway from molecular EST.

P-15

Macrophages play an important role in early angiogenesis and development of endometriosis

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Endometriosis is a common and complex gynecological disorder of unknown pathogenesis. It is characterized by ectopic growth of endometrial tissues. Angiogenesis is a vital for early development and survival of endometriosis. Under hypoxia, pro-angiogenic factors are activated in the poorly diffused ectopic endometrial tissues which stimulate new blood vessels. However, the cellular and molecular mechanism of hypoxia in early angiogenesis and development of endometriosis remains unclear.

Experimental endometriosis murine models and in vivo imaging were used to study the origin and role of oxidative stress in early angiogenesis and development of endometriosis. Reactive oxygen species (ROS) production in the endometrial implants was increased within 2-6 hours. Concurrently, macrophage infiltration and HIF-1α were anticipated. Subsequently, angiogenic cytokines were up-regulated within 1 day and new blood vessels were formed at least after 1 week. Macrophage depletion and lipoxygenase deficiency significantly decreased ROS production, angiogenic factors and under-developed blood vessels, resulting in smaller lesion size. Inhibition of HIF-1α significantly decreased the lesion size and vessel formed. In conclusion, ROS mediated by macrophages play an important role in the early angiogenesis and development of endometriosis.

P-16

Evaluations of *in vitro* embryotoxicity tests for Chinese herbal medicines

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Background: Chinese herbal medicines (CHMs) are widely used to relieve symptoms and treat complications during pregnancy, e.g. miscarriage, preterm labour, low back pain, and common cold. Safety of CHMs is still unknown, there is an urgent need to develop alternative approach to identify the embryotoxic potential of CHMs. *In vitro* embryonic stem cell test (EST) has been validated to screen embryotoxicity of pharmaceuticals, but not yet for CHMs.

Objectives: To evaluate the usefulness of EST for embryotoxicity of CHMs.

Methods: CHMs with well-known in vivo reproductive and developmental toxicity were selected. Mouse embryonic stem cell line (D3) and mouse fibroblast cell line (3T3) were used. Cytotoxicity of the CHMs in 3T3 and D3 and inhibition of cardiomyocyte differentiation were measured. Prediction model was used to classify the embryotoxic potential of each CHM.

Results: 10 CHMs were tested. Embryotoxic potential of 7 CHMs matched with *in vivo* classification. Accuracy of EST on overall prediction of embryotoxicity of CHMs was 70%, but prediction of non-embrytoxic CHMs was 100%.

Conclusions: In vitro EST was inferior to predict strongly embryotoxic CHMs. Specific prediction model or new evaluation parameters for embryotoxicity of CHMs should be established.

P-17

Immunosuppression of myeloid derived suppressor cells during early development of endometriosis

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Endometriosis is a chronic gyneacological disorder characterized by the implantation of endometrium outside the uterus. Its underlying mechanism of ectopic endometrium to escape immune surveillance is still unknown.

In this study, we monitored the dynamic changes of peritoneal immune cells and cytokines in an experimental endometriosis model in mice. Most of the peritoneal immune cells were decreased after the transplantation, except myeloid derived suppressor cells (MDSC), particular granulocytic MDSC (G-MDSC), were significantly increased within 24 hours. Isolated peritoneal MDSC significantly suppressed T cell proliferation probably through arginase activity and reactive oxygen species production, confirming its immunosuppressive functions in endometriosis. Depletion of MDSC significantly reduced the endometriotic growth and angiogenesis, suggesting its role in the development of endometriosis. Concurrently, peritoneal CXCL-1, CXCL-2 and CXCL-5 were significantly increased as early as 6 hours, which induced the mobilization of MDSC *in vitro* and *in vivo*.

Our study suggested that increased MDSC were recruited by CXCL chemokines and might promote the early development of endometriosis.

P-18

Technical and biological variations in RNA-seq data

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RNA-seq is made possible by the latest NGS technology and is most powerful method to analysis gene expression. A large volume of data have been released into public databases in the last 2 years. RNA-seq used the count of reads falling onto a particular gene as an index of gene expression. It is commonly expressed as RPKM or FPKM. As these values are actual counts, there are great potentials to combine various datasets for analysis of gene expression among different clinical conditions. However, there were few studies looked into the technical errors, like between-batch effects, within-batch (duplication) errors, etc and understood how these errors compared to biological variance of gene expression. If these technical errors are much bigger than biological variance that we are interested in, it is not possible to use RNA-seq for understanding of biology of gene expression.

Methods: RNA-seq data from HapMap LCL cell lines were obtained from 3 references (Cheung, et al. 2010; Montgomery, et al. 2010; Pickrell, et al. 2010). These datasets included two ethnic groups (African and Caucasian). And for each ethnic group, there were more than 50 individuals. Some individuals have analysis more than one time. Therefore, it is possible to determine the inter-ethnic and inter-individual biological variance. These biological variation were determined together with technical errors in a mixed linear modeling. QQ plots and variance plots were used to show the global distributions. We confined to gene located in one chromosome for illustration purpose.

Results and Discussions: Simple univariate analysis revealed 57.78 % of genes showed a differential expression between ethnic groups. Different approaches were used to control for type 1 errors. After Bonferroni correction, 21.11% were above the corrected p value of 0.05. On the other hand, QQ plot showed a global inflation of p values and a lambda value of 5.46. After correction by lambda, only 17.08% of gene showed a differential expression between ethnic groups, which was considered a better reflection of the biological situations. The high lambda value was due to unrecognised batch effect (systemic bias).

Mixed linear modeling is applicable to analyse such dataset and could be used to decompose the variance into the inter-ethnic and inter-individual components (inter-class and intra-class). At the same time, replication error was also determined. A comparison of inter-ethnic and inter-individual variance revealed that more genes had a higher inter-individual variance. This is consistent to findings in microarrays and is a long-held consensus.
Large batch effects are likely present in RNA-seq datasets and it must be properly handled in statistical analysis. Poor control of type 1 error is also a potential pitfall.

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P-19

Direct activation of human and mouse Oct4 genes using engineered TALE and Cas9 transcription factors

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The newly developed TALE and CRISPR/Cas9 transcription factors (TF) offered a powerful and precise approach for modulating gene expression. In this study, we systematically investigated the potential of these new tools in activating the stringently silenced pluripotency gene Oct4 (Pou5f1) in mouse and human somatic cells. First, with a number of TALEs and sgRNAs targeting various regions in the mouse and human Oct4 promoters, we found that the most efficient TALE-VP64s bound around −120 to −80 bp, while highly effective sgRNAs targeted −147 to −89 bp upstream of the transcription start sites (TSS) to induce high activity of luciferase reporters. In addition, we observed significant transcriptional synergy when multiple TFs were applied simultaneously. Although individual TFs exhibited marginal activity to up-regulate endogenous gene expression, optimized combinations of TALE-VP64s could enhance endogenous Oct4 transcription up to 30-fold in mouse NIH3T3 cells and 20-fold in human HEK293T cells. More importantly, the enhancement of OCT4 transcription ultimately generated OCT4 proteins. Furthermore, examination of different epigenetic modifiers showed that histone acetyltransferase p300 could enhance both TALE-VP64 and sgRNA/dCas9-VP64 induced transcription of endogenous OCT4. Taken together, our study suggested that engineered TALE-TF and dCas9-TF are useful tools for modulating gene expression in mammalian cells.

P-20

Human embryonic stem cells as tools to study the oncogenic basis of neuroblastoma

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Human embryonic stem cells (hESC) hold great promise as sources of tissue for regenerative medicine and therapeutics. In addition, their utility as tools to study the origins and biology of human disease must not be underestimated. HESC give rise to tissue-specific adult stem cells and, ultimately, to all mature tissues in the body. As such, disruptions to normal stem cell function can have catastrophic consequences and result in life-threatening or devastating disease. Understanding how such diseases arise will afford novel insights into how we can better prevent and treat them.

