School of Biomedical Sciences

Research Day 2013

&

Neuroscience Symposium 2013

6-7 June 2013

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

HONG KONG

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Empower our Future
香港中文大學五十周年
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The Chinese University of Hong Kong

School of Biomedical Sciences Research Day 2013

Members of the Organizing Committee

Professor Wai Yee Chan

Professor Chi Hin Cho

Professor Bo Feng

Professor Yiu Wa Kwan

Professor Alaster H.Y. Lau

Professor Tin Lap Lee

Professor Kingston K.L. Mak

Professor Wing Ho Yung

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Welcome Message from the Dean of Faculty of Medicine

It is my pleasure to welcome you to the School of Biomedical Sciences Research Day 2013 and the joint Neuroscience Symposium.

This year's programme is not only rich but also engaging for participants as it covers two significant developments in medicine – genomics and neuroscience which offer unparalleled opportunities for research.

We are much honored to have invited Professor Jean-Marc Lemaitre, Director of INSERM Laboratory as plenary speaker to present his groundbreaking work on human cell rejuvenation which represents a further step forward in the studies of regenerative medicine. It is also a privilege to have Professor Lin Xu from the Kunming Institute of Zoology to share his interesting findings on the plasticity of the brain of rats. Equally interesting topics to be delivered in the parallel sessions on 6 June include stem cell differentiation, vascular and metabolic biology, ophthalmic research, diseases and treatment, cancer and cell biology.

Special recognition should go to all members of the Organizing Committee who have worked extremely hard to attend to every detail of the programme and the social activities to ensure that the event will be pleasant and interesting.

I trust that you will find this joint event not only a locus of convivial interchange of new ideas and approaches but also a space for discourse and collaborations.

I am looking forward to meeting you at the Opening Ceremony.

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

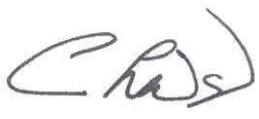
Welcome message from the Director of School of Biomedical Sciences

I am most delighted to welcome you to the School of Biomedical Sciences (SBS) Research Day cum Neuroscience Symposium 2013. The SBS Research Day started 4 years ago with the goal to provide a platform for colleagues to exchange research ideas and familiarize with each other. It grew and improved steadily year by year. Starting last year, aside from the regular Research Day, a second day when a joint Symposium with an invited delegation from another institution was held. This outgrowth of the "internal" Research Day proved to be a success. Apart from continuing this format this year, we added the element of Plenary Speaker this year. We are much honored and proud to have invited two distinguished guests as Plenary Speakers to share with us their research. They are Professor Jean-Marc Lemaitre, Director of INSERM Laboratory, Genome Plasticity and Aging, Institute of Functional Genomics, France, and Professor Lin Xu from the Kunming Institute of Zoology, Chinese Academy of Sciences, Yunnan, China. I would like to extend the warmest welcome to our Plenary Speakers and guests from other institutions for their joining of our annual flagship event.

Similar to the evolving format, this is the second Research Day organized in the Lo Kwee-Seong Integrated Biomedical Sciences Building since we moved into our new home here in early 2012. This new building has taken our School into a new era, in a sense that it does not only mean the enhancement of hardware, but also symbolizes the togetherness of the entire research enterprise of SBS under one roof with the continued evolution and expansion of our research capacity and strength. The annual Research Day is one of the notable examples showcasing our progress in selected areas of biomedical research to the University community each year.

I would like to take this opportunity to express my sincere gratitude to Prof. C.H. Cho, members of the Organizing Committee of the SBS Research Day 2013 and of our Research Team, particularly Mrs. Joresa Ng. I believe that it would not become such a huge success without their careful planning and unfailing dedication. I would also like to express my earnest appreciation to all staffs who help in various aspects of the Research Day and all members and associate members of the School who took an active part in making this event a success.

Whether you are an old or a new friend to our School, we hope you will enjoy our exciting program. As always, your continuous support, advice and sharing will definitely motivate our School members when questing for excellence.



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

Biographies of Plenary Speakers

Dr. Jean-Marc Lemaitre

Director of INSERM Laboratory
Genome Plasticity and Aging
Institute of Functional Genomics
141 rue de la Cardonille
34094 Montpellier Cedex 05, France

Scientific Director of the Stem Cell
Reprogramming Core Facility
Institute of Research in Biotherapies
Saint-Eloi Hospital
80, av. Augustin Fliche
34295 Montpellier Cedex, France

Dr. Jean-Marc Lemaitre, got his Ph.D. of Molecular and Cellular biology of Development in 1995 in Paris 7 University, served as an Engineer at CNRS (Centre National de la Recherche Scientifique) from 1998-2004, and then became an INSERM Researcher and the Research Director from 2004.

Since several years ago Dr. Lemaitre has focused on the molecular mechanisms regulating proliferation, and developed an expertise in development, replication senescence and reprogramming. Among his major achievements, he discovered the missing replication initiating factor responsible for the acquisition of the competence to replicate for embryonic development (*Nature 2002*), which was postulated 30 years ago by J. Gurdon. He also set up a new concept in the DNA replication field, showing that the replication origin selection program for development could be reprogrammed through a mitotic remodeling of the chromosome structure in mimicking nuclear transfer experiments (*Cell 2005*). Currently, the main interest of Dr. Lemaitre's group is to investigate the epigenetics mechanisms in senescence and aging *(Nat. Comms 2011)*. He developed an siRNA to identify target genes involved in senescence induction and reversion, and recently demonstrated the rejuvenated phenotype of senescent and centenarians cells by reprogramming using a specific iPSC strategy (Genes & Dev 2011). His team is developing strategies to produce iPSC safe for clinical applications in human and investigating whether iPSC strategy may be useful for tissue regeneration in animal models. Finally, he also mapped exhaustively the genome-wide distribution and activity of replication origins in human cells and unraveled a cell type specific and reprogrammable signature of replication origins using iPSC technology (*NSMB. 2012*). Now, he further investigates specific changes related to senescence induction and related replicative stress, as possible contributors to the aging process.

Prof. Lin Xu

Chairman of Institute Scientific Committee
Director, Key Laboratory of Animal Models and Human Disease Mechanisms
Professor, Laboratory of Learning and Memory
Kunming Institute of Zoology, the Chinese Academy of Sciences
32 Jiao-Chang Dong Road, Kunming 650223, Yunnan, P.R. China

Professor Xu got MSc in behavioral neuroscience from Kunming Institute of Zoology, the Chinese Academy of Sciences (1987-1990). As a research assistant (1990-1992) and assistant professor (1992-1994), he worked with Professor Jing-Xia Cai to study new drugs for epilepsy treatment. From 1994-1995, he was supported by a visiting scholarship and worked at the Department of Pharmacology, School of Pharmacy of London University to study the mechanisms of a drug called Lamotrigin, which is now marketed in the US and most of Europe as Lamictal, an anticonvulsant drug for treating of epilepsy and bipolar disorder. He then got a PhD fellowship from Ireland government and studied his PhD with Professor Michael Rowan at the Department of Pharmacology and Therapeutic, Trinity College of Dublin University, Ireland, and got this PhD in 1998. From 1998, he has served as Professor at the Laboratory of learning and memory, Kunming Institute of Zoology, the Chinese Academy of Sciences. He also served as Guest Professor at the Institute of Neuroscience, the Chinese Academy of Sciences from 2002 to 2009, Visiting Professor at Mental Health Institute of the 2nd Xiangya Hospital, Central South University from 2004 to 2009 and Guest Professor at the University of Science and Technology of China and Yunnan University since 2005. His research led to "National Science Fund for Distinguished Young Scholars" and "Hundred Talents Program" in 1999.

Professor Xu's major scientific achievements in the field of neuroscience are summarized as the following: 1. Behavioral stress faciliates long-term depression (LTD) in the hippocampus (Nature 1997). The underlying mechanisms are depended on the glucocorticoid receptor and RNA/protein synthesis (PNAS 1998). 2. Novelty exploration induces reversal of long-term potentiation in the hippocampus that is suggested to be a mechanism of interference forgetting (Nature 1998). 3. The hippocampus-dependent mechanism of opioid and CB1 receptors in addiction (J Neurosci 2004, 2010 and 2012, and Cell 2012) and hippocampal mechanisms of contextual fear memory (Hippocampus 2004, 2009 and PNAS 2008) etc. 4. Development of new drugs for the treatment of Alzheimer's disease and major depressive disorder. Both of the drugs are currently on clinical trials. Over 70 SCI papers were published and cited for over 1800 times. Professor Xu's ongoing projects are mainly supported by 973 program from Ministry of Science and Technology of China, Natural Science Foundation of China and Strategic Priority Research Program of Chinese Academy of Sciences.

Programme Summary

SBS Research Day 2013
6 June 2013 (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. Jean-Marc Lemaitre, Room G02, Lo Kwee-Seong Integrated Biomedical Sciences Building

Time	***Site 1 (Room G02)***	***Site 2 (Room G01)***
10:15-11:05	Stem Cell Differentiation (I)	Opthalmic Research
11:05-11:30	*Tea Break & Poster Viewing*	
11:30-12:45	Stem Cell Differentiation (II)	Diseases and Treatment
12:45-13:45	*Lunch Break*	
13:45-14:45	*Poster Presentation Session*	
14:45-16:00	Stem Cells in Skeletal Research	Cancer
16:00-16:25	*Tea Break & Poster Viewing*	
16:25-17:40	Vascular and Metabolic Biology	Cell Biology
17:50-18:45	SBS Tour - Assemble outside Room G02	
19:00	Conference Dinner (by invitation)	

Neuroscience Symposium 2013
7 June 2013 (Friday)

Venue: Room G02, Lo Kwee-Seong Integrated Biomedical Sciences Building

09:00-09:05 Opening Ceremony: Prof. Lin Xu and Prof. Chi Hin Cho

09:05-09:50 Plenary Lecture by Prof. Lin Xu

09:50-10:40 Session I

10:40-11:00 *Photo taking & Tea Break*

11:00-13:05 Session II

13:05-13:15 Closing Ceremony: Prof. Wing Ho Yung

Site 1 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. Jean-Marc Lemaitre (Abstract No. PL-01)
"Reversibility of cellular aging by reprogramming through an embryonic-like state: a new paradigm for human cell rejuvenation"

Time	*Title of Presentation*	*Name of Speaker*	*Abstract No.*
10:15-11:05	**Stem Cell Differentiation (I)** **Chairpersons: Prof. Kingston K.L. Mak and Prof. Huating Wang**		
10:15-10:40	Role of BRE gene in stem cell and embryo development	Prof. Kenneth K.H. Lee	S1-01
10:40-11:05	Functional role of Mst1/Mst2 in embryonic stem cell differentiation	Prof. Ping Yuan	S1-02

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

11:30-12:45	**Stem Cell Differentiation (II)** **Chairpersons: Prof. Kenneth Lee and Prof. Jean-Marc Lemaitre**		
11:30-11:55	Genome-wide survey by ChIP-seq reveals YY1 regulation of LincRNAs in skeletal myogenesis	Prof. Huating Wang	S1-03
11:55-12:20	From pluripotent stem cells to neural stem cells, a study on early neural differentiation	Prof. Bo Feng	S1-04
12:20-12:45	Reprogramming MSCs through dedifferentiation	Prof. Xiaohua Jiang	S1-05

12:45-13:45 ***Lunch Break***

13:45-14:45 ***Poster Presentation Session***

14:45-16:00	**Stem Cells in Skeletal Research** **Chairpersons: Prof. Bo Feng and Prof. Ping Yuan**		
14:45-15:10	Promoting fracture healing through systematic or local administration of allogeneic mesenchymal stem cells	Prof. Gang Li	S1-06
15:10-15:35	Roles of Hippo signaling in bone development	Prof. Kingston K.L. Mak	S1-07
15:35-16:00	The role of insulin/IR signaling in chondrogenesis	Prof. Chao Wan	S1-08

16:00-16:25 ***Tea Break & Poster Viewing***

16:25-17:40	**Vascular and Metabolic Biology** **Chairpersons: Prof. Francis F.Y. Lam and Prof. Suk Ying Tsang**		
16:25-16:50	Bone morphogenic protein-4 attenuates endothelial function	Prof. Yu Huang	S1-09
16:50-17:15	Ion channels and cerebral protective effects of Danshen and Gegen	Prof. Francis F.Y. Lam	S1-10
17:15-17:40	A study on bone anabolic effects, *ex vivo*, of CU207A in osteoblasts of ovariectomized rats	Prof. Yiu Wa Kwan	S1-11

17:50-18:45 **SBS Tour – Assemble outside Room G02**

19:00 **Conference Dinner (by invitation)**

Site 2 Room G01, Lo Kwee-Seong Integrated Biomedical Sciences Building

Time	*Title of Presentation*	*Name of Speaker*	*Abstract No.*
10:15-11:05	**Opthalmic Research** **Chairpersons: Prof. Helen Wise and Prof. Yong Gang Yao**		
10:15-10:40	Suramin stimulates axotomized retinal ganglion cells to sprout axon-like processes	Prof. Eric Y.P. Cho	S2-01
10:40-11:05	The myopia genome	Prof. Calvin C.P. Pang	S2-02

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

11:30-12:45	**Diseases and Treatment** **Chairpersons: Prof. John A. Rudd and Prof. Calvin C.P. Pang**		
11:30-11:55	The potential role of *CAMSAP1L1* in symptomatic epilepsy	Prof. Larry Baum	S2-03
11:55-12:20	Dysregulation of retinoic acid synthesis predisposes embryos of diabetic pregnancy to congenital malformations	Prof. Alisa S.W. Shum	S2-04
12:20-12:45	Translational research in clinical practice	Prof. Gang Lu	S2-05

12:45-13:45 ***Lunch Break***

13:45-14:45 ***Poster Presentation Session***

14:45-16:00	**Cancer** **Chairpersons: Prof. Kwok Wai Lo and Prof. Andrew M. Chan**		
14:45-15:10	Astragaloside IV, a novel CXCR4 antagonist from *Astragalus membranaceus*, inhibits chemoinvasion and chemomigration in breast cancer cell lines	Prof. David C.C. Wan	S2-06
15:10-15:35	TRPC5 is essential for p-glycoprotein induction in drug-resistant cancer cells	Prof. Xiaoqiang Yao	S2-07
15:35-16:00	Exposure risk and potential biomarker of pyrrolizidine alkaloid-induced tumorigenicity	Prof. Ge Lin	S2-08

16:00-16:25 ***Tea Break & Poster Viewing***

16:25-17:40	**Cell Biology** **Chairpersons: Prof. P.S. Leung and Dr. Angel O.K. Chan**		
16:25-16:50	Toll-like receptor 4 (TLR4) signaling in primary sensory neurons	Prof. Helen Wise	S2-09
16:50-17:15	Regulation of COX-2/PGE2 by epithelial sodium channel (ENaC)	Prof. Hsiao Chang Chan	S2-10
17:15-17:40	Inheritable and precise large genomic deletions of non-coding RNA genes in zebrafish using TALENs	Prof. Christopher H.K. Cheng	S2-11

17:50-18:45 **SBS Tour - Assemble outside Room G02**

19:00 **Conference Dinner (by invitation)**

PL-01

Reversibility of cellular aging by reprogramming through an embryonic-like state: a new paradigm for human cell rejuvenation

Jean-Marc Lemaitre

Director of INSERM Laboratory, Genome Plasticity and Aging, Institute of Functional Genomics, 141 rue de la Cardonille, 34094 Montpellier Cedex 05, France; Scientific Director of the Stem cell reprogramming core facility, Institute of Research in Biotherapies, Saint-Eloi Hospital, 80, av. Augustin Fliche, 34295 Montpellier Cedex, France.

