School of Biomedical Sciences Research Day 2011

31 May, 2011

7/F, Mong Man Wai Building
The Chinese University of Hong Kong
HONG KONG

香港中文大學
The Chinese University of Hong Kong

FACULTY OF MEDICINE
THE CHINESE UNIVERSITY OF HONG KONG

School of Biomedical Sciences Research Day 2011

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Welcome Message from the Director of School of Biomedical Sciences

Tomorrow marks the beginning of the 3rd year of the School of Biomedical Sciences. It is the most opportune time for us to reflect on and to re-visit what we have achieved in the two-year existence of our School in this second Annual Research Day.

We came together for the first time to share our joy and discoveries in research at the last Annual Research Day in June of 2010. Albeit doing it only a year after the formation of our School, it seemed to have energized all of us. Aside from increasing collaborations among our staff as well as with colleagues outside the School and the University, there have been obvious positive changes. In the last round of RGC grant cycle, our School submitted 44 applications, which meant 92% of our staff wrote an application. Even though we don't know how many of these will be successful yet, the number by itself is a record. In the last round of NSFC application, one third of the 31 applications from the Faculty of Medicine were from our School. This represents a doubling of the number of such grant applications from our staff. There was also a corresponding increase in the number of scientific publications in the past year. All in all, I can justifiably be proud to say that we continue to excel in scholarly scientific investigations. Research Day, such as this, is indeed a significant sign-post in the development of our young School.

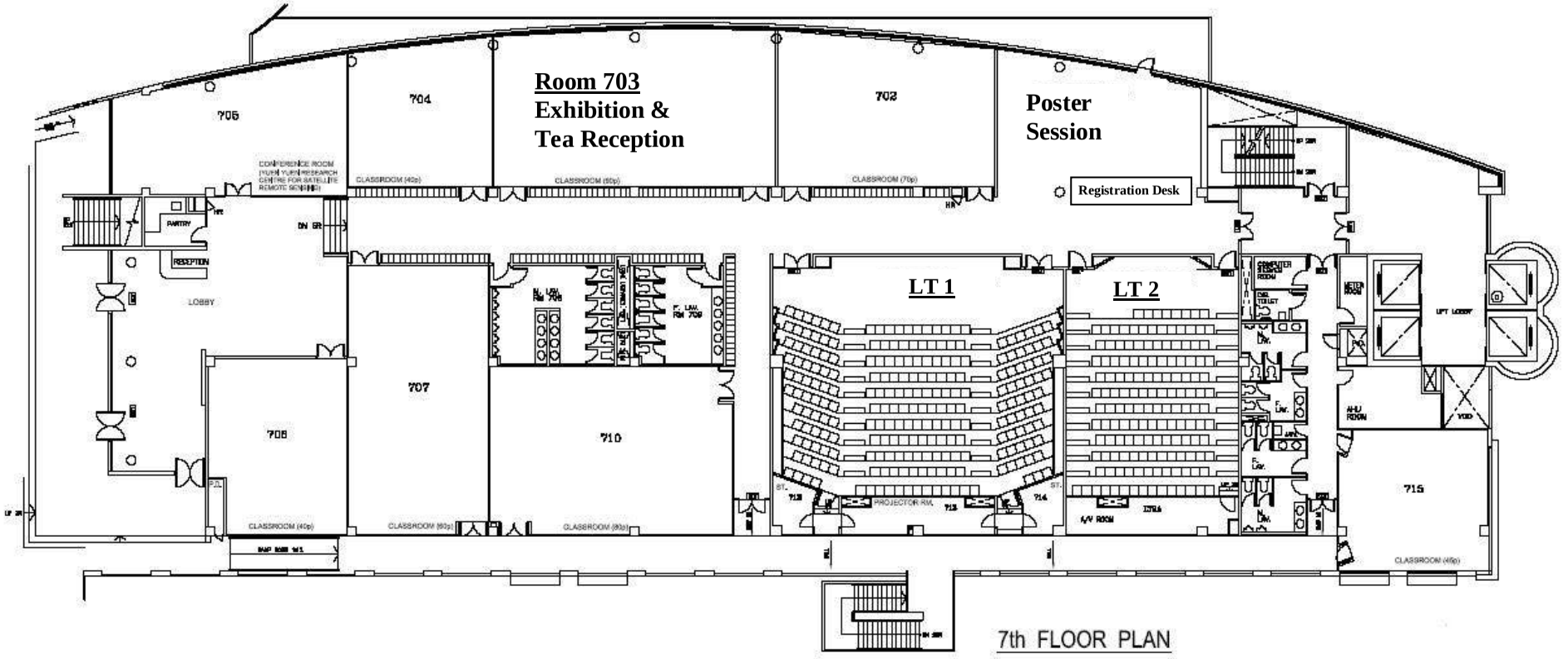
As you may have noticed, our Teaching and Learning Unit is joining the five research themes starting this Research Day. We have a number of teaching award recipients among our colleagues in the Unit. They have also done a large amount of curriculum and pedagogical research. They have contributed significantly to the success of the School and the Faculty. The addition of the Teaching and Learning Unit makes the Research Day a true event for the entire School.