Neuroblastoma is a common childhood malignant tumor of neural crest origin, arising in the sympathetic nervous system. Among the genetic alterations identified in neuroblastoma, amplification of the oncogene *MYCN* is strongly associated with highly malignant behavior and poor prognosis. We have successfully developed an efficient procedure for the rapid differentiation of hESC into human neural crest stem cells (NCSC) using PA6 coculture and HNK1 positive cell isolation. Lentiviral mediated overexpression of *MYCN* in NCSC promotes cell proliferation and self-renewal. Further phenotypic and molecular characterization both in vitro and in vivo are undergoing in the lab.

By creating novel hESC-based models to study the origin and biology of neuroblastoma, we aim to gain novel insights into the origin and biology of these tumors that will aid in the development of more effective, less toxic therapies.

P-21

Acid bath does not induce Oct4 expression in mouse splenocytes

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Two sets of groundbreaking stem cell studies have recently been reported in Nature that "simply" bathing somatic cells in mild acid can reprogramme them to become pluripotent stem cells (Obokata et al., 2014a, b). The researchers harvested spleen cells from 1-week-old Pou5f1-GFP C57BL/6 mice and purified the $CD45^+$ population by flow cytomtery. These $CD45^+$ splenic leukocytes were then treated with pH 5.7 HBSS solution for 25 min at 37ºC and then maintained in DMEM/F-12 culture medium containing B27 and LIF for 1-7 days. It was reported that 2 days after treatment there were $CD45^+$ cells expressing *Oct4-GFP*. The authors named these cells "Stimulus-triggered acquisition of pluripotency (STAP) cell". Furthermore, these cells were capable of forming chimeric embryos when injected into blastocyst. They reported that STAP cells can also contribute to the placenta something that iPS cells and ESC are incapable of doing. In another word, the STAP cells are totipotent. Perhaps these cells should be called stimulus-triggered acquisition of tot totipotency "STAT" cells instead? These blockbuster findings can revolutionize Regenerative Medicine by providing a simple, cheap and immunocompatible source of stem cells for tissue regeneration. Presently, we have attempted to replicate the findings and the results are reported in the poster.

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P-22

The role of CFTR on tenogenic differentiation and tendinopathy

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Introduction: Tendons are subjected to dynamic mechanical stretching. Tendon cells respond to mechanical stretching by altering gene expression, protein synthesis, and cell phenotype. However, how cells sense mechanical stretching and convert them into biochemical signals is not well understood. Recently, it has been demonstrated that Cystic Fibrosis Transmembrane conductance Regulator (CFTR) can be robustly activated by membrane stretch induced by negative pressures. Given that CFTR can also have an unexpected function in mechanosensing, in addition to its roles as a ligand-gated anion channel and a regulator of other membrane transporters. In the current study, we first compare the tendons differences by using the most common CFTR mutation animal model (ΔF508) and its wild type mice, to examine the tendon differences at microstructure, mRNA and histology level respectively. Secondly, we examine the CFTR expression on human tendinopathy, compared its expression level with normal tendon.

Methods: Achilles tendons (AT) and patellar tendons (PT) were collected from 20-24 weeks old male CFTR mutant and wild type mice for examination. Immunofluorescence was performed on paraffin embedded patellar tendons to confirm the expression of CFTR on tendon. Transmission Electron Microscope (TEM) was performed to compare the microstructure of AT. RNA from AT was isolated for comparing the expressions of tendon related markers by qRT-PCR at mRNA level. PT was embedded for paraffin sectioning. The immunohistochemistry was also performed to compare the expressions of tenogenic markers (Tenomodulin) between CFTR mutant and wild type mice at histology level. The expression level of CFTR on human tendinopathy and normal tendons were also performed by immunohistochemistry.

Results: Immunofluorescence showed that CFTR expressed on patellar tendon, especially on the cells in tendon tissue. At microstructure level, TEM indicated that tendon fibrils were loosely organized in mutant mice comparing to wild type mice, and sizes of fibrils were also unevenly distributed in mutant mice. The results of qRT-PCR showed that expressions of Tenomodulin, Scleraxis and Decorin are all lower in mutant mice comparing with wild type mice ($*p<0.05$). Furthermore, the immunohistochemistry also showed lower expression of Tenomodulin in CFTR mutant mouse than that in wild type mouse. In human tendinopathy, the CFTR was highly expressed on vascular region and cells in the matrix with highly degeneration.

Discussion: In the current study, we confirm the expression of CFTR on tendon tissues at microstructure, mRNA and histology level. We found that the CFTR mutant mouse showed lower expression of tenogenic markers and loosely organized tendon fibrils. We also confirmed the high expression level of CFTR on human tendinopathy, especially the region with vascular and cells in the matrix with highly degeneration.

P-23

Systematic or local administration of allogeneic mesenchymal stem cells promoted fracture healing

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Introduction: Mesenchymal stem cells (MSCs) can target specific damaged tissues and allogeneic MSCs do not elicit immediate immune responses. Our previous studies showed that there is a systemic mobilization and recruitment of osteoblastic precursors to the fracture site via the peripheral circulation, on this basis, we hypothesized that systemic administration of allogeneic MSCs promotes fracture healing.

Methods: For cell preparations, bone marrow derived MSCs were isolated from the GFP-SD rats, cultured, characterized by flowcytometry and their multi-potent differentiation potentials confirmed. Skin fibroblasts were also collected from the GFP rat. Rat closed transverse femoral fracture with internal fixation was performed in 48 fourteen-week old male SD rats. Following surgery, the rats were randomly assigned into 4 groups all received injections of various preparation at 5 days following the fracture : A. PBS injection group; B. MSCs injection group ($2x10^6$ MSCs injected through heart puncture), C. Fibroblasts injection group ($2x10^6$ fibroblasts was injected through heart puncture). D. MSCs fracture site injection group ($2x10^6$ fibroblasts was injected through fracture site). Weekly radiographs were taken to monitor the fracture healing. 12 rats from each group were terminatedat 5 weeks after fracture, the fractured femurs were selected for Micro-CT examination, four-point bending mechanical testing and histology examinations.

Results: Both radiographs and 3D-Reconstruction images of Micro CT showed that the gap at fracture sites was larger in control groups than that in the MSC injection groups. The Micro CT analysis showed that the percentage of bone volume over tissue volume (BV/TV) in MSC injection groups were significantly higher than those of the control groups ($p<0.05$), no significant difference between MSC systemic injection group and MSC local injection group ($p>0.05$). E-Modulus, Max Force and energy data from mechanical testing had the same conclusion as microCT results as above.

Discussion: These findings offer therapeutic promise for systemic application of allogeneic MSCs, which could be an alternative therapy for local MSCs administration in conditions such as multi-fractures and osteoporotic fractures.

P-24

GLP1R agonist on hippocampus of aging brain

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Clinical studies have shown that about 80% of AD patients have diabetes or abnormal blood glucose levels, and defects in insulin signaling are associated with the accumulation of NFTs and senile plaques in AD. Age-associated brain insulin abnormalities may contribute to cognitive decline in ageing, as have been documented in older adults with Type 2 diabetes mellitus and hypertension.

Recently, anti-diabetes drugs such as rosiglitazone, metformin, and glucagon-like peptide (GLP)-1 receptor agonists have been suggested to be effective in reducing aging-associated pathologies. There is considerable experimental evidence that a GLP-1 receptor agonist reduces Aβ aggregation and cytotoxicity in AD models. Thus, circumstances in which diabetes is associated with ageing coupled with the fact that anti-diabetes drugs are effective in reducing ageing pathology may further attract researchers to identify additional anti-diabetes drugs as new candidates for ageing-related neurological diseases treatment.

Our works here showed that Exendin-4, a GLP-1 receptor agonist, can improve blood glucose control in both young and aging normal non diabetic mice without the contribution from Bata cells. Moreover, we found that Exendin-4 treatment improved the cognitive deficits in ageing mice. To further explore the detail mechanism of the Exendin-4 on hippocampus related cognitive in ageing, proteomic technology was employed to screening the molecular targets of Exendin-4. At least 23 potential targets were detected which are under investigating now. In our on-going, a series of systematic studies are designed to verify our hypothesis that GLP1 signal is essential for aging cognitive functions and Exendin-4 may contribute for the brain insulin resistance related cognitive defect.