Direct reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) provides a unique opportunity to derive patient-specific stem cells with potential application in autologous tissue replacement therapies and without the ethical concerns of Embryonic Stem Cells (hESC). However, this strategy still suffers from several hurdles that have to be overcome before clinical applications. Among them, cellular senescence which contributes to aging and restricted longevity, has been described as a barrier to the derivation of iPSCs, suggesting that aging might be an important limitation for therapeutic purposes from elderly individuals. Senescence is characterized by an irreversible cell cycle arrest in response to various forms of stress, including activation of oncogenes, shortened telomeres, DNA damage, oxidative stress and mitochondrial dysfunction. To overcome this barrier, we developed an optimized 6 factors based-reprogramming protocol that is able to cause efficient reversing of cellular senescence and reprogramming into iPSCs. We demonstrated that iPSCs derived from senescent and centenarian fibroblasts have reset telomere size, gene expression profiles, oxidative stress and mitochondrial metabolism, and are indistinguishable from hESC. Finally, we further demonstrate that re-differentiation, led to rejuvenated cells with a reset cellular physiology, defining a new paradigm for human cell rejuvenation. We will finally discuss the molecular mechanistic possibly involved in cell reprogramming of senescent cells and propose a model that could provide new insights into iPSCs technology that pave the way for regenerative medicine for aged patients, as well as potential technologies to derived fully reconstructed tissues and organs.

S1-01

Role of BRE gene in stem cell and embryo development

Kenneth Lee

Stem Cell and Regeneration Program, School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, P.R. China.

BRE is also known as TNFRSF1A modulator and BRCC45. This highly conserved gene was initially identified as a stress-responsive gene – whose expression was inhibited following DNA damage and retinoic acid treatment. BRE shares no homology with other known gene products. The protein contains two putative ubiquitin E2 variant domains but lack critical cysteine which is required for ubiquitination. BRE protein is present in both the cytosol and nucleus. In the cytoplasm, BRE binds to the cytoplasmic region of p55 TNF receptors to suppress TNF-α induced activation of NF-κB. The protein can also bind to Fas to inhibit the mitochondrial apoptotic pathway. BRE is also found to be a component in BRISC complex that specifically cleaves lysine63-linked ubiquitin. BRE may function as a key adaptor protein which assembles the different components of the BRISC complex. In the nucleus, BRE is a component of the DNA damage responsive BRCA1-RAP80 complex. BRE protein acts as an adapter that links the interaction between NBA1 and the rest of the complex. This adapter modulates the ubiquitin E3 ligase activity of the BRCA1/BARD1 complex by interacting with MERIT 40 which enhances cellular survival following DNA damage. BRE has been extensively studied in lung tumor, hepatocellular carcinoma and esophageal carcinoma - showing that it promoted cell survival. Nevertheless, the function of BRE in stem cells and embryonic development has never been investigated. In my presentation, I will present our recent finding on BRE in embryo and stem cell differentiation.

BRE is expressed very early on in embryonic development; at the 2-cell stage, in the inner cell mass cells of blastocysts and even in embryonic stem cells. In this context, we want to establish why BRE was expressed so early in development and whether it was involved in maintaining stemness and cell differentiation. It is generally accepted that stress-responsive genes, like BRE, play a crucial role in biological processes such as cell survival, differentiation, apoptosis and regeneration. To address our questions, we employed HUCPV progenitor cells as our experimental cell model [14]. These cells are normally found in the perivascular regions of human umbilical arteries and veins and contained a rich source of commercially valuable mesenchymal stem cells (MSCs). The HUCPV cells were found to have a colony forming unit-fibroblast (CFU-F) frequency of about 1:300, which is far higher than that of bone marrow ($1:10^4$ - $1:10^6$, depending on age) or umbilical cord blood (1:200 million). The HUCPV cells also showed a higher proliferative potential and expressed higher levels of CD146 (a putative MSC marker) in comparison to MSCs obtained from bone marrow [15]. These cells also express surface antigens CD44, CD73, CD90, CD105, CD106 and 3G5 but do not express CD34 or CD45 [14,15,16]. HUCPV cells are multipotent and capable of differentiating into all mesenchymal lineages *in vitro* [15,16,17]. Notably, these cells contribute to rapid connective tissue healing *in vivo* by producing bone, and fibrous stroma [18]. Besides being multipotent, these cells are immunoprivileged making them less likely to be immune-rejected as allografts [16,19]. Furthermore, these fetal cells express the embryonic cell markers SSEA-4, RUNX1 and OCT4 [20]. HUCPV cells can be obtained non-invasively, making them an ideal source for stem cells therapies.

S1-02

Functional role of Mst1/Mst2 in embryonic stem cell differentiation

P. Li[1], Y. Chen[2], K.L.Mak[3], P. Yuan[1,2,3]

[1] Department of Chemical Pathology, The Chinese University of Hong Kong
[2] Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong
[3] The School of Biomedical Sciences, The Chinese University of Hong Kong

The Hippo pathway is an evolutionary conserved pathway that involves in cell proliferation, differentiation, apoptosis and organ size regulation. Mst1 and Mst2 are central components of this pathway and they are essential for embryo development. But their roles in ES cell are yet to exploit. To further understand Mst1/Mst2 function in ES cell pluripotency and differentiation, we derived *Mst1/Mst2* knockout (*Mst*-/-) embryonic stem cells (ES cells). We found that *Mst*-/- ES cells show differentiation resistance after LIF withdrawal. They proliferate faster than wild type cells. Interestingly, although *Mst*-/- ES cells can form embryoid body (EBs) and differentiate into tissues of three germ layers, they are unable to form teratoma. *Mst*-/- ES cells can differentiate into mesoderm lineage, but they are unable to differentiate into cardiac progenitor cells. Microarray analysis revealed that Wnt signaling is significantly repressed in *Mst*-/- EBs. Taken together our results showed that Mst1/2 is required for cardiac progenitor cell development and teratoma formation.

S1-03

Genome-wide survey by ChIP-seq reveals YY1 regulation of LincRNAs in skeletal myogenesis

Leina Lu, Kun Sun, Xiaona Chen, Lijun Wang, Yu Zhao, Liang Zhou, Hao Sun, Huating Wang

Department of Obstetrics and Gynaecology, Li Ka Shing Institute of Health Sciences, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong SAR, P.R. China.

Skeletal muscle differentiation is orchestrated by a network of transcription factors, epigenetic regulators and non-coding RNAs. Yin Yang 1 (YY1) silences several muscle genes and miRNAs in myoblasts through recruiting Ezh2. To elucidate its genome-wide regulation, we performed ChIP-Seq, which revealed 1820 YY1 binding sites in myoblasts with a large portion residing in intergenic regions. Detailed analyses demonstrated that YY1 activates many loci in addition to repressing some. No significant co-occupancy was found between YY1 and Ezh2, suggesting its Ezh2-independent function. Further analysis of the intergenic binding uncovered that YY1 may regulate dozens of lincRNAs (Large Intergenic non-coding RNAs), whose function in myogenesis is under-explored. We characterized a novel YY1 associated muscle lincRNA (*Yam-1*) that is positively regulated by YY1. *Yam-1* is down-regulated upon differentiation and acts as an inhibitor of myogenesis. We demonstrated that *Yam-1* functions through *in cis* regulation of miR-715 which in turn targets Wnt7b. Our studies not only provide the first genome-wide picture of YY1 association in muscle cells but also uncover functional role of *Yam-1* and its interplay with YY1.

S1-04

From pluripotent stem cells to neural stem cells, a study on early neural differentiation

Bo Feng

Stem Cell and Regeneration Program, School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, P.R. China

Primitive neural stem cells (NSCs) define an early stage of neural induction, thus provide a model to understand the mechanism that controls initial neural commitment. In this study, we investigated primitive NSCs derived from mouse embryonic stem cells (ESCs). By genome-wide transcriptional profiling, we revealed their unique signature and depicted the molecular changes underlying critical cell fate transitions during early neural induction at a global level. Together with qRT-PCR analysis, our data illustrated that primitive NSCs retained expression of key pluripotency genes *Oct4* and *Nanog*, while exhibiting gene expression changes associated with ESC differentiation, such as down-regulation of pluripotency-related genes *Zscan4, Foxp1*, *Dusp9* and up-regulation of neural markers *Sox1* and *Hes1*. The early differentiation feature in primitive NSCs was also supported by their intermediate characters on cell cycle profiles. Furthermore, re-plating primitive NSCs back to ESC culture condition could revert them back to ESC stage, as shown by reversible regulation of marker genes as well as cell cycle profiles changes. Other than uncovering the new features of primitive NSCs, our microarray analysis also identified differentially expressed genes whose functions in ESC or neural differentiation have not been reported, thus provided clues to uncover new regulators in early neural induction.

S1-05

Reprogramming MSCs through dedifferentiation

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

R. Chen, X.H. Zhang, Y. Liu, H.C. Chan, X.H. Jiang

Dedifferentiation is a cellular process often seen in more basal life forms such as worms and amphibians in which a partially or terminally differentiated cell reverts to a more primitive developmental stage, usually as part of a regenerative process. In mammals, the differentiation process was thought to be irreversible. However, recent studies have demonstrated that dedifferentiation may take place during mammalian wound repair in vivo or through reprogramming in vitro. Our recent studies show that after *in vitro* induction of differentiation and dedifferentiation, MSCs, which have already committed to a particular lineage, revert to a primitive cell population (De-MSCs) exhibiting a reprogrammed phenotype distinct from their original counterparts. Of therapeutic interest, the De-MSCs exhibit enhanced cell survival and higher efficacy in differentiation compared to unmanipulated MSCs both *in vitro* and *in vivo*. Interestingly, significant upregulation of pluripotency genes was observed in De-MSCs and linked to enhanced cell survival and differentiation, hinting at the possible involvement of reprogramming. Recently, we have revealed that De-MSCs express significantly higher levels of chemokines and cytokines and display enhanced tropism to cancer, indicating its potential application in gene therapy for cancer treatment. Taken together, our findings indicate that MSCs can be reprogrammed in vitro through induced differentiation and dedifferentiation. Our findings also provide a novel method to overcome the hurdles faced by current MSC-based therapy.

S1-06

Promoting fracture healing through systematic or local administration of allogeneic mesenchymal stem cells

Shuo Huang, Gang Li

Department of Orthopaedics & Traumatology, Stem Cell and Regeneration Program, School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong SAR, P.R. China.

Introduction: Mesenchymal stem cells (MSCs) have many advantages for clinical applications: 1) MSCs are easy to obtain by bone marrow aspiration, and culture for expansion in vitro. 2) MSCs can target specific damaged tissues and tumors. 3) MSCs have anti-inflammatory and immunosuppressive characteristics 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: Bone marrow derived MSCs were isolated from the GFP rats, cultured, characterized by flowcytometry. Skin fibroblasts were collected the GFP rat as control cells. 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 through heart puncture of various preparation at 5 days following the fracture : A. PBS injection group (0.5ml PBS); B. MSCs injection group ($2x10^6$ MSCs), C. Fibroblasts injection group ($2x10^6$ fibroblasts). D. MSCs fracture site injection group, ($2x10^6$ MSCs was injected at fracture site). Weekly radiographs were taken, and animals were terminated at 5 weeks following the fracture. 6 fractured femurs per group were selected for Micro-CT examination, 8 paired femurs per group were used for four-point bending mechanical testing; 4 fractured femurs per group were subject to paraffin histology examinations. The localizations of the GFP-MSCs in the fracture callus were determined.

Results: The size of the callus in the MSC injection groups were significant larger than those of the control groups ($p<0.05$), and there was no significant difference between MSC systemic injection group and MSC local injection group ($p>0.05$). 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. Immunohistochemistry results showed that the injected allogeneic GFP-MSCs were still localized within the fracture gap 5 weeks after fracture.

Discussion: In this rat fracture model, the data showed that both systemic and local injection of allogeneic MSCs promoted fracture healing, through enhancing biomechanical properties, bone content, and enlarged callus sizes. 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.

S1-07

Roles of Hippo signaling in bone development

K. Mak, Y. Deng

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

Osteoporosis is one of the most common skeletal diseases frequently found in China and worldwide, particularly for the aged population. However, the current therapeutic approaches are not satisfactory. Current treatments mainly focus on attenuation of bone absorption process without significant improvements in stimulating new bone formation. It is therefore important to have a better understanding on how signaling pathways regulate cell fate commitment for osteoblast lineage and how to efficiently stimulate new bone formation during bone remodeling and bone regeneration processes. Here, we examine the functional roles of Hippo signaling pathway in cell fate commitment, postnatal bone remodeling and regeneration in the skeletal system. It has been previously demonstrated that Hippo signaling regulates cell-cell contact inhibition and organ size development. However, it is unclear whether Hippo signaling is required to regulate skeleton size and bone formation. We hypothesized that inhibition of Hippo signaling promotes osteoblast lineage differentiation from stem/progenitor cells and enhances bone formation during postnatal bone remodeling and bone fracture. We generated genetic engineered mouse models to dissect the molecular mechanism of Hippo pathway. Our findings will inevitably aid the development of novel therapeutic approaches in helping osteoporotic patients.