A party without guests is not a party. We welcome again our friends from our sister institutions, in particular the Hong Kong University of Science and Technology to share the joy with us.

Last, but not the least, I would like to take this opportunity to thank members of the Organizing Committee and all those who help to make this Research Day a success!

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

Map of the Meeting Venue



Program Summary

31 May, 2011 (Tuesday)

09:00-09:15 Opening Ceremony (Dean and Director)

	MMW LT1	*MMW LT2*
09:15-10:30	**Stem Cell (1)** (Chan K.M., Lee K.)	**Neurobiology (1)** (Baum L., Yung W.H.)
09:15-09:40	Prof. Li Gang	Prof. Yung Wing Ho
09:40-10:05	Prof. Mak King Lun Kingston	Prof. Kwong Wing Hang
10:05-10:30	Prof. Wan Chao	Prof. Wise Helen

10:30-11:15 Tea Break

11:15-12:05	**Stem Cell (2)** (Li G., Wu Z.)	**Molecular Tools** (Wan C., Shum A.)
11:15-11:40	Prof. Lee Ka Ho Kenneth	Prof. Wang Huating
11:40-12:05	Prof. Feng Bo	Prof. Wan Chi Cheong David

12:05-12:30 Photo Session

12:30-14:00 Lunch Break

14:00-15:15	**Vascular and Metabolism** (Lam F., Kwan Y.W.)	**Neurobiology (2)** (Yew D.T., Wong Y.H.)
14:00-14:25	Prof. Lam Fu Yuen Francis	Prof. Cho Yu Pang Eric
14:25-14:50	Prof. Yeung Hok Keung John	Prof. Waye Miu Yee Mary
14:50-15:15	Prof. Yao Xiaoqiang	Prof. Chan Sun On

15:15-16:00 Tea Break & Poster Session

16:00-16:50	**Receptors** (Yao X., Wise H.)	**Diseases and Treatment** (Waye M., Poon W.S.)
16:00-16:25	Prof. Kwan Yiu Wa	Prof. Shum Sau Wun Alisa
16:25-16:50	Prof. Rudd John A.	Prof. Poon Wai Sang

Oral Presentations

Abstracts

Site 1 LT1, 7th Floor, Mong Man Wai Building

09:00-09:15 Opening Ceremony: Prof. Fok Tai Fai (Dean of Medicine) & Prof. Chan Wai Yee (Director of School of Biomedical Sciences), LT1, 7th Floor, Mong Man Wai Building

09:15-10:30 **Stem Cell (1)**
Chairpersons: Prof. Chan Kai Ming & Lee Ka Ho Kenneth

Time	Title of Presentation	Name of Speaker	Abstract No.
09:15-09:40	Circulating mesenchymal stem cells and their clinical implications	Prof. Li Gang	S1-01
09:40-10:05	Functional roles of Hippo signaling in the regulation of mesenchymal stem/progenitor cells (MSCs) in the skeletal system	Prof. Mak King Lun Kingston	S1-02
10:05-10:30	The HIF pathway regulates the biological behavior of mesenchymal stem cells	Prof. Wan Chao	S1-03

10:30-11:15 **Tea Break**

11:15-12:05 **Stem Cell (2)**
Chairpersons: Prof. Li Gang & Wu Zhenguo

11:15-11:40	Hair follicle progenitor cells can transdifferentiate into cardiomyocytes when induced by Cardiogenol C	Prof. Lee Ka Ho Kenneth	S1-04
11:40-12:05	Path to understanding the reinstatement of pluripotency	Prof. Feng Bo	S1-05

12:05-12:30 **Photo Session**

12:30-14:00 **Lunch Break**

14:00-15:15 **Vascular and Metabolism**
Chairpersons: Prof. Lam Fu Yuen Francis & Kwan Yiu Wa