P-25

Circulating microRNAs in aneurysmal subarachnoid hemorrhage (SAH) patients

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MicroRNAs (miRNAs) are tiny (18–30 nt) non-coding RNA molecules that work as endogenous physiological and pathological regulators of gene expression. Recent studies have demonstrated that miRNAs were dysregulated in ischemic stroke and can be used as disease biomarkers for diagnosis and prognosis. We hypothesize that there are also distinct blood microRNA expression profiles in SAH patients which could be used to indicate different disease progress.

20 blood samples from 10 SAH patients at time points Day1 and Day14 after onset, and 20 blood samples from 10 healthy donors were employed in this study. Cell-free miRNAs were extracted from blood plasma and loaded to Affymetrix micRNA arrays. Total 5683 microRNA probes that could be analyzed in Affymetrix chip. Only 38 miRNAs could be detected to have larger than two folds change between samples from patients and control. Real-time PCR were further performed for validate in larger samples (40 samples from 20 patients).Bioinformatics analysis showed that 3 of them were brain-enriched, which should be released from injured brain tissue.

Our study provided the first knowledge of blood microRNA expression profile in SAH patients; brain-enriched circulation miRNAs could be used as biomarkers to distinguish different brain injury disease, and to indicate disease progress. More cases from multi-Centre are needed for further verification.

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P-26

Functional roles of Hedgehog signaling in tendon remodeling

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Hedeghog (Hh) signaling has been shown to be an important pathway in regulating skeletal development and remodeling. However, its involvement in tendon remodeling remains largely unclear. Previous findings indicate that Hh signaling components are expressed specifically in bone-tendon junctions, which are common regions of injuries in tendon disorders. Therefore, it is important to better understand the molecular mechanism on how Hh signaling regulates tendon remodeling and maintenance. We found that in addition to bone tendon junctions, Hh signaling components are also expressed in tendon sheaths, but not within the tendon fibers. Interestingly, osteocalcin, a marker for osteoblasts, is also expressed in tendon sheaths. We therefore generated gain-of-function and loss-of-function mutants of Hh signaling specifically in tendon sheath tissues using HOC-Cre transgenic line. The mutant mice showed significant changes in tendon architecture including tendon fiber number, tendon size and tendon strength. The extracellular matrix contents such as *ColIa1*, *Col3a1*, *Fibronectin*, *Biglycan* and *Tenomodulin* were severely affected. We will further investigate the specific targets of Hh signaling in tendon sheaths and examine its effect using tendon injury models.

P-27

Role of lysosomal enzyme cathepsin D in regulation of bone formation

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Insufficiency in nutrient and oxygen availability, oxidative stress, and autophagy failure are fundamental factors for the decline of bone mass and strength with aging. Accumulating evidence indicates that these factors affect normal lysosomal or autophagosomal activities which are the major force in clearance of aggregated or damaged proteins. Cathepsin D (Ctsd), the principal lysosomal aspartate protease and a main endopeptidase, functions in the normal activity of lysosome and autophagysome. It is kown that Ctsd exists in bone tissue during development and injury. However, the evidence on molecular and cellular mechanisms of CD mediated autophagosome or lysosome function in bone cells are lacking. In the present study we show that inactivation of Ctsd causes growth retardation and severe bone loss in the mutant mice compared with their control littermates. This phenotype is characterized by decreased osteoblast numbers and impaired osteoblastic function. Inactivation of Ctsd in osteoblasts in vitro decreases cellular proliferation and attenuates osteogenic differentiation. This is accompanied by alterations in the formation of autophagosome and gene profiles associated with autophagy function. The results suggest that Ctsd mediated autophagy plays an important role in regulating bone acquisition.

P-28

PPARδ involves in the vascular benefits of metformin in obese mice

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Objectives: Anti-diabetic drug metformin is known to activate AMP-activated protein kinase (AMPK) which forms a transcriptional complex with PPARδ and synergistically induces gene expression. The present study investigated whether PPARδ is a critical mediator for metformin in ameliorating endothelial dysfunction in diet-induced obese (DIO) mice.

Methods: Aortae from C57BL/6J mice were cultured with endoplasmic reticulum (ER) stress inducer tunicamycin, metformin, PPARδ antagonist GSK0660, PPARδ agonist GW1516 or AMPK inhibitor compound C. Male *PPARδ* wild-type and knockout mice were fed with high-fat diet for 3 months to induce obesity, followed by oral administration with metformin for one week. Vascular reactivity and protein levels were determined by wire myograph and Western blotting respectively. Levels of reactive oxygen species (ROS) and nitric oxide (NO) were measured by fluorescence imaging.

Results: Tunicamycin impaired endothelium-dependent relaxations (EDR) in response to acetylcholine, and increased the levels of ROS and ER stress markers, such as phosphorylated eIF2α, ATF6 and ATF3 in mouse aortae. These harmful effects of tunicamycin were reversed by co-treatment with metformin while such benefits of metformin were abolished by GSK0660. GW1516 exerted similar beneficial effects as metformin but the benefits were unaffected by compound C. Chronic metformin treatment alleviated EDR and reduced the levels of ROS and above-described ER stress markers in DIO *PPARδ* wild-type but not in *PPARδ* knockout mice. NO production in endothelial cells was also enhanced by metformin.

Conclusions: The present study provides novel evidence that PPARδ is a crucial mediator in the vascular benefits of chronic metformin treatment in restoring the impaired endothelial function and curtailing ER and oxidative stress in obese mice.

P-29

Bone anabolic effects, *ex vivo*, of alendronate and Herba Epimedii extract in osteoblasts of ovariectomized rats

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Background: Osteoporosis is a disease characterized by low bone mass and deterioration of bone structure that increases the risk of fracture. Osteoporosis affects ~15% of the world population, of whom more than 75% are post-menopausal women. In this study, we evaluated whether a combination of alendronate plus Herba Epimedii water extract can provide synergistic bone anabolic effects of primary osteoblasts of ovariectomized rats.

Methods: Primary osteoblasts (bone-building cells) were harvested from trabecular bones in iliac crests of Sprague Dawley (normal and ovariectomized (OVX)) rats (2 months old). Three months after ovariectomy procedures, rats were sacrificed, osteoblasts (~90% confluence) were harvested and treated with alendronate (1 μM) (for 7, 14 and 21 days) alone or in combination with Herba Epimedii water extract (1 μg/ml) before subjecting to osteogenic biomarkers (osteoclacin, p-cortactin/cortactin, F-actin and bone morphogenetic protein 2 (BMP-2)) determinations and wound healing assays.

Results: Before drug treatments, a lower protein expression of osteoclacin, p-cortactin/cortactin, F-actin and bone morphogenetic protein 2 (BMP-2) was observed in osteoblasts harvested from OVX rats compared with controls. Osteoblasts of OVX rats have a slower migration rate as determined by Wound Healing assay compared with controls. A time-dependent increase of all osteogenic biomarkers protein expression measured was observed after drug treatment with a greater magnitude was observed when both drugs were applied in combination. In addition, an improvement of migration rate of osteoblasts of OVX rats was observed especially after drug combination treatment.

Conclusions: Our results demonstrate that a combination of alendronate plus Herba Epimedii water extract offered a synergistic bone anabolic effects, *ex vivo*, with a greater potency in primary osteoblasts of OVX rats.

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P-30

The anti-arthritis effects of Aconitum vilmorinianum, a folk herbal medicine in Southwestern China

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Introduction*:* Aconiti Radix and Aconiti Kusnezoffii Radix are two traditional Chinese medicines commonly used to treat arthritis. In Southwestern China, Huangcaowu, the root of Aconitum vilmorinianum Kom., has been used as a local substitute. However, its pharmaceutical value on anti-arthritis has not yet been explored. This study investigated the anti-arthritis effects of Aconitum vilmorinianum and compared it with the anti-arthritis efficacies of the other two aconitum herbs.

Methods*:* Rats were orally administered with 70% ethanol extract of Aconitum vilmorinianum (10 mg/kg and 100 mg/kg), Aconiti Radix (100 mg/kg) and Aconiti Kusnezoffii Radix (100 mg/kg) for 14 consecutive days while arthritis was induced by unilateral intra-articular injection of Freund's complete adjuvant at the seventh day. Anti-arthritis effects were assessed by measuring allodynia, swelling, hyperaemia and vascular permeability of the knee joints.