S1-08

The role of insulin/IR signaling in chondrogenesis

F. Zhang[1], J. Liu[2], Q. He[3], W.T. Garvey[2], T.L. Clemens[4], C. Wan[1]

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Insulin/insulin receptor (IR) signaling is a key mediator to regulate energy and glucose metabolism, growth and body composition. Insufficient insulin production or action associated with diabetic states is accompanied clinically or experimentally with impaired bone healing. Both IR and IGF-1 receptor (IGF-1R) exist in the cartilage, and IGF-I stimulates growth plate development. However, the function of insulin/IR in chondrocytes remains unclear. In this study, we first confirmed localization of IR in chondrocytes using immunostaining. In a mouse embryo metatarsal bone culture system, treatment with insulin increased the total length of metatarsals over that of untreated controls. Histological analysis shows that insulin increases the size of the proliferating zone in the cartilage rudiments, accompanied by increased cellular proliferation indexed by positive immunostaining for proliferating cell nuclear antigen. Primary chondrocytes from mice carrying floxed IR alleles were infected with adenoviral vectors expressing the Cre recombinase or green fluorescent protein as control. Deletion of IR elevated IGF-1R mRNA and protein levels. IGF-1 stimulated Akt and ERK phosphorylation in the control cells, the effects were further enhanced in the IR mutant cells. The cell mass culture and Alcian Blue staining showed that deletion of IR decreased chondrogenic differentiation and proteoglycan synthesis, and the effects were rescued by IGF-1 treatment. In vivo phenotyping showed that the cell numbers in the proliferating zone of the growth plate is increased and the cell size is smaller in mice lacking IR in chondrocytes compared with the control littermates though the length of the growth plate was maintained. This change was associated with activation of TSC2 signaling. The data suggests that deletion of IR in chondrocytes sensitizes IGF-1R signaling and action.

S1-09

Bone morphogenic protein-4 attenuates endothelial function

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BMP4 is a proinflammatory gene induced by disturbed flow in endothelial cells. The limited data available indicate that BMP4 reduces endothelium-dependent relaxations and induces hypertension by activating NADPH oxidase. Our study shows that BMP4 and its downstream oxidative stress–dependent upregulation of the expression and activity of COX-2 are important in vascular dysfunction. These findings suggest that strategies specifically targeting this signaling cascade could be potentially useful in treating vascular dysfunction related to human hypertension.

S1-10

Ion channels and cerebral protective effects of Danshen and Gegen

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We have previously demonstrated that Danshen and Gegen, used individually or as a combined formulation (DG), have vasodilator activities that could benefit patients with obstructive cerebrovascular diseases. This study investigates their active ingredients on vascular tone, and on K^+ and Ca^{2+} channels in rat cerebral basilar arteries. The cerebral protective effects of DG were also examined in rat models of stroke.

Two active ingredients of Danshen (danshensu and salvianolic acid B) and three active ingredients of Gegen (puerarin, daidzin, and daidzein) were investigated. Glibenclamide (a K_{ATP} channel blocker) inhibited the vasodilator effects of all these agents, except salvianolic acid B. Moreover, all these agents inhibited $CaCl_2$-induced contraction, but not puerarin. Accordingly, patch-clamp studies on smooth muscle cells of rat basilar artery showed that all these agents enhanced K_{ATP} currents, except salvianolic acid B. Furthermore, all these agents inhibited Ca^{2+} influx in con-focal microscopic studies, but not puerarin. In a global ischaemia model, DG reduced malondialdehyde (MDA) and nitric oxide (NO) contents, and restored superoxide dismutase (SOD) and catalase (CAT) activities. DG also reduced cerebral infarct volume and improved neurological deficits in an MCAO model. These findings confirmed DG as a potential therapeutic drug for treatment of cerebral ischaemia.

This research was supported by a grant from the University Grant Committee of Hong Kong Special Administrative region, China (Project No. AoE/B-10/11).

S1-11

A study on bone anabolic effects, *ex vivo*, of CU207A in osteoblasts of ovariectomized rats

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Background: Osteoporosis affects ~15% of the world population, of whom more than 75% are post-menopausal women. Current therapeutic strategies such as estrogen replacement therapy and bisphosphonates are associated with serious side effects especially after a long-term treatment. In this study, we evaluated whether CU207A can provide bone anabolic effects of primary osteoblasts and the underlying mechanisms involved.

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 CU207A (1, 3 and 10 nM) (for 7, 14 and 21 days) before subjecting to osteogenic biomarkers (osteoclacin, osteopontin, bone morphogenetic protein 2 (BMP-2), calbindin and type 1 collagen) determinations.

Results: Femur head sections illustrated that the bone harvested from OVX rats, in contrast to normal rats, is porous - a typical characteristic of osteoporosis. Before drug treatments, there was no apparent difference in protein expression of osteoclacin, osteopontin, bone morphogenetic protein 2 (BMP-2), calbindin and type 1 collagen of osteoblasts harvested from normal and OVX rats. At Day 7, CU207A (1 nM) increased, with a similar magnitude, the expression of osteopontin, BMP-2 and type 1 collagen in osteoblasts of normal and OVX rats. However, no apparent effect on osteoclacin and calbindin expression was observed. Moreover, no further increase by CU207A (1 nM) of the expression of osteopontin, BMP-2 and type 1 collagen was observed at Day 14 and Day 21 compared with Day 7. A similar profile of increase in the expression of osteopontin, BMP-2 and type 1 collagen was observed with higher concentrations of CU207A (3 and 10 nM) and with a longer incubation periods (14 and 21 days) compared with Day 7.

Conclusions: Our results demonstrate that CU207A, at nano-molar concentrations, elicited potential bone anabolic effects *ex vivo*, with an equal potency, of primary osteoblasts of normal and OVX rats.

S2-01

Suramin stimulates axotomized retinal ganglion cells to sprout axon-like processes

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Background
Retinal ganglion cell (RGC) axons, like other CNS axons, exhibit poor regeneration after injury. We have previously found that suramin (a P2 receptor antagonist), when injected intravitreally after optic nerve (ON) injury, was able to stimulate robust regeneration of RGC axons into a peripheral nerve (PN) grafted to the cut ON. The regenerative propensity of suramin was even higher than that of CNTF (a potent trophic factor for RGCs). It has also been demonstrated that RGCs could be stimulated by an intravitreally transplanted PN to sprout axon-like processes from dendrites instead of the cut axon tip: an alternative mode of CNS regeneration. In this study, we examined whether suramin could stimulate axotomized RGCs to sprout axon-like processes, and to compare its efficacy against that induced by an intravitreal PN. Moreover, we tested whether a combined intravitreal suramin and PN treatment could potentiate more RGCs to undergo sprouting than either of the two procedures performed separately.

Methods
The ON of adult hamster was cut by microscissors to induce RGC axonal injury. Immediately after ON injury, animals were treated with either an intravitreal injection of 20 nmol suramin or the vehicle (NaCl), or were transplanted intravitreally with a 2 mm autologous PN segment (or a nonviable PN as control). In a separate group of animals, suramin was injected intravitreally together with the PN transplant. At 2 weeks post-ON injury, the retina was quantified for the number of RGCs that had exhibited sprouting of axon-like processes by anti-NF200 (clone RT97) staining.

Results
Compared to the vehicle control, suramin injected intravitreally after ON injury stimulated RGCs to sprout axon-like processes from dendrites. These sprouting cells could be identified by the characteristic appearance of the axon-like processes (long wavy growth in different retinal laminae with the formation of loops). The number of sprouting RGCs induced by suramin treatment was comparable to that treated by intravitreal PN grafting. When suramin was co-injected with intravitreal PN, the number of sprouting cells seen was greater than that induced by the 2 protocols performed separately, and was even greater than their numbers added together. This suggests that combined intravitreal suramin and PN could boost different populations of RGCs to sprout axon-like processes probably via different mechanisms.

Conclusion
Besides stimulating RGCs to undergo axonal regeneration into a PN grafted to the cut ON, suramin also activates RGCs to sprout axon-like processes from dendrites, highlighting its potency in switching on cellular mechanisms for regeneration. Combined suramin and intravitreal PN grafting can further up-regulate RGC sprouting which may be of benefit in repair after CNS injury.
Supported by GRF grant CUHK463309.

S2-02

The myopia genome

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Myopia, or short-sightedness, is a common ocular refractive error that causes visual impairment worldwide, and is more prevalent in Asian than other populations. Heredity is a factor to the development of myopia based on family and twins studies. At present still no gene that causes myopia directly has been identified. However more than 40 chromosomal loci have been reportedly mapped for myopia at chromosome 1p, 1q, 2q, 3q, 4q, 5p, 7p, 7q, 8p, 10q, 11p, 11p, 12p, 12q, 13q, 14p, 15q, 16q, 17q, 18p, 18q, 19q, 20q, 21q, 22q, and Xq. In recent years a number of myopia associated SNPs have been identified by genome wide association study (GWAS). SNP rs577948 on chromosome 11q24.1 in Japanese has identified to associate with pathological myopia. Two other GWAS reports on study subjects of European ancestries have shown rs634990 on 15q14 and rs8027411 on 15q25.1 are linked with myopia. *RASGRF1*, located in 15q25.1, has been proposed to be a myopia gene. GWAS in Chinese, including Chinese in Hong Kong, have yielded several myopia associated SNPs: 5p15 rs12716080 and rs6885224, 1q41 rs4373767 in *ZC3H11B,* and 13q12.12: rs9318086. While further validation studies, such as deep sequencing of these regions, should reveal important genetic information in the near future for myopia, the phenotypic features of patients and controls have to be more stringently defined. Apart from the two major parameters, axial length and refractive errors, information such as the course of development of the refractive errors, central cornea thicknesses, age of myopia onset, should be attended to. Consideration should also be given to the living environment and reading habits of all the study subjects. While ethnic differences in prevalence are well accepted, are the differences really unaffected by environment? Genotype data based on a myopia gene, once identified, should throw light on these issues.

S2-03

The potential role of *CAMSAP1L1* in symptomatic epilepsy

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Our recent genome-wide association study (GWAS) of symptomatic epilepsy in the Chinese population identified the most significant association as the single nucleotide polymorphism (SNP) rs2292096 ($P=1.0\times10^{-8}$, OR=0.63 for the G allele), in the *CAMSAP1L1* gene. We sought to elucidate CAMSAP1L1 function and to compare expression among genotypes. Epilepsy surgery patients with the rs2292096 GG genotype tended towards higher expression of *CAMSAP1L1* RNA in the temporal lobe (p=0.024) and hippocampus (p=0.173). Double immunofluorescence of human SH-SY5Y neuroblastoma cells showed CAMSAP1L1 expression on neurites, partially overlapping with β-tubulin. *CAMSAP1L1* siRNA transfection of human SH-SY5Y neuroblastoma cells treated with or without retinoic acid reduced the CAMSAP1L1 protein level nearly 60% and stimulated neurite outgrowth, as measured by total length, number of processes and number of branches. The rs2292096 GG genotype of CAMSAP1L1, which was associated with reduced risk of symptomatic epilepsy, tended to associate with increased expression of CAMSAP1L1, which represses neurite outgrowth. Greater neurite growth in response to brain insults may increase formation of ectopic neural circuits and thus the risk of epileptogenesis. Treatments blocking this pathway might be developed to prevent post-injury epilepsy.

S2-04

Dysregulation of retinoic acid synthesis predisposes embryos of diabetic pregnancy to congenital malformations

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Although it is well-documented that babies of mothers with diabetes mellitus are more prone to have malformations, such as neural tube defects, knowledge of the underlying mechanisms remains limited. Here, we identify that the biosynthesis and endogenous levels of all-*trans* retinoic acid (RA), a key signaling molecule that controls the transcription of hundreds of genes important for embryo development, show significant reduction in embryos of streptozotocin-induced diabetic mice, which can be normalized by lowering maternal blood glucose levels. Culturing rodent embryos in varying concentrations of D-glucose demonstrate a dose-dependent reduction of RA synthesis and RA levels, concomitant with an increase in malformations. Notably, replenishing RA in vitro rescued embryos from glucose-induced malformations including neural tube defects. Conversely, further in vivo reduction of RA levels in embryos of diabetic mice by maternal treatment with a RA synthesis inhibitor increases malformations. Together, our results indicate a previously undescribed pathogenic role of dysregulation of RA levels in elevating risk of malformations in diabetic pregnancies, which provides insights into new preventive and therapeutic strategies for diabetic embryopathy.

S2-05

Translational research in clinical practice

Qiang Liu[2], Wai Sang Poon[2], Xian Lun Zhu[2], Kam Sze Kent Tsang[2], Gang Lu[1,2]

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Translational research has emerged as the emphasis of modern medical research, which refers to transforming scientific discoveries arising from the laboratory, clinical, or population studies into clinical applications.

The science research is traditionally divided into Basic Research and Applied Research. Due to the compartmentalization of science, there are barriers to transfer knowledge and findings from basic science to practical application. Translational research is the key component to integrate the basic and applied research together, and focus on multi-disciplinary collaboration. There are generally five steps for translational research, Basic Scientific Discovery, Early Translation, Late Translation, Dissemination and Adoption. The process of translational research requires collaboration, data sharing, data integration and standards for the communication. The seamlessly integration of all parts of the whole scope is critical to accelerate the transition.

In this presentation, the speaker will mainly talk about two leaps of transforming including the "bench-to-bedside" research, which translates basic sciences knowledge into new clinical practice, and the application of findings from clinical trials into multi-centre practice to improve population health. Cases about HA hospital nets everyday practice from clinical translation studies are presented, Strategies of how to make use of the advantage of University-Hospital-Government complexes is discussed.

S2-06

Astragaloside IV, a novel CXCR4 antagonist from *Astragalus membranaceus*, inhibits chemoinvasion and chemomigration in breast cancer cell lines

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The chemokine receptor CXCR4 and its natural ligand CXCL12 have been demonstrated to be involved in breast cancer metastasis. Overexpression of CXCR4 increased both bone and lung cancer metastasis. This CXCR4-mediated metastasis can be reduced by blockade of CXCR4 signaling thru drug intervention, indicating that specific CXCR4 antagonists can serve as anti-cancer drugs. Astragaloside IV (ASN IV) is a major saponin purified from *Astragalus membranaceus* (Fisch) Bge, which has been reported to exhibit anti-tumor activities. Our recent study showed that ASN IV blocked CXCL12-induced CXCR4 internalization by competitive binding to CXCR4, therefore inhibiting downstream intracellular signaling. ASN IV inhibited CXCL12-induced chemoinvasion and chemomigration in MDA-MB-231 cells that express high levels of CXCR4. Overexpression of CXCL12 sensitized MDA-MB-231 cells to the inhibition of ASN IV, which was abolished by CXCR4 knockdown in the same cells. The inhibition of ASN IV was also observed in MCF-7/CXCR4 cells but not in MCF7 cells that express low levels of CXCR4. ASN IV also significantly decreased tube formation in HUVEC cells, which can be attenuated by knockdown of CXCR4. Taken together, we have provided here for the first time a novel mechanistic explanation of anti-tumor activity of *Astragalus membranaceus* through CXCR4 antagonsim by astragaloside IV.