14:00-14:25	NF3, a formulation of traditional Chinese medicine, on disease states of arthritic rats with diabetes	Prof. Lam Fu Yuen Francis	S1-06
14:25-14:50	Effects of tanshinones on mitochondrial reactive oxygen species (ROS) in HepG2 cells	Prof. Yeung Hok Keung John	S1-07
14:50-15:15	Heteromeric coassembly of TRPV4, TRPC1 and TRPP2 to form a flow-sensitive channel	Prof. Yao Xiaoqiang	S1-08

15:15-16:00 **Tea Break and Poster Session**

16:00-16:50 **Receptors**
Chairpersons: Prof. Yao Xiaoqiang & Wise Helen

16:00-16:25	Roles of Ca^{2+}-sensing receptors in mediating the bone anabolic effects, *ex vivo*, of CU409B1 and 1,25α-dihydroxyvitamin D_3 in osteoblast-like cells of rats	Prof. Kwan Yiu Wa	S1-09
16:25-16:50	Control of emesis: Modulation of tachykinin systems using TRPV1 ligands	Prof. Rudd John A.	S1-10

Site 2 LT2, 7th Floor, Mong Man Wai Building

09:00-09:15 Opening Ceremony: Prof. Fok Tai Fai (Dean of Medicine) & Prof. Chan Wai Yee (Director of School of Biomedical Sciences), LT1, 7th Floor, Mong Man Wai Building

09:15-10:30 **Neurobiology (1)**
Chairpersons: Prof. Baum Larry & Yung Wing Ho

Time	Title of Presentation	Name of Speaker	Abstract No.
09:15-09:40	Mechanism of GABA ionic plasticity: the tale of two proteins	Prof. Yung Wing Ho	S2-01
09:40-10:05	Effect of Ginsenoside Rg1 on some Alzheimer processes and learning in ovariectomized rats	Prof. Kwong Wing Hang	S2-02
10:05-10:30	Regulation of dorsal root ganglion glial cell proliferation by dorsal root ganglion neurons	Prof. Wise Helen	S2-03

10:30-11:15 **Tea Break**

11:15-12:05 **Molecular Tools**
Chairpersons: Prof. Wan Chao & Shum Sau Wun Alisa

11:15-11:40	The role of microRNA-29 in Duchenne Muscular Dystrophy	Prof. Wang Huating	S2-04
11:40-12:05	DW-IN15, a small molecule, functions as a novel anti-HIV-1 inhibitor targeting the interaction between integrase and cellular *Lens epithelium-derived growth factor*	Prof. Wan Chi Cheong David	S2-05

12:05-12:30 **Photo Session**

12:30-14:00 **Lunch Break**

14:00-15:15 **Neurobiology (2)**
Chairpersons: Prof. Yew Tai Wai David & Wong Yung Hou

14:00-14:25	P2X7 receptor blockade by Brilliant Blue G promotes retinal ganglion cell survival and regeneration after optic nerve injury	Prof. Cho Yu Pang Eric	S2-06
14:25-14:50	Study of two dyslexia candidate genes: KIAA0319 and KIAA0319L	Prof. Waye Miu Yee Mary	S2-07
14:50-15:15	The role of Nogo on the guidance of axons in the mouse optic chiasm	Prof. Chan Sun On	S2-08

15:15-16:00 **Tea Break and Poster Session**

16:00-16:50 **Diseases and Treatment**
Chairpersons: Prof. Waye Miu Yee Mary & Poon Wai Sang

16:00-16:25	Subnormal level of retinoic acid increases the risk of birth defects in diabetic pregnancy	Prof. Shum Sau Wun Alisa	S2-09
16:25-16:50	Phase I/II randomized controlled trial of autologous bone marrow-derived mesenchymal stem cell therapy for chronic intracranial hemorrhagic stroke	Prof. Poon Wai Sang	S2-10

S1-01

Circulating mesenchymal stem cells and their clinical implications

G. Li[1,2], C. Wan[1], Q.L. He[3], L.L. Xu[2], F.B. Meng[2]

[1]Stem Cell and Regeneration Program, School of Biomedical Sciences, [2]The Department of Orthopaedics and Traumatology, The Chinese University of Hong Kong; [3]Department of Microbiology, University of Alabama at Birmingham, USA.

Introduction: MSCs were demonstrated to exist within peripheral blood (PB) of several mammalian species including human, guinea pig, mice, rat, and rabbit. We have found increased numbers of circulating MSCs in human peripheral blood after fracture and in patients with cancers. We have also compared the difference between circulating MSCs and bone marrow derived MSCs and evaluated their potential clinical applications in tissue engineering and cell therapy.