Results*:* All three aconitum herbs suppressed joint allodynia. Aconiti Kusnezoffii and Aconitum vilmorinianum also reduced joint swelling and hyperaemia, but Aconitum vilmorinianum was the only herb that attenuated vascular permeability.

Conclusion*:* Aconitum vilmorinianum improved all arthritis parameters measured including allodynia, swelling, hyperaemia and vascular permeability of the knee joints. It showed the highest anti-arthritis efficacy among the three tested Aconitum herbs.

P-31

Potential role of TM9SF4 in starvation-induced autophagy

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Autophagy is a highly regulated process responsible for the bulk degradation of unnecessary and dysfunctioned proteins and organelles, and it is associated with several forms of human diseases including cancer, neurodegenerative disease and ischemia injury. However, the molecular mechanism involved in autophagy in mammalian cells remains poorly understood. TM9SF4 belongs to the transmembrane 9 superfamily, a highly conserved family of proteins that are characterized by the presence of a large variable hydrophilic amino-terminal domain and 9–10 putative transmembrane domains. Here, we report a potential role of TM9SF4 proteins in autophagy.

Tissue distribution TM9SF4 proteins in rats were examined. The results showed that TM9SF4 is abundantly expressed in rat heart, kidney, liver and aorta tissues using western blot and immunohistochemistry. Furthermore, subcellular localization was determined in HEK293 cells, and the results showed localization of TM9SF4 in the late endosome and Golgi body. In functional studies, overexpression of TM9SF4 markedly increased the LC3II expression level as well as the punctate distribution of LC3. On the other hand, TM9SF4-transfected cells displayed a decreased viability in response to starvation. Suppression of TM9SF4 by small interference RNA inhibited the starvation-induced autophagy, suggesting that TM9SF4 could be an important regulator of autophagy. Taken together, these findings show for the first time that TM9SF4 is a novel regulator involved in autophagy.

P-32

HOTAIR mediates tumorigenicity by suppressing glucose-regulated protein 78 (GRP78) in nasopharyngeal carcinoma

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Nasopharyngeal carcinoma (NPC) is an unique head and neck cancer that is most common in eastern Asia. Until now, the therapeutic options have been limited mainly to radiotherapy or chemotherapy. The treatment remains not satisfactory even with combined therapies. Therefore, it is urgently needed to develop effective novel therapies for NPC. Our preliminary data showed that HOTAIR was extremely abundant in NPC cells and its knockdown suppressed cell growth. The furthering proteomics analysis showed that CRP78 was a promising protein target for HOTAIR in NPC cells. Based on these findings, we provide our hypothesis that HOTAIR may mediate tumorigenesis through anti-proliferation by suppressing GRP78 expression in NPC. In this proposal, we will further characterize the role of HOTAIR on NPC in vivo tumorigenicity in xenograft models. In addition, we will attempt to elucidate the underlying mechanism. The results gained from this project will provide new insights into pathology of NPC development and explore the potential of HOTAIR as a promising therapeutic target for NPC patients.

P-33

RRP15, a nucleolar protein, controls ribosomal biogenesis and cell proliferation in mammalian cells

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Nucleolus is the sub-organelle that controls ribosome biogenesis in cells, and ribosome biogenesis is a highly regulated process that ensures cell growth (increase in biomass) coordinated with cell proliferation. In the nucleolus, ribosomal RNA precursor (47S rRNA) is initially transcribed from rDNA and then subsequently processes into 5.8S, 18S and 28S rRNAs, together with numerous ribosome proteins and nonribosomal factors, to form 60S and 40S pre-ribosomal particles before they are exported to the cytoplasm. A large body of evidence demonstrates that perturbation of rRNA synthesis, process and/or pre-ribosomal factor function impairs ribosome biogenesis and induces cell cycle arrest. This is attributed to nucleolar disruption and diffusion of ribosomal proteins, such as Rpl11 and Rpl5, into the nucleoplasm in which these ribosomal proteins bind to and inhibit MDM2 function, resulting in p53 induction and activation. In this study, we described the identification and characterization of a human protein, hRrp15, that has sequence homology with yeast pre-ribosomal factor, Rrp15p. Previous studies showed that Rrp15p is a ribosome RNA processing protein required for 60S pre-ribosomal particle formation and/or maturation in yeast. Using immunofluorescence and sucrose gradient centrifugation analyses, we demonstrated that hRrp15 was mainly localized at nucleoli in human cells. Depletion of hRrp15 expression disrupted nucleolar structure, inducing diffusion of nucleolar protein, nucleolin, in the nucleus. In contrast to Rrp15p, hRrp15 mainly associated with 60S pre-ribosomal particle but it also presented in 40S preribosomal particle. Consistently, inhibition of hRrp15 expression perturbed nucleolar formation and impaired ribosomal biogenesis in both 60S and 40S preribosomal subunits. In addition, depletion of hRrp15 in human non-transformed cells, RPE1, resulted in the induction of p53 and p21 proteins and cell cycle arrest at G1-G1/S whereas depletion of hRrp15 in human tumor cells, HeLa and MCF7, caused DNA damage response with detectable γ-H2AX and apoptosis. These results indicated that hRrp15 plays a critical role in regulating formation of both 60S and 40S pre-ribosomal particles, ribosome biogenesis, construction of nucleoli, cell growth and cell proliferation in human cells. Hence, hRrp15 may serve as a potential target for cancer therapy.

P-34

Genomic rearrangements and chromosomal aneuploidies for the detection and prognostic prediction of esophageal squamous cell carcinoma

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Esophageal squamous cell carcinoma is a common cancer type in China. We investigated genomic rearrangements and chromosomal aneuploidies to develop possible genetic biomarkers for diagnosis and prognosis of ESCC. Gene rearrangements were analyzed in 6 cell lines and 59 tumors by array-CGH, and frequent splittings of *SHANK2*, *LRP1B*, *SAPS3*, *TPCN2* and *CDKAL* genes were observed. Chromosomal aneuploidies were examined in 493 tumors and 61 precancerous lesions by fluorescence in situ hybridization with chromosome enumeration probes (CEP). Common gains of CEP3, 8, 10, 12, 17 and 20 as well as loss of CEPY were detected in tumors and precancerous lesions. An optimal four-probe panel CEP3/12/17/20 was established for detecting ESCC, and CEP3/10/12/20 for precancerous lesions. Kaplan-Meier survival curves indicated that patients with positive *SHANK2*/*CDKAL1* splitting, CEP10/17 (pT1+pT2) and CEP8/17 (stages IIb+III+IV) gains had poor overall survival. Moreover, *SHANK2* and *CDKAL1* splittings were correlated with both overall survival and disease-free survival. Combinations of LNM/stage and CEP or gene panels, such as LNM+*SHANK2*/*CDKAL1* and stage+CEP3/17, could divide patients into subgroups with different survivals. Multivariate Cox regression analysis confirmed that the above combinational models were independent prognostic factors. Our data provide potential chromosomal and genomic biomarkers for the detection and prognostic prediction of ESCC.

P-35

Identification of serum peptide biomarkers for esophageal squamous cell carcinoma by MALDI-TOF mass spectrometry

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Esophageal squamous cell carcinoma (ESCC) is one of the most common malignant neoplasms worldwide, especially in China. Because of the absence of obvious early symptoms, most patients were diagnosed at the advanced stage with poor prognosis. To improve the early diagnosis of ESCC, a two-stage serum peptide profiling analysis was performed in 397 unique samples from 201 ESCC patients and 196 healthy controls. Among them, 100 ESCC patients and 98 healthy controls were used as a training set and the rest was validation set. Serum peptides were purified using weak cationic exchanger magnetic beads (WCX-MB), and then MALDI-TOF MS was used to obtain peptide expression profiles from the serum samples with and without ESCC. The spectra were analyzed using ClinProt™ and BioExplore™ bioinformatics software. Finally, three most significant peaks were selected out to establish a genetic algorithm model to diagnose ESCC. The sensitivity and specificity were 97% and 96% in the training set, and 96% and 97% in the validation set, respectively. The three peaks were successfully identified via LTQ Obitrap XL MS. In conclusion, the classified pattern is helpful for improve the diagnosis of ESCC and these proteins could be served as potential serological biomarkers.