S2-07

TRPC5 is essential for p-glycoprotein induction in drug-resistant cancer cells

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An attractive strategy to overcome multidrug resistance in cancer chemotherapy is to suppress P-glycoprotein (P-gp), which is a pump overproduced in cancer cells to remove cytotoxic drugs from cells. In the present study, a Ca^{2+}-permeable channel TRPC5 was found to be overproduced together with P-gp in Adriamycin (ADM)-resistant breast cancer cell line MCF-7/ADM. Suppressing TRPC5 activity/expression reduced the P-gp induction and caused a remarkable reversal of adriamycin resistance in MCF-7/ADM. In an athymic nude mice model of adriamycin-resistant human breast tumor, suppressing TRPC5 decreased the growth of tumor xenografts. NFATc3 was the transcriptional factor that links the TRPC5 activity to P-gp production. Together, we demonstrated an essential role of TRPC5-NFATc3-P-gp signaling cascade in P-gp induction in drug-resistant cancer cells.

S2-08

Exposure risk and potential biomarker of pyrrolizidine alkaloid-induced tumorigenicity

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Pyrrolizidine alkaloids (PAs) are widespread substances in plant kingdom. Hepatotoxic PAs have been detected in herbal plants, teas, dietary supplements, honeys, and milks. Our recent study observed that about 80% of retail honeys purchased in Hong Kong markets contained PAs. PAs are considered as a group of natural toxins with a high risk of acute poisonings, prolonged toxicities and potential tumorigenicity. PAs are pro-toxins and induce toxicity via metabolic activation to generate toxic pyrrolic metabolites, which are chemically reactive and react rapidly with cellular macromolecules to form pyrrole-protein adducts and pyrrole-DNA adducts leading to hepatotoxicity and tumorigenicity, respectively. PAs were found to induce liver tumors by U.S. National Toxicology Program chronic tumorigenicity studies on one representative toxic PA, and the results demonstrated that the tumorigenic potencies correlated closely with hepatic pyrrole-DNA adducts. Recently, we have developed an UPLC-ESI-MS method and successfully determined pyrrole-DNA adducts in both liver and blood samples in PA-intake animals. These studies will facilitate the better understanding of the mechanism underlying the PA-induced tumorigenicity, and also provide a rational for further development of a potential biomarker for the evaluation of tumorigenic risk of PAs. [Supported by RGC GRF grants (471310 and 469712) and CUHK Direct Grant (2041744)]

S2-09

Toll-like receptor 4 (TLR4) signaling in primary sensory neurons

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The purpose of the current study is to examine the characteristics of Toll-like receptor 4 (TLR4) in rat dorsal root ganglion (DRG) cells, comprising a mixture of primary sensory neurons and associated glial cells. 65 ± 5% of neurons showed cell surface expression of TLR4-ir, and approximately 70% of these cells co-expressed the associated signaling molecules CD14 and MD-2; none of these TLR4 signaling molecules were detected on glial cells. When DRG cells were incubated with the TLR4 agonist lipopolysacharride (LPS), we detected a TLR4-dependent increase in the expression of IL-1β and TNFα mRNA immediately preceding expression of COX-2 mRNA, with no increase in IFNβ mRNA. These results suggest activation of the MyD88-dependent, but not MyD88-independent, signaling pathway in DRG neurons. Although inhibition of NF-κB attenuated LPS-stimulated cytokine expression by 73%, COX-2 expression only decreased by 32%, suggesting an additional route for activating COX-2 gene expression. In conclusion, DRG neurons express functionally active TLR4 which signals via the MyD88-dependent cell signaling pathway to regulate expression of mediators of inflammation. (This work was supported by a grant from the Research Grants Council of the Hong Kong SAR (GRF476710)).

S2-10

Regulation of COX-2/PGE2 by epithelial sodium channel (ENaC)

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Embryo implantation remains a poorly understood process. The implantation is initiated by embryo attachment to endometrial epithelium followed by the so-called decidualization process in the stroma beneath the epithelium into large, round or polygonal decidual cells, which is considered a maternal prerequisite for embryo implantation and successful pregnancy. It remains unclear how signals from the embryo (either physical or chemical) at the endometrial surface are converted into chemical signals, such as PGs, leading to decidualization in the stromal cells that do not have direct contact with the embryo.

Our previous work has demonstrated that ENaC is functionally expressed in the endometrial epithelial cells with maximal expression detected at peri-implantation period. Our recent study has also revealed a previously unrecognized role of ENaC in signal transduction leading to decidualization. We demonstrate that the activation of ENaC in mouse endometrial epithelial cells by an embryo-released serine protease results in membrane depolarization leading to activation of L-type Ca^{2+} channels and Ca^{2+} influx. This Ca^{2+} influx can lead to PGE_2 release, and induce phosphorylation of the transcription factor CREB targeting COX-2, the enzyme important for PGE2 production and implantation. Maximum ENaC activation is detected at the time of implantation in mice. Blocking or knocking down uterine ENaC in mice results in implantation failure with altered implantation markers and microRNAs targeting COX-2. These results indicate a previously undefined role of ENaC in regulating PGE_2 production and release required for embryo implantation. More interestingly, similar regulatory mechanism is found in other tissues such as the lung. Taken together, it is clear that ENaC plays an important role in regulation of COX-2/PGE2 and associated physiological functions.

S2-11

Inheritable and precise large genomic deletions of non-coding RNA genes in zebrafish using TALENs

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Transcription activators like effectors nucleases (TALENs) have so far been applied to disrupt protein-coding genes which constitute only 2-3% of the genome in animals. The majority (70-90%) of the animal genome is actually transcribed as non-coding RNAs (ncRNAs), yet the lack of efficient tools to knockout ncRNA genes hinders studies on their *in vivo* functions. Here we have developed novel strategies using TALENs to achieve precise and inheritable large genomic deletions and knockout of ncRNA genes in zebrafish. We have demonstrated that individual miRNA genes could be disrupted using one pair of TALENs, whereas large microRNA (miRNA) gene clusters and long non-coding RNA (lncRNA) genes could be precisely deleted using two pairs of TALENs. We have generated large genomic deletions of two miRNA clusters (the 1.2 kb *miR-17-92* cluster and the 79.8 kb *miR-430* cluster) and one long non-coding RNA (lncRNA) gene (the 9.0 kb *malat1*), and the deletions are stably transmitted through the germline. Taken together, our results establish TALENs as a robust tool to engineer large genomic deletions and knockout of ncRNA genes, thus opening up new avenues in the application of TALENs to study the genome *in vivo*.

Key words: TALENs; Non-coding RNAs; Zebrafish; Gene knockout.

Poster Presentation Session

6 June 2013 (Thursday) 13:45 – 14:45

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

Title of Presentation	*Abstract No.*
Regulatory role of an orphan nuclear receptor LRH-1 in castration-resistant growth of prostate cancer cells Lijia Xiao, Shan Yu, Wendy W.L. Hsiao, Franky Leung Chan	P-01
Roles of a Ras-related GTPase in anti-tumor immunity: a lesson from characterizing an R-Ras knockout mouse G. Singh, D. Hashimoto, X. Yan, Y. He, J. Hel6, P. Park, G. Ma, R.F. Qiao, C.A. Kennedy, S.H. Chen, M. Merad, C.A. Hillary, A.M. Chan	P-02
Small molecule microRNA-34a modulators identified through library screening inhibited hepatocellular tumor growth Z.G. Xiao, C.H.K. Cheng, Y.C. Chen	P-03
GPCR MAS suppresses purinergic P2Y2 receptor and glucose transport 1 activity via interacting with a ligand-like motif J.X. Sun, M.H.K. Yeung, M.K. Teng, E.Y.M. Chan, L. Zhang, C.Y. Yip, S.S.T. Lee, N.M. Zhou, Y.Y. Ho, W.H. Ko, W.T . Cheung	P-04
Cathelicidin is a host defense peptide in controlling *Helicobacter pylori* survival and infection C.H. Cho, L. Zhang, William K.K. Wu	P-05
Study of the probable use of photodynamic therapy for treatment of breast cancer and Methicillin-Resistant *Staphylococcus Aureaus* (MRSA) infection S.W.H. Hoi, J.Y.W. Chan, N.H. Bui-Xuan, J.L. Jiang, H.M. Wong, K.K.Y. Cheung, B.Y. Wang, C.L. Chan, C.B.S. Lau, C.K. Wong, M. Ip, K.P. Fung	P-06
Differential regulation of substance P induced human mast cell activation by the Toll-like receptor 2 agonists, peptidoglycan and tripalmitoyl-S-glycero-Cys-(Lys)4 H.Y.A. Lau, Y.Y. Yu	P-07
Anti-melanoma activity of an isomalabaricane triterpene W.K. Liu, F.W.K. Cheung, Y.H. Ling, C.T. Che	P-08
A potential human hepatocellular carcinoma inhibitor from *Bauhinia purpurea* seeds: from purification to mechanism exploration T.B. Ng, E.F. Fang, J.H. Wong, W.L. Pan, Y.S. Chan, X.L. Dan, C.M. Yin, R.C.F. Cheung	P-09
FHL2 regulates interleukin-6 expression through p38 MAPK mediated NF-κB pathway in muscle cells C.H. Wong, G.W.Y. Mak, M.S. Li, S.K.W. Tsui	P-10
MiR-218 suppressed tumorigenesis in hepatocellular carcinoma through targeting oncogene Bmi-1 and activating P14 and P16 signaling Jin-fang Zhang, Wei-mao Wang, Hua Wang, Hsiang-fu Kung	P-11
Treatment of giant cell tumor of bone by the combination of nitrogen-bisphosphonates and prenyl transferase inhibitors: an in vivo study Carol P.Y. Lau, Wayne Y.W. Lee, Gang Li, Stephen K.W. Tsui, Lin Huang Shekhar M. Kumta	P-12
Sorafenib – an opportunity in translational research in hepatocellular carcinoma Paul B.S. Lai, George G. Chen	P-13
Role of Smad3-dependent microenvironment in cancer progression Shuang Zhou, Xiaoming Meng, Guangyu Lian, Xiaoru Huang, Yongjiang Tang, Huiyao Lan	P-14

Title of Presentation	*Abstract No.*
CUDC-101, a hybrid molecular targeted agent, reverses multiple mechanisms of anticancer drug resistance Kenneth K.W. To, Daniel C. Poon, X.G. Chen, Ge Lin, Li-wu Fu	P-15
microRNA-29b prevents liver fibrosis by attenuating hepatic stellate cell activation and inducing apoptosis in vitro and in mice Jia Wang, Eagle S.H. Chu, H.Y. Lan, Joseph J.Y. Sung, Jun Yu	P-16
Decreased basal and postprandial plasma acylated ghrelin in female patients with Functional Dyspepsia (FD) Cynthia K.Y. Cheung, Ying Ying Lee, Yawen Chan, Pui Kwan Cheong, Wai Tak Law, Joseph J.Y. Sung, Francis K.L. Chan, Justin C.Y. Wu	P-17
Molecular mechanism of regulation and action of microRNA-199a in development and differentiation S. Gu, H.H. Cheung, X. Chen, G. Lu, W.S. Poon, W.Y. Chan	P-18
Sacral neural crest cell migration and their genome-wide expression in the *Dominant megacolon* mouse mutant Y.H. Hou, C.F. Wang, T.D. Wang, T.L. Lee, W.Y. Chan	P-19
Activation of the novel G protein-coupled receptor 30 (GPR30) inhibits P2Y receptor-mediated Ca^{2+} signalling in human bronchial epithelia M.Y. Hao, A.W.M. Chow, W.C.Y. Yip, W.T.F. Wan, W.W.Y. Chan, C.H.K. Cheng, W.H. Ko	P-20
Novel lncRNA regulations in lung cancer Jiajie Tu, Alfred Chun Shui Luk, Shuk Han Ng, Tin-lap Lee	P-21
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P-01

Regulatory role of an orphan nuclear receptor LRH-1 in castration-resistant growth of prostate cancer cells

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The advanced development of castration-resistant prostate cancer (CRPC) in patients upon androgen-deprivation therapy is generally believed to be mostly mediated by reactivation of androgen receptor (AR) signaling or its bypass with mechanisms involved, including clonal selection of androgen-independent or stem cell-like cell populations, AR hypersensitivity due to AR overexpression, and ligand promiscuity or independence by AR mutations, intratumoral conversion of adrenal androgens to high affinity AR ligands. Besides, the de novo steroid synthesis from cholesterol in CRPC has also been proposed. In this study, we found that the nuclear receptor liver receptor homolog-1 (LRH-1) might contribute to the CRPC growth or de novo steroid synthesis in prostate cancer cells through its positive regulation on expression of several critical enzymes in steroidogenesis, including CYP17A1, HSD3B1, HSD3B2 and StAR. Functional analyses showed that overexpression of LRH-1 could induce higher resistance to antiandrogen bicalutamide and steroid-depleted culture condition in AR-positive androgen-sensitive (LNCaP) but not in AR-negative androgen-insensitive (DU145) prostate cancer cells. *In vivo* tumorigenicity study showed that LNCaP-LRH-1 cells grew more aggressively in castrated or intact SCID mice, contrary to LNCaP-vector control cells that did not grow in castrated mice. Importantly, liquid chromatography-tandem mass spectrometry showed that intratumoral androgen concentrations (testosterone and DHT) were significantly higher in tumors formed by LNCaP-LRH-1 cells than those by LNCaP-vector control cells. Together, our results indicated that LRH-1 might play a regulatory role in advanced castration-resistant growth of prostate cancer via positive control on de novo steroid synthesis in prostate cancer cells.