Methods and Findings: Using culture conditions similar to those defined for bone marrow derived mesenchymal stromal cells (BMMSCs), we have isolated and expanded multi-colony and single colony derived PBMSCs strains from the GFP transgenic rats. Aspects of molecular, cellular and developmental properties of this poorly characterized peripheral blood subpopulation were examined. PBMSCs share some common phenotypic characteristics with BMMSCs, but are distinguishable in gene expression profile by cDNA microarray analysis, with 84 up-regulated and 83 down-regulated genes (>2 fold, E-B/B-E>100, $P<0.05$). Most of these genes are related to cell proliferation, differentiation, cytoskeleton, and calcium/iron homeostasis. Differentially expressed genes with fold change ≥ 10 were further confirmed with quantitative real time RT-PCR, and these genes are: retinol-binding protein 1 (CRBP1), cadherin 2, bone morphogenetic protein 6 (BMP6), SRY-box containing gene 11 (Sox11), the aquaporin 1 (AQP1), and so on, and they can be potential targets for further investigations. We have demonstrated that single colony derived PBMSCs strains possess extensive proliferation and multipotent differentiation potentials into osteoblasts, adipocytes, chondrocytes, endothelial cells and neuronal cells. In terms of potential clinical implications of PBMSCs, we have demonstrated that allogenic PB-MSCs enhance bone regeneration in rabbit ulna critical-sized bone defect model. We also demonstrated that BM-MSCs can be recruited towards the sites of bone fracture and participate fracture healing. We are now working on using MSCs as a gene delivery vehicle for management of wound healing or cancer therapy, and ways of enhancing the homing and recruitment MSCs towards to specific sites after their systemic delivery.

Conclusion: Taken together, PB-MSCs may be a new cell source for cell therapy, tissue engineering and gene therapy strategies.

S1-02

Functional roles of Hippo signaling in the regulation of mesenchymal stem/progenitor cells (MSCs) in the skeletal system

K.K.L. Mak

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Hippo signaling has been implicated in the control of organ size and tumorigenesis. Overexpression of Yes-associated protein (YAP) in the mouse liver, the effector of the Hippo pathway, results in a dramatic increase of liver mass, and eventually leads to the development of hepatocarcinoma. In addition, it has been shown that Hippo signaling is also involved in promoting epidermal stem cell compartment and leads to the development of squamous cell carcinoma. All these data suggest that Hippo signaling has a critical function in regulating mesenchymal stem cell and cancer stem cell renewal and maintenance in multiple organs. In particular, the regulation of the skeleton size remains unclear. We hypothesize that Hippo signaling has a functional role in regulating the size of the skeleton. Here, we have generated $Mst1^{c/c}$ and $Mst2^{c/c}$ floxed alleles, which are upstream components of the YAP transcription factor in the canonical Hippo signaling pathway, to study its functional roles in the regulation of the skeleton size. By using specific *Cre* mouse lines, we remove Hippo signaling specifically in the developing skeleton *in vivo* in our genetic engineered mouse models. Surprisingly, the skeleton size is not severely affected, albeit an increase of chondrocyte proliferation. This data suggest that additional signaling pathways are involved in the regulation of the skeleton size, in addition to the Hippo pathway.

S1-03

The HIF pathway regulates the biological behavior of mesenchymal stem cells

C. Wan, F. Zhang, J. Shao, L. Deng, W.P. Tsang, Q. Li, E. Schipani, F. Long, T.L. Clemens

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

Hypoxia inducible factor-1α (HIF-1α) is an essential mediator of cellular adaptation to oxygen fluctuations and is crucial for proliferation and differentiation of stem/progenitor cell populations, organogenesis and tissue regeneration. Our recent studies show that HIF-1α serves as a key to couple angiogenesis to osteogenesis during skeletal development and regeneration. Osteoblast restricted overexpression or deletion of HIF-1α produces marked changes in the vascularization and formation of long bones but not in the flat bones of the skull. These observations suggest a different relationship between angiogenesis and osteogenesis at this skeletal site. In this study, we analyzed the effects of disrupting HIF-1α in the condensing mesenchyme using the Cre/loxp strategy. Interestingly, the distribution and pattern of vascular networks in the developing cranium were not different between the mutants and controls, as visualized by immunostaining for endothelium in both whole mount and frozen cranial sections. And cranial bones of the mutant mice were smaller and less mineralized compared to controls, with disorganized mesenchymal condensation. Primary calvaria preosteoblasts deficient in HIF-1α had reduced expression of osteogenic marker genes, in accord with our findings *in vivo*. Chromatin immunoprecipitation assays showed direct occupancy of the osterix promoter by HIF-1α. These findings suggest that HIF-1α is required for normal cranial bone development but functions independent of angiogenesis to control the biological behavior of mesenchymal stem cells.