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P-36

Detection of circulating tumor cells from patients with hepatocellular carcinoma

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Aim: Hepatocellular carcinoma (HCC) is one of the most common malignant tumors in China. Although hepatectomy and other therapeutic schemes have been widely used, cancer recurrence and metastasis are still a problem. Detecting circulating tumor cells (CTCs) may be a new way to understand recurrence and metastasis. Here we explored the detection of CTCs for patients with HCC.

Methods: Negative enrichment by nanometer magnetic beads and Captor^{TM} label-free capture system were used to isolate and enrich CTCs from peripheral blood of HCC patients, and epithelial and HCC markers were applied to identify CTCs by immunofluorescence staining. A total of 50 HCC patients receiving hepatectomy were enrolled to check CTCs. The correlations between CTC numbers and clinical parameters were analyzed by SPSS19.0 software.

Results: Negative enrichment by nanometer magnetic beads and Captor^{TM} label-free capture system were both suitable for HCC CTCs' isolation and enrichment. The positive ratio of CTCs' detection via negative enrichment was 96.0% (48/50), and the mean number was 24.0 per 7.5mL blood. AFP was a significant relevant factor for CTC enumeration, while CEA, ALT, AST and tumor size did not show the similar associations. The expressions of epithelial-mesenchymal transition (EMT) biomarkers were observed at different levels, indicating EMT changes may happen on some CTCs.

Conclusion: Negative enrichment and Captor^{TM} system were both suitable for CTCs' isolation and enrichment of HCC patients. The CTCs with EMT biomarkers' expressions probably gave us a view on cancer metastatic biology. CTCs' detection will provide helpful information for doctors in clinics.

This work was supported by grants of the National High-tech R & D Programs (No. 2012AA020206, 2012AA02A503) and the Key Project for the Infectious Diseases (No. 2012ZX10002-017) of China.

P-37

A Mir-182/snail feedback loop regulates epithelial-mesenchymal transition

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Epithelial-mesenchymal transition (EMT), which describes the molecular reprogramming and phenotypic changes characterizing the conversion of polarized immotile epithelial cells to motile mesenchymal cells, is implicated in the promotion of tumor invasion and metastasis. To investigate the genes and microRNAs (miRNAs) participating in this process, we induced an EMT model by overexpressing transcription factor Snail in MCF-10A cells. Subsequently, microarray-based microRNA and gene expression profiling was performed to find out different miRNAs and genes. During the verification of those miRNAs and genes, we found that mir-182 maintains breast cancer cells in an epithelial-cell phenotype, with repression of Snail. Luciferase reporter assay identified that there is a binding site of mir-182 on Snail-3'UTR, which means mir-182 directly targets on Snail. On the other hand, two binding sites of Snail on mir-182 promoter were predicted by bioinformatics, and the ChIP assay verified our prediction. Meanwhile, either knocking down Snail or mir-182 in breast cancer cells, the expression of the other one reaches a higher level after the treatment of TGF-β. Next, we tested the expression level of mir-182 and Snail in breast cancer tissues, and found that mir-182 and Snail appears a negative linear correlation. These findings implicate that there exist a negative feedback loop between mir-182 and Snail during the EMT process.

P-38

YM155 induced caspase-independent cell death in esophageal squamous cell carcinoma

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Many Esophageal squamous cell carcinoma (ESCC) is one of the most common malignant neoplasms worldwide, especially in China. Survivin, a member of the inhibitor of apoptosis (IAP) family, implicated in both cell survival, cell cycle regulation and DNA damage repairing. High expression of survivin exits in many types of cancer, including esophageal squamous cell carcinoma (ESCC), which also associates with poor prognosis and multiple drug resistance. YM155, a novel small-molecule survivin suppressant, inhibited cell growth and induced cell death in relapsed/refractory diffuse large Bcell lymphoma (DLBCL), neuroblastomas and human leukemia, but it still remains unknown the mechanism underlying YM155 induced cell death. In this study, we investigate whether YM155 can suppress the proliferation or induce cell death in esophageal cancer cell. A dose-response curve was determined by MTT assay after treatment with YM155 for 24 hours, suggesting that ESCC cell growth was inhibited. Cell death and sub-G1 population significantly increased after treatment with YM155 by flow cytometry. Furthermore, we found that the expression of PARP, c-FOS was increased after treatment with YM155, but p53, survivin and XIAP partially decreased. The active fragments of Caspase-3 and Caspase-9 were not found with western blot analysis. Taken together, our data reveal that YM155 can inhibit cell proliferation, induce Caspase-independent cell death.

This work was supported by grants of the National High-tech R & D Program (No.2012AA02A503, 2012AA020206,) and NSFC of China (No. 81372385).

P-39

Cdk1 phosphorylated chromokinesin Kif4A is critical for chromosome condensation and alignment in early mitosis

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Chromokinesins play important roles in regulating genomic stability that requires faithful segregation of sister-chromosomes determined by maturation of chromosome condensation and accuracy of chromosomes alignment in early mitosis. Previously, we and others showed that a chromokinesin, Kif4A, controlled chromosome condensation, alignment and segregation as well as cytokinesis. However, the regulation of Kif4A function in mitosis remains obscure. Here, we report that Kif4A is phosphorylated at threonine 1161 (T1161) by cyclin-dependent kinase (Cdk1). We determine the molecular mechanism underlying Cdk phosphorylation of Kif4A. Mutagenesis and functional studies demonstrate that Cdk phosphorylated form of Kif4A interacts with condensin MSC2 and localizes on chromosomes whereas non-phosphorylated form of Kif4A localizes on the mitotic spindle and cytoplasm in early mitosis. Cdk phosphorylation of Kif4A is critical for Kif4A promoting chromosome condensation, spindle formation and chromosome alignment. Given the fact that Kif4A is a chromokinesin that controls chromosome condensation and chromosome alignment via cooperation with MSC2 function and regulation of spindle microtubule dynamics, our results indicate that Cdk phosphorylation of Kif4A in early mitosis spatiotemporally regulates Kif4A function and localization for proper chromosome condensation and segregation that is essential for genomic stability.

School of Biomedical Sciences

Cancer and Inflammation Symposium 2014

6 June 2014 (Friday)

This is a joint meeting with the State Key Laboratory of Molecular Oncology, Chinese Academy of Medical Sciences

Cancer and Inflammation Symposium 2014
6 June 2014 (Friday)

Room G02, Lo Kwee-Seong Integrated Biomedical Sciences Building

09:00-09:05 **Opening Ceremony: Prof. Jie Ma and Prof. Chi Hin Cho**

09:05-09:50 Plenary Lecture by Prof. Wei Jiang (Abstract No. PL-02)
"DNA replication, checkpoint surveillance and cancer"
Chairperson: Prof. Franky L. Chan

09:50-09:55 **Photo taking**

Time	*Title of Presentation*	*Speaker*	*Abstract No.*
	Liver and Esophageal Cancers ***Chairpersons: Prof. Stephen K.W. Tsui and Prof. Hongying Wang***		
09:55-10:15	Molecular mechanisms underlying male predominance of hepatocellular carcinoma: Linking androgen receptor signaling to cancer epigenome	Prof. Alfred S.L. Cheng	S3-01
10:15-10:35	Neonatal hepatitis B vaccination decreased primary liver cancer incidence and mortality in Qidong, China	Prof. Chunfeng Qu	S3-02
10:35-10:55	Receptor interactive protein kinase 3 promotes cisplatin-triggered necrosis in apoptosis-resistant esophageal squamous cell carcinoma cells	Prof. Yang Xu	S3-03
10:55-11:15	The signal pathways induced by methylseleninic acid in esophageal squamous cells	Prof. Hongxia Zhu	S3-04