P-02

Roles of a Ras-related GTPase in anti-tumor immunity: a lesson from characterizing an R-Ras knockout mouse

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R-Ras is a member of the *RAS* superfamily of small GTP-binding proteins with a restricted pattern of expression. We found that mouse melanoma cells from an allogeneic source induced tumors in *Rras*$^{-/-}$ mice with shorter latency and greater in size. This finding has prompted the investigation of a role for R-Ras in the immune system. We uncovered that Dendritic cells (DCs) from *Rras*$^{-/-}$ mice were impaired in priming allogeneic and antigen-specific T-cell responses. *Rras*$^{-/-}$ DCs expressed lower levels of surface MHCII and CD86 in response to lipopolysaccharide (LPS) when compared to wild type. These defects were associated with an attenuated capacity of *Rras*$^{-/-}$ DCs to form stable immunological synapses with T-cells. Further studies also revealed multiple defects in T cells. *In vivo* T cell trafficking studies have detected a 1.5-fold reduction in homing to lymph nodes but not to the spleen. This is correlated with significant decreases in lymph node size, celluarity, and maturation of high endothelial venues. These observations were correlated with reduced surface expression of L-selectin/CD62L on $CD4^+$ and $CD8^+$ T-cells. Indeed, *Rras*$^{-/-}$ T cells have lowered rolling and firm adhesion capacity on endothelial cells under low shear stress conditions. Consistently, *Rras*$^{-/-}$ T-cells have attenuated binding capacity to Intercellular Adhesion Molecule 1 upon chemokine stimulations. These data unravel a novel small G-protein signaling pathway in anti-tumor immunity.

P-03

Small molecule microRNA-34a modulators 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. In this study, we established the miR-34a luciferase report system for screening small molecule activators of miR-34a. Two compounds named **1** and **3** were identified to be microRNA-34a activators. These two compounds dramatically and specifically activated microRNA-34a expression in HCC cells with microRNA-34a silencing. We further demonstrated that these two compounds exhibited growth inhibiting activities on various HCC cell lines but not in non-tumorigenic human hepatocytes. These two compounds also downregulated the expression of microRNA-34a target proteins cyclin D1 and Bcl-2. Compound **1** and **3** induced HCC cell cycle arrest and inhibited HUVEC cell tube formation in vitro. Furthermore, compound **1** and **3** dramatically inhibited tumor growth in xenografted HCC mouse model without any obvious side effects. These two compounds activated mir-34a expression in HCC cells with wild type or mutated p53 but not in p53-deleted HCC cell, suggesting that p53 may play important roles underlying the anticancer activities of compound 1 and 3. CHIP assay showed that these two compounds significantly enhanced p53 activitie the occupancy of p53 on miR-34a promoter.

P-04

GPCR MAS suppresses purinergic P2Y2 receptor and glucose transport 1 activity via interacting with a ligand-like motif

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Orphan G protein-coupled receptor (GPCR) *MAS* was initially isolated from a human epidermal carcinoma, and expression studies suggest MAS can be activated by angiotensin peptides and RFamide neuropeptides. Using phage-displayed peptide library, our lab identified a surrogate ligand MBP7 (MAS Binding Peptide 7) for MAS. To characterize the ligand-independent receptor activity of MAS, several stable CHO cell lines expressing native *MAS* at different levels were used to examine its effect on purinergic receptor-mediated calcium mobilization. It is of interest to note that the higher the levels of *MAS* expression, the larger the right shift of the dose-response curve of ATP-stimulated calcium mobilization. However, in calcium-free buffer, only the maximal response of ATP-induced calcium mobilization was reduced but there was no change in the potency of ATP. These results suggested MAS overexpression suppressed P2Y receptor-mediated calcium mobilization. Expression of P2Y2 receptor was not significantly altered by overexpression of MAS in CHO cells as evidenced by Western and RT-PCR. Intriguingly, a sequence motif similar to the surrogate ligand MBP7 was found in the putative C-terminal tail of P2Y2 receptor, implying MAS suppressed P2Y2 activity probably via interacting with the MBP7-like motif. In line with this hypothesis, an MBP7-like motif was also identified in glucose transporter 1 and 7 (GLUT1 and 7), and GLUT1-mediated glucose uptake in cells overexpressing *MAS* was lower than that of cells stably transfected with empty vector. By contrast, disrupting the MAS - GLUT 1 interactions in the presence of MBP7, GLUT1-mediated glucose uptake was elevated in cells over-expressing *MAS* but not in cells stably transfected with empty vector.

P-05

Cathelicidin is a host defense peptide in controlling *Helicobacter pylori* survival and infection

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Cathelicidin, a host defense antibacterial peptide in humans can eradicate different kinds of microbial infection. This study sought to elucidate the actions of cathelicidin in protection against *Helicobacter pylori* (*H. pylori*) infection both *in vitro* and *in vivo*. To examine the direct antimicrobial action of cathelicidin, *H. pylori* survival, biofilm formation and morphology change were determined after exposure to different doses of cathelicidin *in vitro*. Intracellular *H. pylori* in gastric epithelial cells and in cathelicidin wild-type ($Cnlp^{+/+}$) and knockout ($Cnlp^{-/-}$) mice stomachs were also investigated. Results showed that exogenous cathelicidin could affect *H. pylori* growth, destroy bacteria biofilm and cause pore formation in *H. pylori* membranes. To further study the role of intrinsic cathelicidin in controlling *H. pylori* infection, we induced the synthesis of endogenous cathelicidin in gastric epithelial cells by the active form of vitamin D (1,25D3). Indeed 1,25D3 significantly increased the cellular level of cathelicidin and reduced the intracellular *H. pylori in vitro*. Furthermore, $Cnlp^{-/-}$ mice exhibited stronger *H. pylori* colonization after both acute and chronic *H. pylori* infection when compared to $Cnlp^{+/+}$ mice. To further confirm this antibacterial action was due to endogenous cathelicidin, a high level of mouse gastric cathelicidin (CRAMP) production was found in $Cnlp^{+/+}$ mice but not in $Cnlp^{-/-}$ animals. This finding could partially explain why there was less bacterial infection in the wild type mice. Taken together, these results indicate that human cathelicidin plays a significant role as a host defense peptide against *H. pylori* infection and can promote clearance of the bacteria from the stomach.

Grant support: Research Fund for the Control of Infectious Diseases, Food and Health Bureau, Hong Kong SAR, P.R. China.

P-06

Study of the probable use of photodynamic therapy for treatment of breast cancer and Methicillin-Resistant *Staphylococcus Aureaus* (MRSA) infection

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Photodynamic therapy (PDT) in defined as the administration of non-toxic agent known as photosensitizer either systemically, locally or topically into a patient, followed by illumination with visible light, which, in the presence of oxygen, leads to the generation of cxytotoxic reactive oxygen species (ROS) and subsequent cell death. We have purified a photosensitizer, Pheophorbide a (Pa) from Traditional Chinese Medicine *Scutelleria barbata* and examined its probable use in combination with visible light to treat human breast cancer and MRSA-infected wound. We found that IC_{50} value on human breast tumour MCF-7 cells was found to be 0.5 μM when incubated with the cells for 24 hours after photodynamic therapy with Pa (Pa-PDT). Mechanistic studies demonstrated that Pa was localized in the mitochondria and ROS were found to be released after Pa-PDT on MCF-7 cells. Apoptosis was found to be the major mechanism for the tumour cell death. Mitochondrial membrane depolarization and cytochrome *c* release revealed the role of mitochondria in the apoptotic mechanism. Increased expression of tumour suppressor protein p53, cleavage of caspase-9, caspase-7 and Poly (ADP-ribose) polymerase indicated the involvement of caspase-dependent pathway. On the other hand, apoptosis-inducing factor release demonstrated the mediation of caspase-independent mechanism. *In vivo* study using the mouse xenograft model showed a significant inhibition of tumour growth by Pa-PDT in nude mice bearing MCF-7 cells. Pa-PDT may also exhibit anti-angiogenesis and immunomodulatory activities. We further investigated whether Pa-PDT could be a modality to treat MRSA-infected wound. The *in vitro* efficacies of Pa-PDT in inhibiting the growth of MRSA strains and its probable wound healing and immunomodulatory activities in a mouse model of MRSA-infected wound will be studied. Preliminary results will be presented.

P-07

Differential regulation of substance P induced human mast cell activation by the Toll-like receptor 2 agonists, peptidoglycan and tripalmitoyl-S-glycero-Cys-(Lys)4

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Human mast cells express Toll-like receptors (TLRs) and serve as key players in innate immunity. The neuropeptide substance P (SP) is well known for its role in evoking neuroimmunological responses and in participating in allergic disorders by activation of pertussis toxin (PTX)-sensitive Gαi/o protein in mast cells. The study aims to investigate the effects of TLR2 agonists peptidoglycan (PGN) and tripalmitoyl-S-glycero-Cys-(Lys)4 (Pam3CSK4) on human mast cell line LAD2 cells activation and the modulatory effects of TLR2 ligands on LAD2 cells activation in response to SP. TLR2 agonists did not cause degranulation, but induced the release of IL-8. Pretreatment of PGN and Pam3CSK4 inhibited SP induced degranulation. Pam3CSK4 acted by blocking the calcium mobilization induced by SP, while PGN did not. In the case of IL-8 release, PGN acted in synergy with SP, but Pam3CSK4 failed to demonstrate similar effect. Synergistic release of IL-8 induced by PGN and SP required the activation of Ca^{2+}/calcineurin/NFAT, Erk, NF-κB and PI3K signaling networks, while Pam3CSK4 acted by occupying the binding site for SP on $G\alpha_{i/o}$ proteins to inhibit the effect of SP. These findings suggest that activation of human mast cells can be differentially modified by different TLR2 agonists via distinct signaling pathways.

P-08

Anti-melanoma activity of an isomalabaricane triterpene

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Melanoma is one of the most malignant cancers that causes over 70% of the mortality by skin cancers in Caucasian populations, but effective treatment is limited by its rapid metastasis, low response rates and fast development of resistance to chemotherapy. New modalities and more potent chemotherapeutic agents are urged to attenuate this highly fatal disease. About 50% of proteins in melanoma cells are synthesized and glycosylated in the rough endoplasmic reticulum (ER) before they are secreted to the Golgi complex and target organelles. Glycosylation is an enzymatic process through which an oligosaccharide is conjugated to a protein for proper physiological events. Aberrant glycosylation not only interferes with protein maturation but also initiates ER stress and unfolded protein responses, and triggers cell death. Disruption of glycosylation has been a novel therapeutic strategy for melanoma. In a continuing search for bioactive natural products, it was found that isomalabaricanes, a small class of rearranged triterpene metabolites obtained from marine sponges, inhibited the growth of melanoma cells by an induction of abnormal protein glycosylation, ER stress and cell death.

P-09

A potential human hepatocellular carcinoma inhibitor from *Bauhinia purpurea* seeds: from purification to mechanism exploration

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A 20-kDa Kunitz-type trypsin-chymotrypsin inhibitor, *Bauhinia purpurea* trypsin inhibitor (BPLTI), has been isolated from the seeds of *B. purpurea* L. by using liquid chromatography procedures that involved ion exchange chromatography on SP-Sepharose and Mono S and gel filtration on Superdex 75. BPLTI demonstrated protease inhibitory activities of 7226 BAEE units/mg and 65 BTEE units/mg toward trypsin and α-chymotrypsin, respectively. BPLTI was relatively thermal (0 – 60 ℃) and pH (3 - 10) stable and its activity could be decreased by dithiothreitol treatment. BPLTI exhibited a wide spectrum of anti-proliferative and pro-apoptotic activities especially on human hepatocellular carcinoma Hep G2 cells. However, it was devoid of a significant antiproliferative effect on immortal human hepatic WRL 68 cells. We show here that BPLTI stimulates apoptosis in Hep G2 cells, including (1) evoking DNA damage including the production of chromatin condensation and apoptotic bodies; (2) induction of cell apoptosis/necrosis; (3) mitochondrial membrane depolarization; and (4) increasing the production of cytokines. Taken together, our findings show for the first time that purified protease inhibitor from *B. purpurea* L. seeds is a promising candidate for the treatment of human hepatocellular carcinoma.

P-10

FHL2 regulates interleukin-6 expression through p38 MAPK mediated NF-κB pathway in muscle cells

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Interleukin 6 (IL-6) is pleiotropic cytokine playing an important role in inflammatory response. Other than classical immune tissues, IL-6 is also produced in muscle cells under specific conditions. Four-and-a-half LIM-only protein 2 (FHL2) is preferentially expressed in skeletal and cardiac muscle cells compared to other tissues indicating it has an important role in skeletal muscle and cardiovascular system. In this report, the regulation of IL-6 by FHL2 in muscle cells was investigated. We demonstrated that FHL2 overexpression increased IL-6 mRNA level and its protein secretion in skeletal myoblasts. In contrast, the IL-6 secretion was significantly decreased after FHL2-knockdown by siRNA in response to TNFα stimulation. We further showed that FHL2-mediated induction of IL-6 was regulated by the activation of IL-6 promoter through stimulating NF-κB and p38 MAPK signaling pathway. Our results further illustrated the molecular mechanisms of IL-6 production, which provides new insights in the roles of FHL2 in post-injury inflammation or cytoprotection of muscle cells.

P-11

MiR-218 suppressed tumorigenesis in hepatocellular carcinoma through targeting oncogene Bmi-1 and activating P14 and P16 signaling

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Oncogene Bmi-1 regulates cell cycle and senescence in multiple cancers and our preliminary data displayed that it was up-regulated in hepatocellular carcinoma (HCC), indicating that it mediates carcinogenesis in HCC. Although several miRNAs have been reported to suppress Bmi-1 expression, the pathophysiological function of miRNA involved in HCC tumorigenesis by regulating Bmi-1 expression remains largely unknown. In the present study, online programs predicted that there are 6 miRNAs which could directly target Bmi-1. Among these candidates, miR-218 was identified to be the most promising one by using specific qPCR analyses. And we also demonstrated that Bmi-1 was a real target of miR-218 in HCC cells. Further investigation displayed that ectopic miR-218 dramatically inhibited cell viability and colony formation by inducing G1 phase arrest in hepatoma cells *in vitro*. We also found it suppressed the *in vivo* tumorigenicity in both xenograft and orthograft models, thereby suggesting that miR-218 may act as a tumor suppressor in carcinogenesis. Tumor suppressor $P16^{Ink4a}$ and $P14^{ARF}$ are the main down-stream targets of Bmi-1 and our results revealed that miR-218 promoted the activation of P14-P53 and P16-Rb signaling. Finally, the down-regulation of miR-218 was found in 53.1% of HCC tissues and its expression was inversely associated with Bmi-1 expression.

Conclusion: Our data suggest that miR-218 may be a promising candidate against HCC and implicate its potential application in cancer therapy.

P-12

Treatment of giant cell tumor of bone by the combination of nitrogen-bisphosphonates and prenyl transferase inhibitors: an in vivo study

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Giant Cell Tumor of Bone is the most common bone tumor reported in the Asian population. This is an aggressive tumor that primarily affects the younger patient at 20-40; and is characterized by a destructive lesion that affects the major joints at the end of long bones. Often the tumor recurs even after repeated surgery with significant morbidity to the young patients. The use of some novels drugs may help reduce the aggressive potential of this tumor with obvious advantages to the patient. However, a major barrier towards the study of the effects of drugs and their combinations on GCT has been the lack of an animal model.

In this study, we have piloted a novel animal model of the Giant Cell Tumor in which we propose to study the effects of a novel combination of drugs, Bisphosphonates (Zoledronate) and Prenyl Transferase Inhibitors (GGTI-298), and to evaluate their effects on the tumor. The use of this combination of drugs bases on the experimental results in our previous study, which showed the combination of bisphosphonates and GGTIs significantly inducing S-phase cell cycle arrest and caspase-3/7 activity. Furthermore, this combination also increased the OPG/RANKL ratio, which favored bone formation and may reduce bone resorption caused by the osteoclast-like giant cells in the tumor. In the long-term, the reduced recurrence following adjuvant drug therapy may translate to major economic benefits given that the scale and magnitude of surgery may be reduced in these patients.

P-13

Sorafenib – an opportunity in translational research in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is a common cancer in Hong Kong. Overall prognosis is poor as a result of a number of factors, including late presentation, high recurrence rate after curative surgery, lack of adjuvant therapies to reduce post-resection recurrence, relatively ineffective palliative treatment, poor tumor responses to systemic chemotherapy and target therapy.

Sorafenib, the only approved target therapy for the systemic treatment of HCC, has aroused a lot of interest because the molecular mechanisms to account for the clinical effectiveness of Sorafenib are rather unclear. Furthermore, while some patients displayed objective disease control, a significant proportion of HCC patients showed very little response to Sorafenib. Thus, more understanding of the complex interactions of Sorafenib is desperately needed to better explain the molecular mechanisms. With such understanding, we may be able to design better strategies (e.g. combination of other chemotherapeutic agents) to improve the anti-tumor effects of Sorafenib.

We have recently showed that sorafenib reduced tumor vascularisation and the growth of HCC via inhibiting HIF-1α synthesis and such an inhibition is associated with previously undefined pathways in which mTOR/p70S6K/4E-BP1 and ERK phosphorylation are downregulated. One group, however, has recently reported that the hypoxic micro-environments induced by sustained sorafenib treatment may occur in a subset of HCCs conferring sorafenib resistance to HCC through HIF-1α and NF-κB activation. Further experiments should aim to identify markers that can be used to define sorafenib resistance and sorafenib sensitivity.

P-14

Role of Smad3-dependent microenvironment in cancer progression

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Cancer microenvironment is a complex system involving the interaction between cancer cells and the surrounding stromal cells. TGF-β1 is a potent tumor promoter and may act through the cancer microenvironment to drive cancer progression. However, mechanisms governing this malignant process remain yet largely unclear. We hypothesized that cancer-derived TGF-β1 may drive the malignant progression through the Smad3-dependent tumor microenvironment. This was tested in two syngeneic mouse tumor models of lung carcinoma (LLC) and melanoma (B16F10) in Smad3 gene KO mice. We found that mice null for Smad3 were protected against cancer growth, invasion, metastasis, and death of the mouse, which was associated with angiogenesis blockade, MMP system inhibition and Treg cells reduction, also associated with enhanced NK functional activity, leading to a marked upregulation of IFN-γ and IL-2, and granzyme B within the cancer microenvironment and plasma. Furthermore, the role of bone marrow-derived Smad3-dependent microenvironment in cancer progression was determined by GFP-Smad3 WT or KO bone marrow chimeric mice LLC and B16F10 tumor models. Targeted therapeutic remodeling of Smad3-dependent microenviroments using Smad3 inhibitor prevented cancer progression in vivo. The results suggested that bone marrow-derived Smad3-dependent cancer microenvironments may determine cancer progression or regression, and Smad3-mediated suppression of NK cell immunity may be a key mechanism.

P-15

CUDC-101, a hybrid molecular targeted agent, reverses multiple mechanisms of anticancer drug resistance

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CUDC-101 is a potent molecular targeted anticancer agent, rationally designed to simultaneously inhibit HDAC, EGFR, and HER2. It exhibits potent antiproliferative and proapoptotic activities *in vitro* and *in vivo*, and it is expected to enter Phase II clinical trial this year.

Multidrug resistance (MDR) is a major unresolved obstacle to successful cancer chemotherapy. It is often associated with increased ABC transporters-mediated efflux of substrate anticancer drugs out of the cells. 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 upon their administration, which can be caused by overexpression of 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 enhance the cytotoxicity and accumulation of substrate anticancer drugs preferentially in MRP-1 (doxorubicin) or MRP-2 (methotrexate) overexpressing cells than 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 their ATPase activity. In Pt-resistant cancer cells, CUDC-101 appears to circumvent the resistance through inhibition of MRP-2 and DNA repair-mediated mechanisms. The combination of CUDC-101 with cisplatin or oxaliplatin was found to display synergistic cytotoxic effect in Pt-resistant cancer cell lines, but not in the sensitive cells. 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 levels of DNA repair genes (e.g. ERCC1) was inhibited in Pt-resistant cells. The data advocates further development of CUDC-101 as a novel MDR reversal agent for use in combination cancer chemotherapeutic regimens.

P-16

microRNA-29b prevents liver fibrosis by attenuating hepatic stellate cell activation and inducing apoptosis in vitro and in mice

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Background & Aims: microRNA-29b (miR-29b) is known to be associated with transforming growth factor-β (TGF-β)-mediated liver fibrosis, but the mechanistic action of miR-29b in liver fibrosis remains unexplored. We aimed to examine the role and molecular regulators of miR-29b in liver fibrosis.
Methods: Male C57BL6 mice were injected with carbon tetrachloride (CCl_4) to induce liver fibrosis. The anti-fibrotic effect of miR-29b was evaluated by ultrasound-mediated gene transfer of miR-29b through tail vein injection into the liver. Primary hepatic stellate cell (HSC) culture of rat origin was established to evaluate the change of miR-29b expression in quiescent and activated HSCs. Human HSC cell line (LX-1) and rat HSC cell line (HSC-T6) were transfected with miR-29b to study its effect on HSCs activation in vitro. The upstream regulator of miR-29b was identified by CHIP-PCR. The downstream targets of miR-29b were screened using *in silico* searches and validated by luciferase reporter assay. The role of miR-29b-modulated targets in liver fibrosis was examined by loss-of-function assays.
Results: miR-29b was significantly downregulated i) in human fibrotic liver tissues compared with normal liver tissues ($P<0.01$); ii) in activated HSCs compared with quiescent HSCs ($P<0.05$); and iii) in CCl4-induced liver fibrotic tissues compared to the normal liver tissues in mice ($P<0.05$). Gene transfer of miR-29b into the liver prevented fibrogenesis induced by CCl_4 ($P<0.01$). Such delivery of miR-29b in liver decreased expression of α-SMA, collagen I and TIMP-1, indicating that the anti-fibrotic effect by miR-29b was associated with the suppression of HSC activation. This was further confirmed by *in vitro* experiments. Ectopic expression of miR-29b in activated HSCs (LX-1, HSC-T6) inhibited cell viability ($P<0.05$) and colony formation ($P<0.05$), and caused cell cycle arrest in G1 phase ($P<0.01$). miR-29b also induced apoptosis in HSCs ($P<0.05$). The underlying mechanisms of miR-29b in liver fibrosis were therefore investigated. CHIP-PCR assay revealed that Smad3 bound to the promoter of miR-29b and down-regulated its expression. Whilst, miR-29b could in turn suppress Smad3 expression. TargentScan prediction and luciferase reporter assay showed that miR-29b targeted the 3'UTR of PIK3R1 and AKT3, and inhibited the expression of these two genes at translation level. A significant up-regulation of PIK3R1 and AKT3 was detected in human fibrotic liver tissues ($P<0.05$). Knockdown of PIK3R1 or AKT3 which blocked the phosphorylation of AKT induced apoptosis in HSCs ($P<0.05$).
Conclusions: miR-29b prevents liver fibrogenesis by inhibiting HSC activation and inducing HSC apoptosis though down-regulation of PIK3R1 and AKT3 to block AKT phosphorylation. These results provide novel mechanistic insights for the anti-fibrotic effect of miR-29b in liver.

P-17

Decreased basal and postprandial plasma acylated ghrelin in female patients with Functional Dyspepsia (FD)

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Background: The role of ghrelin in the pathogenesis of FD is unclear.

Aim: To compare the plasma ghrelin profile in female FD patients and healthy controls.

Methods:
Consecutive female FD patients (Rome III criteria) were recruited. After an overnight fast, they underwent caloric drinking test (Ensure©, 1.06 kcal/ml at 30ml/min) and 13C-Octanoic acid breath test for gastric emptying measurement. Serial blood samples were collected at fasting and 30, 60, 90, 120 min postprandially for plasma acylated ghrelin (AG) assay. Asymptomatic female healthy volunteer controls were recruited.

Results:
35 patients and 16 controls were studied with mean age of 45.1(10.3) and 44.7 (13.7) respectively. 30 patients had postprandial distress syndrome (PDS) and 5 had both PDS and epigastric pain syndrome. There was no difference in total calorie intake (FD: 721.6 ± 53.0, Control: 792.3 ± 88.7, $p = 0.48$) and gastric emptying rate (T1/2) (FD: 109.6 ± 27.5 min; Control: 73.1 ± 3.7 min, $p = 0.37$). However, FD patients had significantly lower basal AG ($p = 0.006$), at postprandial 30 min ($p = 0.002$), 60 min ($p = 0.01$), 90 min ($p = 0.002$), and 120 min ($p < 0.0001$) and area under curve ($p = 0.001$). Repeated measures of ANOVA revealed high correlation between FD and AG profile across 120 min ($p = 0.0004$).

Conclusion:
Female FD patients have significantly lower basal and postprandial plasma AG concentrations. The findings suggest (1) Ghrelin may contribute to the pathophysiology of FD and (2) modulation of AG system may have therapeutic value in treatment of FD.

P-18

Molecular mechanism of regulation and action of microRNA-199a in development and differentiation

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MicroRNA-199a (miRNA-199a) has been shown to have comprehensive functions and behave quite differently in different systems and diseases. It is encoded by two loci in the human genome, miR-199a-1 on chromosome 19 and miR-199a-2 on chromosome 1. Both loci give rise to the same miRNAs (miR-199a-5p and miR-199a-3p). The cause of the diverse action of the miRNA is not clear. However, it is likely caused by different regulation of the two genomic loci and variable targets of the miRNA in different cells and tissues. Here we studied promoter methylation of miR-199a in testicular germ cell tumors (TGCTs) and glioblastomas (gliomas) and discovered that hypermethylation in TGCTs for both miR-199a-1 and -2 resulted in its reduced expression, while hypomehylation of miR-199a-2 but not -1 in gliomas resulted in its elevated expression. In addition to DNA methylation, we also studied the functions of transcription factors in controlling the expression of miR-199a during stem cell differentiation. The action of miR-199a was also affected by its downstream targets. We applied both genomic and proteomic approaches to study the targets of miR-199a-5p in TGCTs. We identified two putative targets of the miRNA 199a-5p and confirmed that the tumor suppression activity of the microRNA is the results of its action on PODXL and MAFB. By studying the mechanisms that control the expressions of miR-199a and its various downstream targets in different systems, we hope to use miR-199a as a model to illustrate the complexity of miRNA biology.

P-19

Sacral neural crest cell migration and their genome-wide expression in the *Dominant megacolon* mouse mutant

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Hirschsprung's disease (HSCR) is a congenital disease with an incidence of 1 in 5000 live births. It is characterized by the reduction or absence of ganglia in the enteric nervous system. Abnormal migration of neural crest cells (NCCs, precursors of enteric ganglia) during embryonic development has been considered as one of the possible causes of the disease. In this study, we analyzed the gene expression profile of migrating sacral NCCs from the mouse mutant *Dominant megacolon (Dom)*, a HSCR model expressing a truncated *Sox10*. Delayed migration and aggregation of sacral NCCs from the explanted neural tube of homozygous embryos were found *in vitro*. The gene signature of migrating sacral NCCs from the mutant revealed that genes associated with myelination, melanogenesis and adhesion were significantly over-represented. Luciferase reporter assay and chromatin immunoprecipitation results suggested that one of the genes encoding adhesion molecules was regulated directly by Sox10 through the Sox10 responsive region on its promoter. Transwell migration assay showed that down-regulation of the molecule by shRNA led to reduced migration of NIH3T3 cells. Our results implicate that the mutation of *Sox10* in the *Dom* mutant down-regulates the expression of the adhesion molecule resulting in the abnormal migration of sacral NCCs.

P-20

Activation of the novel G protein-coupled receptor 30 (GPR30) inhibits P2Y receptor-mediated Ca^{2+} signalling in human bronchial epithelia

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The airway epithelium plays a central role in respiratory physiology through its transport and immunological functions. Our previous study suggested that P2Y receptors are expressed in airway epithelia and play a significant role in regulating transepithelial ion transport. P2Y receptors belong to the family of purinergic receptors, which can be stimulated by nucleotides such as UTP and UDP. P2Y receptors are G protein-coupled receptors and classically signal through Gq, resulting in an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and thereby in the activation of Ca^{2+}-dependent ion channels and downstream signalling pathway(s). Furthermore, P2Y receptors may be involved in asthmatic inflammation.

Oestrogen (or 17β-oestradiol, E2) is an important hormone for both women and men, not only because of its capability to regulate a multitude of biological processes, but also because it is a significant target in many diseases, such as cancer and inflammation. In addition to the classical nuclear hormone receptors ERα and ERβ, a novel oestrogen receptor, G protein-coupled receptor 30 (GPR30), was recently identified and found to be involved in both rapid signalling and transcriptional regulation. The action of GPR30 is unclear, but it has been implicated in mediating anti-inflammatory responses. Our study aimed to investigate the interaction between GPR30 and P2Y receptor-mediated signalling pathways.