11:15-11:30 ***Tea Break***

	Cancer Associated MicroRNAs ***Chairpersons: Prof. Wing Tai Cheung and Prof. Simon Ng***		
11:30-11:50	Looking for valuable plasma microRNA as diagnostic tool for pancreatic cancer	Prof. Jie Ma	S3-05
11:50-12:10	Identification of tumor-suppressive microRNA-490-3p through a mouse model of gastric cancer	Prof. Chi Hin Cho	S3-06
12:10-12:30	Small molecule modulator of microRNA-34a identified through library screening inhibited hepatocellular tumor growth	Prof. Yangchao Chen	S3-07
12:30-12:50	MicroRNA, DNA methyltransferase and aberrant DNA methylation in testicular germ cell tumor	Prof. Wai Yee Chan	S3-08

12:50-13:50 ***Lunch Break***

13:50-14:35 Plenary Lecture by Prof. Phillip Nagley (Abstract No. PL-03)
"Mitochondrial contributions to neuronal autophagy: Links to energetics and mitophagy"
Chairperson: Prof. Mary Waye

14:35-15:10 **Poster Presentations**

	Cancer Metastasis & Cell Cycle Regulation ***Chairpersons: Prof. Ge Lin and Prof. Hui Yao Lan***		
15:10-15:30	Regulation of mitotic progression by Cdk1 and Plk1 phosphorylation of Kif18A	Prof. Lina Pan	S3-09
15:30-15:50	Orphan nuclear receptor ERRα and ion channel TRPM8 as hypoxic growth regulators in prostate cancer	Prof. Franky L. Chan	S3-10
15:50-16:10	Role of tumor-derived Schwann cells in bone marrow metastasis of neuroblastoma	Prof. Andrew M. Chan	S3-11

16:10-16:20 **Closing Ceremony: Prof. Franky L. Chan**

16:20-16:35 ***Tea Break***

16:35-17:00 **Memorandum of Understanding (MOU) Signing Ceremony**

PL-02

DNA replication, checkpoint surveillance and cancer

W. Jiang

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Genome duplication is stringently regulated and restricted to occur only once per cell cycle to prevent anomalous gains or losses that can trigger downstream pathological ramifications, such as cancer. One level of control is the recruitment of replication machinery to freshly post-mitotic DNA in a process known as “licensing”. Licensing begins at replicating origins in early G1 with the formation of pre-RCs (pre-replicative complex), which involves sequential recruitment of the origin recognition complexes (ORCs), the loading factors Cdc6 and Cdt1, and the component of DNA replicative helicase, MCM complex. Although necessary, formation of pre-RC is not sufficient to initiate DNA replication. The initiation of DNA replication requires the activation of two S-phase promoting kinases, Cdks (cyclin-dependent kinases) and Ddk (Dbf4-dependent kinase, Cdc7) during the G1/S transition. Hence, deregulation of pre-RC proteins by either overexpression or functional deficiency could result in perturbation of cell cycle control, genomic instability, and tumorigenesis.

We have investigated pre-RC proteins in regulating the initiation of the DNA replication, checkpoint surveillance, genome stability and carcinogenesis. Our results showed that depletion of pre-RC components yields distinct outcomes in human non-transformed cells and cancer cells. G1-G1/S arrest is an outcome observed in both non-transformed and cancer cells and is triggered by profound abrogation of origin licensing and/or a G1-checkpoint that blocks S phase entry. The second outcome is S phase specific; cell cycle arrest is observed in non-transformed cells, whereas abnormal DNA replication and ultimately cell death are observed in cancer cells. We show that in S phase-non-transformed cells, deficiency of pre-RC results in activation of the ATR-dependent S phase checkpoint that halts replication fork progression. Codepletion of pre-RC and ATR in these cells abrogates checkpoint responses; restores fork progression at extremely reduced rates; and ultimately causes cell death, recapitulating the phenotype of cancer cells. The selective cytotoxic effect of pre-RC inhibition on cancer cells suggests that the pre-RC might be an attractive target for the development of drugs that kill proliferating malignant cells but spare actively proliferating host cells. Thus, the exploitation of the differences in normal and cancer cells through the selective targeting of pre-RC proteins could lead to anticancer therapies with increased selectivity and efficacy.

PL-03

Mitochondrial contributions to neuronal autophagy: Links to energetics and mitophagy?

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Dysfunctional mitochondria are recognized as a common theme amongst various neuropathologies. Recent genetic studies of Parkinson's disease have revealed mutations of PINK1 and parkin, which regulate degradation of damaged mitochondria through mitophagy. Here we investigated neuronal autophagy in general, and mitophagy in particular, in primary neurons. Primary cultures of cerebellar granule cells (Swiss mice) were exposed to insults targeting mitochondrial respiratory chain complexes I-V (rotenone, 3-nitropropionic acid, antimycin A, KCN and oligomycin, respectively) to induce dysfunctional mitochondria. The extent of bioenergetic failure was determined by observing the level of ATP, depolarisation of mitochondrial membrane potential and decrease in oxygen consumption rate measured by the Seahorse XF24 extracellular flux analyzer. All stressors produced mitochondrial dysfunction as shown by concentration-dependent and time-dependent decline in ATP over 4-24 h ($n \geq 3$). Neurons with inhibited complexes I, III or IV showed rapid loss of mitochondrial membrane potential and concentration-dependent and time-dependent decrease in oxygen consumption rate over 4-24 h (n=5). Neurons with dysfunctional complex II showed significant reduction (~80% reduction) in mitochondrial reserve capacity and oxygen consumption, showing the most significant disruption to mitochondrial bioenergetics overall. Investigation of autophagy was followed by observing significant accumulation of punctate acidic vacuoles in cytoplasm after 4 h of drug treatment ($p<0.05$) labelled by monodansylcadaverine. Detection of PINK1 antibody by cellular immunofluorescence revealed cytoplasmic translocation of PINK1, indicating likely involvement of mitophagy. Immunoblotting of cell extracts for microtubule-associated protein 1 light chain 3 (LC3-I/II) showed overall increase in LC3-II bands 24 h after inhibition of respiratory complexes, especially for inhibition of complex I ($p<0.05$; n=3) and complex II ($p<0.01$; n=3), suggesting the involvement of more general autophagic mechanisms.

S3-01

Molecular mechanisms underlying male predominance of hepatocellular carcinoma: Linking androgen receptor signaling to cancer epigenome

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Hepatocellular carcinoma (HCC) is one of the commonest and deadliest cancers worldwide. A striking epidemiological characteristic of HCC regardless of localities and etiologies is prominent male predominance, which is even more pronounced in hepatitis B virus (HBV)-endemic areas. Androgen receptor (AR) is a ligand-activated nuclear receptor that regulates the development of male sexual phenotype. Aberrant AR signaling, however, has detrimental consequences in the development of male-predominant cancers. Using genome-wide location and functional analysis, we have uncovered *cell cycle-related kinase* (*CCRK*) as an AR direct transcriptional target that drives aberrant hepatocellular proliferation and tumorigenicity through β-catenin/T cell factor signaling (*J Clin Invest* 2011; 121(8):3159-75). We have further demonstrated that CCRK mediates the interaction between AR signaling and the HBV X protein to form a viral-host oncogenic circuitry, thus validating the critical role of CCRK in male hepatocarcinogenesis (*Gut* 2014).

Chromatin remodeling has emerged as a hallmark of male-predominant HCC; however, it is unknown whether mechanistic links exist between chromatin regulators and sex hormone signaling. Our previous integrative epigenomics analysis has established Polycomb-mediated histone H3 lysine 27 trimethylation (H3K27me3) as a repressive histone modification that silences genes independently of promoter methylation in cancer (*Nat Genet* 2008;40(6):741-50). In HCC, we have demonstrated that the Polycomb protein Enhancer of zeste homolog 2 (EZH2) concordantly represses Wnt antagonists via H3K27me3, thus providing an epigenetic mechanism for constitutive β-catenin activation during hepatocarcinogenesis (*Cancer Res* 2011;71(11):4028-39). Here, we elucidate a new role for AR signaling in the regulation of EZH2-mediated chromatin remodeling through the activities of CCRK. These data have not only advanced our fundamental understanding of the gender disparity in HCC, but may also provide a new paradigm for nuclear receptor regulation of cancer epigenome. Elucidating the detailed signaling network of AR/CCRK/EZH2 will lead to the discovery and development of novel targeted therapies for HCC and other male-predominant cancers.