Our results 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. Expression of GPR30 receptors was localized in the human bronchial epithelial cells by immunofluorescence staining and western blotting of fractionated cell lysates. $[Ca^{2+}]_i$ was examined using a calcium imaging technique in Fura-2-loaded cells grown on glass coverslips. Stimulation of epithelial cells with E2 or with the specific agonist of GPR30, G1, rapidly attenuated a UDP- and UTP-evoked increase in $[Ca^{2+}]_i$. Furthermore, the specific GPR30 antagonist, G15, reversed the GPR 30-mediated inhibition of nucleotide-evoked Ca^{2+} increase. The inhibitory effects were concentration-dependent and oestrogen-specific, because only E2, but not testosterone or progesterone, could inhibit the P2Y receptor-evoked Ca^{2+} response. Our results provide the first evidence that human bronchial epithelia express GPR30, which interact with the P2Y receptor-mediated calcium signalling pathway. 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.

The work was supported by a Research Grant Council General Research Fund (Ref. No.: 466611) awarded to W. H. Ko.

P-21

Novel lncRNA regulations in lung cancer

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Lung cancer is a leading cause of cancer deaths worldwide. While coding genes remain the primary focus of current genomic and proteomic studies, dysregulation of non-coding RNAs has emerged to be critical in normal and disease development. In particular, the biological and regulatory roles of long non-coding RNAs (lncRNAs) in lung cancer development remain elusive. By performing meta-analysis on published genomic data, we have identified twelve putative lncRNAs in the lung cancer samples. The candidates were then validated in thirteen paired lung cancer samples with normal controls, and twelve lung cancer cell lines. Two promising lncRNA candidates demonstrated consistent and significant differential expression patterns in both sample groups. Unbiased examination on genomic structures and protein-RNA prediction led to a number of important regulators and pathways highly associated with lung cancer development. The results suggested a novel lncRNA circuitry might mediate lung cancer development.

P-22

Human fetal liver stromal cell co-cultures enhance the differentiation of pancreatic progenitor cells into islet-like cell clusters

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Recent advance in directed differentiation of pancreatic stem cells has potential for the development of replacement therapy for diabetes patients. Existing differentiation protocols, however, are complex, time-consuming, and costly; thus there is a critical need for alternative protocols. Given the common developmental origins of the liver and pancreas, we sought to develop a novel protocol, devoid of growth factors, by using a microenvironment established by liver stromal cells (LSCs) derived from human fetal liver. We examined the effects of this LSC microenvironment on the differentiation of established pancreatic progenitor cells (PPCs) into islet-like cell clusters (ICCs). PPCs and LSCs isolated from 1st and 2nd trimester human fetal tissues underwent co-cultures; differentiation and functionality of ICCs were determined by examining expression levels of critical markers and insulin secretory ability. Co-cultures with 2nd but not 1st trimester LSCs enhanced ICC differentiation and functionality without the use of exogenous differentiation 'cocktails'. Differential expression profiles of growth factors from 1st vs. 2nd trimester fetal liver were compared. Many morphogenic factors were expressed by LSCs, and insulin-like growth factor 1 (IGF1) was identified as being responsible for ICC differentiation. This is the first report showing that an LSC-induced microenvironment can enhance ICC differentiation from PPCs while also enhancing ICC functionality. Further modifications of the stroma microenvironment may offer an alternative, efficient and cost-effective approach to providing islets for transplantation.

P-23

Dragon (RGMb) inhibits E-Cadherin expression and 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 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 PARP 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 Dragon inhibited E-Cadherin expression through the neogenin receptor but not the BMP pathway in IMCD3 cells. Dragon expression in the kidney was upregulated by unilateral ureteral obstruction (UUO) in mice. Compared with wild-type mice, heterozygous Dragon knockout mice exhibited 45-66% reduction in Dragon mRNA expression, decreased epithelial apoptosis, increased tubular E-Cadherin expression, and had attenuated tubular injury after UUO. Our results suggest that Dragon may impair tubular epithelial integrity and induce epithelial apoptosis both in vitro and in vivo.

P-24

p150Glued-interacting domains of TRAPPC9 reveal potential function of TRAPPC9 at the centrosome

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The vesicle tethering factor TRAPP (Transport protein particle) complex has been implicated in cellular processes other than vesicle trafficking. Increasing evidence suggests that TRAPP has microtubule-related functions. We have recently discovered TRAPPC9, a subunit of TRAPPII, interacts with CT^{Glued}, the cargo-binding, carboxyl terminal domain of $p150^{Glued}$, a subunit of dynactin. In the present study, we have identified three domains on TRAPPC9 that mediate this interaction. They are called $p150^{Glued}$-interacting domain 1, 2 and 3 (GID1, 2 3). GID1 contains 52 amino acids. Purified recombinant proteins of GID1 and CT^{Glued} could interact in vitro, demonstrating a direct, physical interaction. Both GID2 and GID3 can bind to CT^{Glued} in the presence of GID1, suggesting they bind to CT^{Glued} at different sites. Unexpectedly, GFP-GID1 and GFP-GID3 target to the centrosome and this finding leads to the discovery of the endogenous TRAPPC9 signal at the centrosome when its Golgi-localized signal is first removed with the detergent digitonin. This and data from others indicates the presence of TRAPPC9 in microtubule-based axonemes.

P-25

Efficient targeted gene disruption in *Xenopus* embryos using engineered transcription activator-like effector nucleases (TALENs)

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Transcription activator-like effector nucleases (TALENs) are a novel approach for directed gene disruption and have been proved to be effective in various animal models. Here we report that TALENs can induce somatic mutations in *Xenopus* embryos with reliably high efficiency and that such mutations are heritable through germline transmission. We modified the Golden Gate method for TALEN assembly to make the product suitable for RNA transcription and microinjection into *Xenopus* embryos. Eight pairs of TALENs were constructed to target eight *Xenopus* genes, and all resulted in indel mutations with high efficiencies of up to 95.7% at the targeted loci. Furthermore, mutations induced by TALENs were highly efficiently passed through the germline to F1 frogs. Together with simple and reliable PCR-based approaches for detecting TALEN-induced mutations, our results indicate that TALENs are an effective tool for targeted gene editing/knockout in *Xenopus*.

P-26

Zebrafish *foxk2* is required for neuronal survival during embryonic development

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While many of the forkhead transcription factors have been implicated in various aspects of development, the "K" subfamily members of forkhead factors are less characterized. Using zebrafish as a genetic model, here we showed that *foxk2* is essential in maintaining neuronal survival during early development. *foxk2* is a maternal transcript and it is expressed in all stages of zebrafish development. *foxk2* is expressed strongly in the central nervous system (CNS), suggesting it may play a critical role in CNS development. *foxk2* knockdown in zebrafish embryos by antisense morpholino resulted in severe apoptosis in CNS and defective neuronal circuit. The apoptotic phenotype of the *foxk2* morphant can be rescued by simultaneous knockdown of the tumor suppressor *p53*, indicating that the loss of *foxk2* directly induces a *p53* dependent apoptotic pathway. Our data suggest that *foxk2* may play an important role in maintaining neuronal cell survival and inhibiting apoptosis in-vivo.

P-27

Interaction between genetic polymorphisms in the regulation of Insulin-like growth factor 1 (IGF1) expression

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Insulin-like growth factor 1 (IGF1) is an important growth hormone in the body. The level of circulating IGF1 is controlled by multiple factors, among which genetic factor contributes to 38% of inter-individual variation of IGF1 expression. Our previous results showed that the circulating IGF1 level was regulated by haplotypes, which were consisted of microsatellite and SNPs. In this study, we used in vitro assays to provide details of regulatory mechanism of the haplotype effects. We found a length effect of the microsatellite exclusively in the C-T-T haplotype, in which a longer microsatellite length had a lower transcriptional activity. We proposed a regulatory model to explain the interaction between the microsatellite and SNPs in the regulation of gene expression. This model is based on the exclusive binding of C/EBPD transcription activation complex to the C allele of rs35767: T>C, but not the T allele. As the C/EBPD complex is a common interaction partner of FOXA3 in the liver, the length of the microsatellite, which is in the middle of the upstream C/EBPD complex and the downstream FOXA3, may regulate the interaction of the two transcription complexes. Preliminary results of serial deletion, site-directed mutagenesis and transcription factor depletion tests all agreed with this model.

P-28

Low intensity pulsed ultrasound enhanced mesenchymal stem cell recruitment through stromal derived factor-1 signaling in fracture healing

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We examined the effect of low intensity pulsed ultrasound (LIPUS) on the recruitment of mesenchymal stem cells (MSCs) and the pivotal role of stromal cell-derived factor-1/C-X-C chemokine receptor type 4 (SDF-1/CXCR4) pathway in response to LIPUS stimulation. Fractured rats received intracardiac administration of the green fluorescent protein (GFP) labeled MSCs were assigned to LIPUS treatment, LIPUS+AMD treatment or vehicle control groups. The dynamic migration of transplanted MSC to the fracture site in response to LIPUS treatment was investigated by *ex vivo* fluorescent imaging and immunohistochemical staining; serum SDF-1 level was quantified. Fracture healing parameters, such as callus morphology was assessed by weekly radiographs, micro computed tomography (μCT) and histological studies; the biomechanical properties of the healing bone were examined. Results showed that LIPUS promoted MSCs migration to the fracture site, which was correlated with an increase of serum SDF-1 level, changes in callus formation, and improvement of callus microarchitecture and mechanical properties; whereas the blockade of SDF-1/CXCR4 signaling attenuated the LIPUS effects on the fractured bones. These results suggested SDF-1 mediated MSCs migration might be one of the crucial mechanisms through which LIPUS exerted influence on fracture healing.

P-29

From quadrupedal to bipedal animal models in osteonecrosis research involving hip joint collapse

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Our group established core-decompression model in steroid-osteonecrosis in qudrapedal rabbits and prevention of joint collapse in bipedal emu model to test a bioactive porous scaffold incorporating osteogenic and anti-adipogenic phytomolecule Icaritin for prevention of osteoporosis and osteonecrosis using a poly (L-lactide-co-glycolide)/ tricalcium phosphate (PLGA/TCP)-based porous scaffold. A fine spinning technology was used for fabricating the scaffolds. In vitro release of Icaritin from PLGA/TCP scaffold was quantified by high-performance liquid chromatography (HPLC). Both in vitro cytotoxicity test and in vivo test via muscular implantation were conducted to confirm its biocompatibility. The attachment, proliferation and osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs) on composite scaffold were evaluated. The results showed that the PLGA/TCP/Icaritin composite scaffold was porous with interconnected macro-pores (about 480 μm) and micro-pores (2-15 μm). The mechanical properties of PLGA/TCP/Icaritin scaffold were comparable to those of pure PLGA/TCP scaffold, yet was direction-dependent. Icaritin content was detected in medium and increased with time, confirming its sustained release from the scaffold. The *in vitro* cytotoxicity test and in vivo intramuscular implantation showed that the composite scaffold had no toxicity and good biocompatibility. The PLGA/TCP/Icaritin scaffold facilitated the attachment, proliferation and osteogenic differentiation of BMSCs. Animal experiments confirmed its treatment efficacy bone defect repair in a core-decompression model in steroid-osteonecrosis in qudrapedal rabbits and prevention of joint collapse in bipedal emu model. The underlying mechanisms are associated promotion of osteogenesis and anti-adipogeneis as well as promotion of BMSCs migration towards bone defect repair region with presence of bioactive scaffold. In conclusion, an osteopromotive phytomolecule Icaritin could be successfully incorporated into PLGA/TCP to form an innovative porous composite scaffold with sustained release of osteopromotive Icaritin and this scaffold had good biocompatibility and osteoinduction, suggesting its potential for orthopaedic applications.

Keywords: Quadrupedal rabbit, Bipedal emu, Icaritin; Poly (L-lactide-co-glycolide)/ tricalcium phosphate; Scaffold; Osteogenesis; Biocompatibility; Cytotoxicity, Rabbits; Emus.

School of Biomedical Sciences

Neuroscience Symposium 2013

7 June 2013 (Friday)

This is a joint meeting with the Kunming Institute of Zoology Chinese Academy of Sciences

Neuroscience Symposium 2013
7 June 2013 (Friday)

Site: Room G02, Lo Kwee-Seong Integrated Biomedical Sciences Building

09:00-09:05 Opening Ceremony: Prof. Lin Xu and Prof. Chi Hin Cho

09:05-09:50 Plenary Lecture by Prof. Lin Xu (Abstract No.PL-02)
"Forced swim test induces long-term potentiation in the rat hippocampus"

Time	*Title of Presentation*	*Speaker*	*Abstract No.*
09:50-10:40	**Session I** **Chairpersons: Prof. Mary M.Y. Waye and Prof. Larry Baum**		
09:50-10:15	Dopamine depletion impairs synaptic plasticity in the primary motor cortex and motor skill consolidation	Prof. Wing Ho Yung	S3-01
10:15-10:40	Decreased mitochondrial DNA copy number in hippocampus and peripheral blood during opiate addiction is mediated by autophagy and can be salvaged by melatonin	Prof. Yong Gang Yao	S3-02

10:40-11:00 ***Photo taking & Tea Break***

11:00-13:05	**Session II** **Chairpersons: Prof. Wing Ho Yung and Prof. Lin Xu**		
11:00-11:25	Construction and analysis of four-dimensional metabolic network in human brain	Prof. Jing Fei Huang	S3-03
11:25-11:50	Regulation of inflammation in the Central Nervous System: Platelets as sensors of neuronal damage	Prof. Eugene Ponomarev	S3-04
11:50-12:15	The therapeutic effect of hepcidin in Parkinson's disease via regulation of brain iron and α-synuclein accumulation	Prof. Ya Ke	S3-05
12:15-12:40	Gastric myoelectrical activity during ageing and in a mouse model of Alzheimer's disease	Prof. John A. Rudd	S3-06
12:40-13:05	Genes and proteins: Much Ado about developmental dyslexia	Mary M.Y. Waye	S3-07

13:05-13:15 Closing Ceremony: Prof. Wing Ho Yung

PL-02

Forced swim test induces long-term potentiation in the rat hippocampus

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Immobility in the forced swim test is widely used to evaluate the efficacy of antidepressant, but the underlying mechanism remains unclear. Here we find that immobility is resulted from hippocampus-dependent learning, because intrahippocampal injection of the drugs that are known to impair learning effectively prevent the formation of immobility. Remarkably, this hippocampus-dependent learning induces a AP-5 sensitive long-term potentiation (LTP) in the hippocampus, which shares the known mechanisms of long-term potentiation expression induced by high frequency stimulation. Thus, our study demonstrates that forced swim test is a model of emotional learning that directly induces LTP in the hippocampus.