S3-02

Neonatal hepatitis B vaccination decreased primary liver cancer incidence and mortality in Qidong, China

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Neonatal hepatitis B vaccination has been implemented worldwide to prevent hepatitis B virus (HBV) infection. However, its efficacy on infant fulminate hepatitis (IFH) and primary liver cancer in young adults is challenging to evaluate in observational studies.

We conducted a population-based randomized controlled trial between 1983-1990 in Qidong, China involving approximately 80,000 newborns randomly assigned to vaccination or control groups. Neonates in the vaccination group received a three-dose HBV vaccination series, while those in the control group received neither vaccine nor placebo. Information on incidence of primary liver cancer and mortality due to IFH was obtained from a well-established population-based registry in December 2012. Two cross-sectional surveys on sero-HBsAg prevalence were conducted in 1994-2000 and 2006-2012, respectively.

The incidence rate of primary liver cancer and mortality rate of IFH were significantly lower in the vaccination group than control group with efficacy of 69% for primary liver cancer and 59% for IFH. The protective effect of neonatal vaccination against primary liver cancer was also supported by the low sero-HBsAg prevalence rates in the vaccination group as compared to the rates in the control group. The protection efficacy of neonatal HBV vaccination against sero-HBsAg positivity was 76.15% and 72.81% respectively.

Neonatal HBV vaccination dramatically decreased HBsAg seroprevalence in childhood as well as in young adulthood and subsequently reduced incidence of primary liver cancer in young adults. Additional follow-up in this population is warranted to elucidate its long-term efficacy on reducing liver cancer in adults.

S3-03

Receptor interactive protein kinase 3 promotes cisplatin-triggered necrosis in apoptosis-resistant esophageal squamous cell carcinoma cells

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Cisplatin-based chemotherapy is the current standard treatment for locally advanced esophageal cancer. Cisplatin has been shown to induce both apoptosis and necrosis in cancer cells, but the mechanism by which programmed necrosis is induced remains unknown. In this study, we provided evidence that cisplatin induces necrotic cell death in apoptosis-resistant esophageal cancer cells. Such cell death is dependent on RIPK3 and the formation of necrosome via autocrine production of TNFα. More importantly, we identified that RIPK3 is necessary for cisplatin-induced killing of esophageal cancer cells as inhibition of RIPK activity by necrostantin or knockdown of RIPK3 significantly attenuates necrosis and leads to cisplatin resistance. Moreover, microarray analysis confirmed an anti-apoptotic molecular expression pattern in esophageal cancer cells in response to cisplatin. Take together, our data indicate that RIPK3 and autocrine production of TNFα contribute to cisplatin sensitivity by initiating necrosis when the apoptotic pathway is suppressed or absent in esophageal cancer cells. They provide a new insight into the molecular mechanisms underlying cisplatin-induced necrosis, and suggest that RIPK3 is a potential marker for predicting cisplatin sensitivity in apoptosis-resistant and advanced esophageal cancer.

S3-04

The signal pathways induced by methylseleninic acid in esophageal squamous cells

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Esophageal cancer occurs at a very high frequency in certain areas of China. Supplementation with selenium-containing compounds was associated with a significantly lower cancer mortality rate in a study conducted in Linxia,China. Thus, selenium could be a potential anti-esophageal cancer agent. In our study, MSA (an organic type of selenium compound) could inhibit esophageal carcinogenesis both i*n vitro* and *in vivo*. MSA could inhibit histone deacetylases (HDACs) activity and upregulate GCN5 activity (a histone acetyltransferase). The level of H3K9 acetylation (H3K9ac) was increased after MSA treatment. The elevated level of acetylated histone H3 at Krüppel-like factor 4 (KLF4) promoter was observed and KLF4 was upregulated. By using microRNA array-based screening, we found that MSA induced miR-200a expression in ESCC cell lines. We also demonstrated that miR-200a directly targeted Keap1 3'-untranslated region (3'-UTR). The reduction of Keap1 protein leaded to Nrf2 nuclear translocation and activation of Nrf2-dependent gene transcription. In conclusion, our study indicated that MSA could exert its anti-tumor effects, at least in part, by the following two pathways: MSA-HDAC(↓)/GCN5(↑)-Histone H3 acetylation(H3K9ac↑)-KLF4 pathway; MSA-miR-200a(↑)-Keap1(↓)-Nrf2 pathway. Our results will demonstrate that MSA can inhibit esophageal carciogenesis and also support that selenium will potentially be used as a chemopreventive agent against ESCC.

S3-05

Looking for valuable plasma microRNA as diagnostic tool for pancreatic cancer

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Pancreatic cancer (PC) is a highly malignant tumor with a poor prognosis because of lack of symptoms at early stage and short of effective systemic therapies at advanced stage. Thus effective diagnostic tool was in urgent need to improve PCa patients' survival. To look for valuable plasma diagnostic tool for pancreatic cancer and identify their tissue specificity, we measured the expression of 6 miRNAs (miR-21, miR-210, miR-155, miR-20a, miR-25, miR-196a） and a candidate protein Mic-1 with plasma samples of 64 PCa patients and 33 normal controls. The correlation of their expression with the clinical characteristics and their dignositic value were analyzed. All of 6 miRNAs and Mic-1 were significantly upregulated in the PCa group, among which miR-20a and miR-196a have poor diagnostic value (AUC<0.7). The expression of the other 4 miRNAs and Mic-1 were further measured in 30 colorectal cancer（CRC）patients, 22 gastric cancer (GC) patients and 22 liver cancer patients to identify their tissue specificity. The result showed that Mic-1, miR-25 and miR-21 (AUC=0.89, 0.829 and 0.862) can not only distinguish PCa from normal control but also from other digestive system cancer.

S3-06

Identification of tumor-suppressive microRNA-490-3p through a mouse model of gastric cancer

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MicroRNA (miRNA) dysregulation is implicated in gastric tumorigenesis. However, the identification of novel pathogenic miRNAs has been confounded by the heterogeneity of clinical specimens used for miRNA profiling. We aimed to identify dysregulated miRNA(s) associated with gastric cancer through an animal model. In this study, miRNA expression in pre-neoplastic and neoplastic lesions in murine stomachs induced by *Helicobacter pylori* and N-methyl-N-nitrosourea (MNU) was profiled by genome-wide miRNA expression array. Expression and promoter hypermethylation of miRNA(s) in human clinical specimens were assessed by real-time PCR and methylation specific-PCR, respectively. *In vitro* and *in vivo* effects of candidate miRNA(s) were assessed by functional assays. Results showed that *H. pylori* plus MNU induced intestinal metaplasia and adenocarcinoma in murine stomachs. Various miRNAs exhibited progressive downregulation during murine gastric tumorigenesis. Among these significant downregulation of miR-133b and miR-490-3p was confirmed in human gastric cancer tissues in which miR-490-3p promoter DNA was found to be hypermethylated. Restored expression of miR-490-3p inhibited oncogenic phenotypes of gastric cancer cells, including cell proliferation, anchorage-independent growth, cell migration and invasion, and *in vivo* tumorigenicity. In conclusion, making use of a mouse model led to the discovery of miR-490-3p as a novel tumor-suppressive miRNA in human gastric cancer. MiR-490-3p mediated its anti-cancer effect through targeting novel oncogenes in the stomach.

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S3-07

Small molecule modulator of microRNA-34a identified through library screening inhibited hepatocellular tumor growth

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MicroRNA-34a (miR-34a) functions as a tumor suppressor and is downregulated or silenced in various cancers including hepatocellular carcinoma (HCC). MiR-34a therefore represents a promising therapeutic target for cancer. A miR-34a luciferase report system was established for library screening of miR-34a modulators. One small molecule named Compound 3 was identified as a specific miR-34a modulator. Compound 3 specifically activated miR-34a expression in HCC cells lines with wild type or mutated p53 but not in HCC cells lines with p53 deletion. Compound 3 inhibited the growth of HCC cells but not non-tumorigenic human hepatocytes. Compound 3 decreased the expressions of miR-34a targets including Cyclin D1, Bcl-2 and others. Rubone inhibited human umbilical vein endothelial cells (HUVECs) tube formation *in vitro* and reduced HCC angiogenesis *in vivo*. Furthermore, Compound 3 dramatically inhibited xenografted HCC tumor growth in mice model. Compound 3 exhibited stronger anti-HCC activities than Sorafenib both *in vitro* and *in vivo*. ChIP assay showed Compound 3 significantly enhanced p53 activities by increasing the occupancy of p53 on miR-34a promoter. Compound 3 was the firstly identified specific miR-34a modulator with strong anti-cancer activities and warrants further investigation as a potential effective anti-HCC agent. Compound 3 modulated miR-34a expression by increasing p53 occupancy on miR-34a promoter.

S3-08

MicroRNA, DNA methyltransferase and aberrant DNA methylation in testicular germ cell tumor

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It was previously demonstrated that miR-199a was down-regulated in testicular germ cell tumor (TGCT) probably caused by hypermethylation of its promoter. miR-199a is encoded by two loci in the human genome, miR-199a-1 on chromosome 19 and miR-199a-2 on chromosome 1. Both loci encode the same mature hsa-miR-199a. Another microRNA, miR-214, also locates on chromosome 1. Previous study revealed that it is co-transcribed with miR-199a-2. However, the biological significance of co-expression of miR-199a and miR-214 remains largely unknown. In this study, we determined that DNA methylation was involved in the down-regulation of miR-214 in NT2 cells. After 5-aza-dC treatment, miR-199-3p/5p and miR-214 expression was significantly increased. Silencing of DNMT1 [DNA (cytosine-5) -methyltransferase T1] with siRNA restored the expression of miR-199a and miR-214, which was accompanied by de-methylation of the promoters of miR-199a-1/2. Tumor protein p53 (TP53) down-regulated the expression of DNMT1 in NT2 cells and overexpression of TP53 restored the expression of miR-199-3p/5p and miR-214. In addition, silencing of PSMD10 (gankyrin) up-regulated the expression of TP53, while miR-214 over-expression resulted in PSMD10 down-regulation and TP53 up-regulation. Collectively, our findings highlighted a miR-199a/miR-214/TP53/DNMT1 self-regulatory network, which caused the down-regulation of miR-199a, miR-214 and TP53, as well as the up-regulation of DNMT1 in TGCT. This partially explains the mechanism of miR-199a hypermethylation in TGCT. These findings suggest a potential therapeutic approach in targeting the miR-199a/miR-214/TP53/DNMT1 regulatory network for the treatment of TGCT.

S3-09

Regulation of mitotic progression by Cdk1 and Plk1 phosphorylation of Kif18A

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Kif18A is a processive microtubule (MT) plus-end-directed kinesin that has a length-specific MT depolymerase activity. Kif18A controls spindle function, chromosome congression and alignment during mitosis. However, the regulation of Kif18A function and activity in mitosis remains elusive. Previously, our studies showed that the expression and phosphorylation of Kif18A was regulated during cell cycle, peeking in mitosis. In vitro and in vivo analyses indicated that Kif18A was phosphorylated by cyclin-dependent kinase 1 (Cdk1) and polo-like kinase 1 (Plk1) in mitosis. In this study, we utilized mass-spec analysis to identify potential Cdk1 and Plk1 phosphorylation sites in Kif18A. Mutagenesis and functional studies reveal that abrogation of potential Cdk1 and Plk1 phosphorylation of Kif18A induces aberrantly mitotic spindle formation and function, resulting in the defects of chromosome congression and alignment. Now, the studies focus on determining the molecular mechanisms by which Cdk1 and Plk1 phosphorylation of Kif18A controls mitotic progression and cell division.

S3-10

Orphan nuclear receptor ERRα and ion channel TRPM8 as hypoxic growth regulators in prostate cancer

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Adaptation of cancer cells to grow in a hypoxic microenvironment is one of the major hallmarks of cancer. This growth adaptation not only can facilitate the survival advantages of cancer cells in the O_2-reduced microenvironment but also render cells resistance to various therapies. Compared to other cancers, hypoxia is particularly significant in prostate cancer and also closely associated with its clinical aggressiveness and resistance to therapies. One major mechanism mediating the hypoxic responses is via up-regulation of hypoxia-inducible factor 1 (HIF-1), a key transcription factor controlling the transcriptional networks involved in reprogramming of carbohydrate metabolism and angiogenesis. HIF-1 is a heterodimeric transcription factor and its activity is primarily controlled by the protein stabilization of its HIF-1α subunit, which is strictly regulated by an O_2-dependent ubiquitin-proteasomal degradation pathway and also an O_2-independent pathway involving the interaction of HIF-1α with HSP90 and a multi-functional scaffold protein RACK1. In this presentation, the author will present some recent findings in his laboratory how the orphan nuclear receptor ERRα and also an ion channel TRPM8 can regulate the levels of HIF-1α in prostate cancer cells via two distinct molecular mechanisms and also their potential therapeutic values as drug targets for prostate cancer will also be discussed.

S3-11

Role of tumor-derived Schwann cells in bone marrow metastasis of neuroblastoma

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Neuroblastoma (NB) is the second most common malignancy among infants. With 40% survival in patients > 5 year old, novel treatments are needed especially for high risk NB that are refractory to conventional therapies. Treatment of an *ALK*-positive neuroblastoma cell line, SK-N-SH, with a kinase inhibitor, TAE684, led to the emergence of resistant cells comprising of a mixture of predominantly Schwann-like cells and a few neuroblastic tumor cells. As neural crest progenitors are cells of origin, this observation raises the possibility that tumor-derived Schwann-like cells (TDSC) confer survival to neuroblastic tumor cells. Indeed, co-culture experiments demonstrated the ability of Schwann-like cells to protect neuroblastic tumor cells from the apoptotic effects of TAE684. Also, co-injection of neuroblastic tumor cells with TDSC drastically enhanced tumor development in nude mice. Mechanistically, this protective effect was mediated by cell-cell interaction and paracrine factors. Cell-cell interaction upregulated Stat3 signaling while heparin-binding growth factors (HBGFs) secreted by TDSC enhanced the survival of neuroblastic tumor cells through the PI3-K pathway. Attempts to isolate relevant HBGFs have identified a Wnt modulator, secreted Frizzled-Related Protein 1 (sFRP1) that may be involved in this crosstalk. Furthermore, TDSC expressesd human mesenchymal stem cell marker and conditioned medium harbored osteogenic activity.

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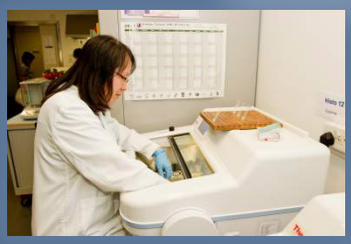

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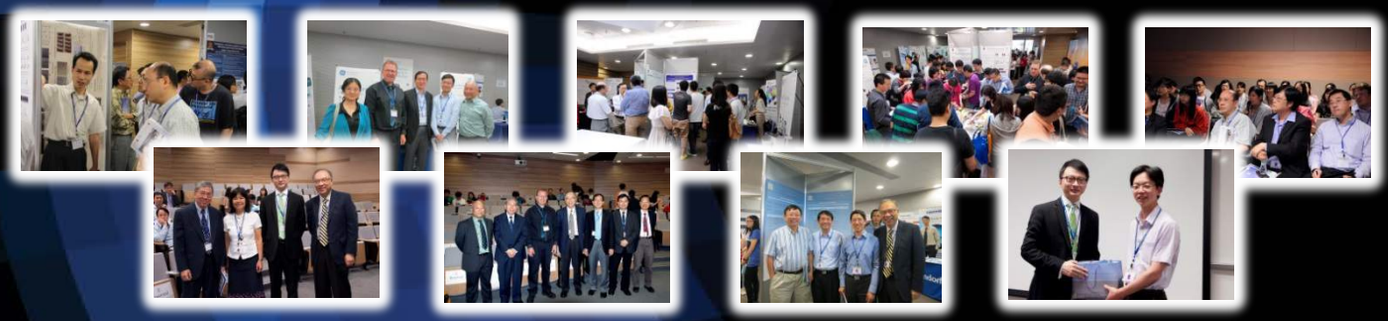

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