S3-01

Dopamine depletion impairs synaptic plasticity in the primary motor cortex and motor skill consolidation

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The ability to learn and retain new motor skills is important in daily life. After repeated exposure to a specific procedure, the motor skill becomes more accurate and automatic, reducing the demand on conscious recollection of prior learning episodes or the rules underlying the task. In Parkinson's disease (PD), in addition to the cardinal motor symptoms like akinesia and bradykinesia, there were studies revealing that PD patients have deficits in motor skill learning but the neurobiological basis is not certain. Impairment in the synaptic plasticity of the basal ganglia circuitry has been suggested to be involved in this phenomenon. In PD, dopamine innervation originating from the mesocortical pathway is reduced in the primary motor cortex (MI), an area that also contributes to motor learning. However, the impact of dopamine depletion in MI on synaptic plasticity and motor skill learning is not well studied. The present study addresses these questions based on in vivo recordings in the MI and motor training on PD rats. Local depletion of dopamine in the primary motor cortex resulted in reduced performance in the arm reaching for food learning task. Although the performance of the PD rats in the initial learning phase was comparable to that of the sham-operated group, as training continued, these animals exhibited deficit in consolidating the motor skill. These deficits closely paralleled the impairment in training-enhanced synaptic connections in layer V neurons, and the in vivo long term potentiation (LTP) of evoked field excitatory postsynaptic potentials induced by intermittent high frequency stimulation. Our study therefore revealed that dopamine depletion confined to the MI could lead to impairment in cortical synaptic plasticity which may preferentially affect the consolidation, but not the acquisition, of motor skills. These findings shed light on the cellular mechanisms of motor skill learning and could explain the decreased ability of PD patients in learning new motor skills.

This work was supported by the Research Grants Council of Hong Kong (T13-607/12R; HKU6/CRF/11G) and the National 973 Program (2011CB510004).

S3-02

Decreased mitochondrial DNA copy number in hippocampus and peripheral blood during opiate addiction is mediated by autophagy and can be salvaged by melatonin

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Drug addiction is a chronic brain disease that is a serious social problem and causes enormous financial burden. Because mitochondrial abnormalities have been associated with opiate addiction, we examined the effect of morphine on mtDNA levels in rat and mouse models of addiction and in cultured cells. We found that mtDNA copy number is significantly reduced in the hippocampus and peripheral blood of morphine-addicted rats and mice compared to control animals. Concordantly, decreased mtDNA copy number and elevated mtDNA damage were observed in peripheral blood from opiate-addicted patients, indicating detrimental effects of drug abuse and stress. In cultured PC12 cells and neurons, morphine treatment caused many mitochondrial defects, including a reduction in mtDNA copy number that was mediated by autophagy. Knockdown the *Atg7* gene could counteract the loss of mtDNA copy number induced by morphine. The mitochondria-targeted antioxidant melatonin restored mtDNA content and neuronal outgrowth and prevented the increase in autophagy upon morphine treatment. In mice, co-administration of melatonin with morphine ameliorated morphine-induced behavioral sensitization, analgesic tolerance, and mtDNA content reduction. During drug withdrawal in opiate-addicted patients and improvement of protracted abstinence syndrome, we observed an increased level of serum melatonin. Taken together, our study indicates that opioid addiction is associated with mtDNA copy number reduction and neurostructural remodeling. These effects appear to be mediated by autophagy and can be salvaged by melatonin.

S3-03

Construction and analysis of four-dimensional metabolic network in human brain

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The metabolic network in human brain is an important basis of brain function, and the network is changeable during brain development from the fetal period to adulthood, and to old age. Thus, the construction and analysis of brain metabolic network based on the brain development data will be helpful for the understanding the functional change during brain development. Here, 1340 context-specific (period- and region-specific) metabolic networks have been constructed, and 16 over-expressed and 37 under-expressed metabolic genes can be observed in aging people brain. These differential expression genes are involved in some metabolic disease pathways, such as, Parkinson's, and Alzheimer's disease; and these changes in aging people will probable result in increasing ROS (reactive oxygen species) and IP3 (inositol 1,4,5-trisphosphate), and decreasing PH. Thus, the environment change in brain may be an important factor resulting in some brain diseases.

S3-04

Regulation of inflammation in the Central Nervous System: Platelets as sensors of neuronal damage

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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 involved P-selectin (CD62P). During neuroinflammation, platelets accumulated in the central nervous system and secreted proinflammatory factors such as IL-1 and PF4. Thus the study determines a new role of platelets that directly recognize a neuronal damage and contribute to inflammation in the central nervous system.

S3-05

The therapeutic effect of hepcidin in Parkinson's disease via regulation of brain iron and α-synuclein accumulation

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Experimental and clinical evidence suggest that abnormal iron accumulation is involved in the pathogenesis of Parkinson's disease (PD). Since the hormone hepcidin is the main regulator of body iron level, including that in the brain, we hypothesize that hepcidin offers therapeutic potential in Parkinsonism. We tested our hypothesis based on two different animal models of PD, namely 6-hydroxydopamine (OHDA) and rotenone models. In 6-OHDA lesioned hemi-Parkinsonian rats, pre-treatment with adenovirus-hepcidin (Ad-hep) could reduce loss of dopamine neurons and iron accumulation in the substantia nigra pars compacta (SNc). Ad-hep also reduced apomorphine-induced rotation in these animals. In vitro studies revealed that hepcidin effectively suppressed 6-OHDA induced apoptosis in mescencephalic neurons. In the rotenone model, which captures the clinical features of PD with respect to α-synuclein accumulation as well as the progressive nature of the disorder, we found that chronic rotenone treatment (up to 30 days) resulted in selective accumulation of α-synuclein and iron in the SNc, which was accompanied by degeneration of dopamine neurons. The motor ability assessed by the open field test and grid test was also significantly reduced. Injection of Ad-hep starting at day 5 into the lateral cerebral ventricle could significantly rescue the motor deficit induced by rotenone. Post-mortem examination and in vitro experiments revealed that the over-expressed hepcidin could suppress α-synuclein and iron accumulation, and reduced neuronal toxicity. Together, these results strongly suggest that manipulating the level of the hepcidin is a promising therapeutic strategy for PD.

This work was supported by the National 973 Program (2011CB510004).

S3-06

Gastric myoelectrical activity during ageing and in a mouse model of Alzheimer's disease

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Alzheimer's disease is classically associated with a loss of cholinergic neurones in the central nervous system. A similar loss of neurons in gastrointestinal (GI) tract would be expected to affect the control of electrical slow wave activity and motility patterns. Studying the mechanism of the control of slow waves in a transgenic mouse model of Alzheimer's disease (AD) may provide novel insights into the progression of the disease.

Radiotelemetry was used to record slow waves in 6- and 12-month old Tg2576 mice and their wild-type age-matched controls; a study was also conducted using ICR mice. There was no difference in baseline dominant frequency (DF) between Tg2576 and wild-type controls (DFs of 6-month-old Tg2576 and age-matched wild-type were 6.9 ± 0.8 and 6.8 ± 0.8 cpm, respectively; DFs of 12-month-old Tg2576 and age-matched wild-type were 6.7 ± 0.7 and 6.8 ± 0.7 cpm, respectively; $P > 0.05$). However, nicotine (3 mg/kg, i.p.) reduced the DF in 6-month old wild-type and Tg2576 mice ($P < 0.05$), but not in 12-month-old Tg2576 and or age-matched wild-type mice ($P>0.05$). In normal ICR mice, there was also a reduction in the effectiveness of nicotine during aging. Whilst no differences in baseline DF were detected between wild-type and Tg2576 mice, the use of nicotine revealed subtle changes in the control of gastric slow wave activity. Further studies are required to elucidate the mechanisms involved.

S3-07

Genes and proteins: Much Ado about developmental dyslexia

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Developmental dyslexia is a persistent difficulty in learning to read despite the absence of sensory or cognitive deficits, and the presence of normal intellectual ability and adequate education. It affects 5–17% of children and interferes with their learning. Linkage and association studies have pinpointed at least nine quantitative trait loci (DYX1-9) for dyslexia, and several genes have been proposed as susceptibility candidates at some of these loci, including KIAA0319 on chromosome 6 and more recently KIAA0319L on chromosome 1. This lecture will discuss some frontiers of research on genetic studies of developmental dyslexia. Our recent findings on the functional role of these two proteins related to susceptibility of developmental dyslexia will be presented. Furthermore, some of their protein interaction partners and related signaling pathways will also be described.

ACKNOWLEDGEMENTS

The Organizing Committee gratefully acknowledges the generous support of the following organizations:

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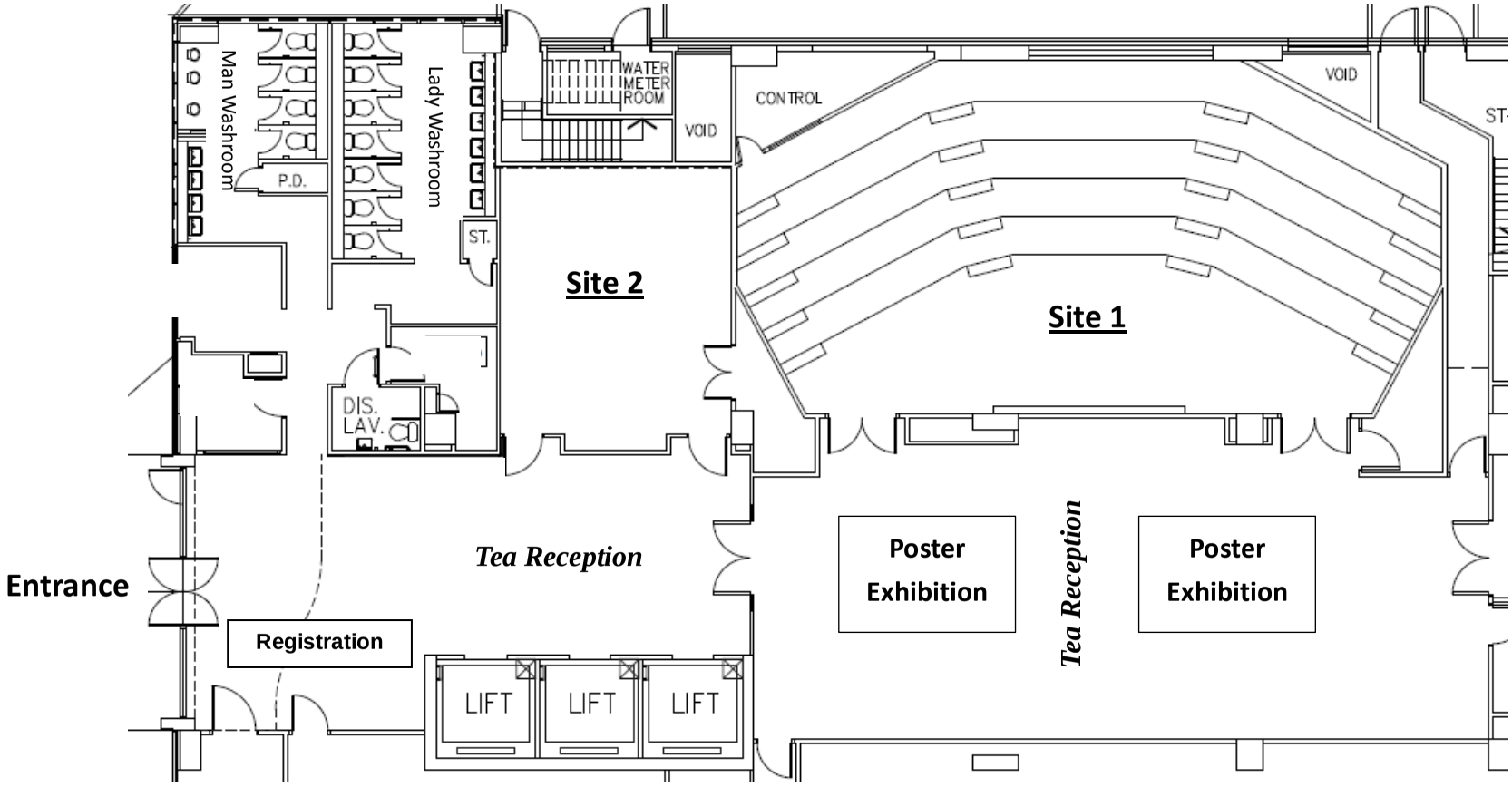

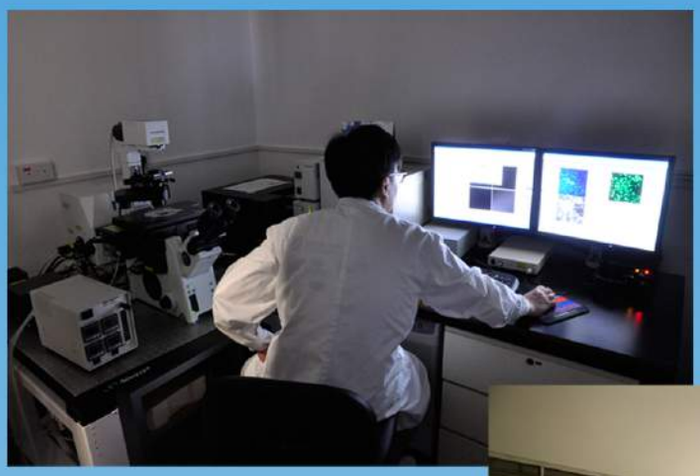

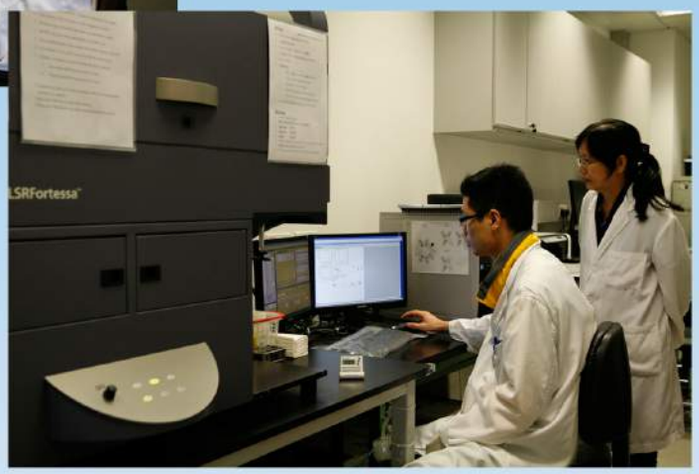

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