S1-04

Hair follicle progenitor cells can transdifferentiate into cardiomyocytes when induced by Cardiogenol C

E. Chen[1], Y. Yao[1], L.Y. Wang[1], K.K.H. Lee[1,2]

[1]Stem Cell and Regeneration Program, School of Biomedical Sciences, The Chinese University of Hong Kong; [2]Key Laboratory for Regenerative Medicine, Ministry of Education.

One area of interest in our laboratory is studying the developmental potentials of hair bulge progenitor cells (HBPC). These cells are localized on the hair sheath and are easily isolated, thus making them extremely useful for use regenerative medicine. We demonstrated that these cells could be readily induced to transdifferentiate into adipocytes and osteocytes. We also examined the possibility of using a small cell permeable molecule called Cardiogenol C to induce HBPCs to become cardiomyocytes. It was established that Cardiogenol C activates HBPCs to express transcriptional factors GATA-4 and NKx2-5, which are both early markers for pre-cardiac cells. In more mature cultures, the Cardiogenol C-induced cells also expressed sarcomeric myosin heavy chain and cardiac-specific troponin I. To understand how the small molecule works, we conducted comparative proteomic analysis on Cardiogenol C-treated HBPCs and compared it with control HBPCs. We identified eighteen different proteins that were differentially expressed. These proteins were involved in promoting cell differentiation, cardiomyocyte development and for the normal function of striated muscles. Furthermore, our data also suggests that Cardiogenol C exerts its effect by activating the Wnt signal transduction pathway and by altering the expression of several key chromatin remodeling proteins.

S1-05

Path to understanding the reinstatement of pluripotency

B. Feng

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The mammalian developmental process involves a progressive restriction of developmental potential as the totipotent zygote transits through the pluripotent inner cell mass and eventually gives rise to a multitude of differentiated cell types. Although, cellular differentiation is irreversible under normal physiological contexts, methods have been developed to reprogram somatic cells into undifferentiated states in culture. Interestingly, the reinstatement of pluripotency can be triggered using defined transcription factors, such as Oct4, Sox2, Klf4 and c-Myc; and the resulting induced pluripotent stem cells (iPS cells) possess characteristics of the undifferentiated embryonic stem cells.

In order to uncover the mechanism underpinning the reprogramming process, we have initiated a study to screen for new reprogramming factors. Through the screening, we identified nuclear receptor Esrrb and Nr5a2 that can replace Klf4 and Oct4, respectively, to mediate the reprogramming of mouse embryonic fibroblasts (MEFs). However, the low efficiency and slow progress of current reprogramming methodologies still indicate that some important reprogramming factors are yet to be identified. Therefore, we will continue to screen for new factors that can promote more rapid reprogramming, hoping to further unravel the mechanism that controls pluripotency and to promote the generation of clinical-grade iPS cells.

NF3, a formulation of traditional Chinese medicine, on disease states of arthritic rats with diabetes

F.F.Y. Lam [1,*], E.S.K. Ng [1], D.T.W. Lau [2], V.K.M. Lau [3], C.B.S. Lau [3,4], P.C. Leung [3,4], K.P. Fung [1,3,4]

[1]School of Biomedical Sciences, The Chinese University of Hong Kong, Shatin, Hong Kong; [2]Department of Chemical Engineering, The Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong; [3]Institute of Chinese Medicine, [4]State Key Laboratory of Phytochemistry and Plant Resources in West China, The Chinese University of Hong Kong, Shatin, Hong Kong, China.

NF3 is a formulation of traditional Chinese medicine composed of *Radix Rehmanniae* (RR) and *Radix Astragali* (RA) in 1:2 ratio. In this study, we have investigated the anti-arthritic and anti-diabetic potentials of NF3 and its two constituents in arthritic rats with diabetes.

Neonatal streptozotocin (70 mg/kg) (STZ)-induced diabetic rats were randomized to receive 2 weeks of daily oral administration of NF3 (0.98 g/kg/day or 4.91 g/kg/day), RR (0.98 g/kg/day), RA (0.98 g/kg/day), or vehicle. After one week of oral treatment, all rats were injected with 125 μl Freund's complete adjuvant (FCA) into one knee to induce monoarthritis. Symptoms of arthritis were assessed daily until termination of the experiment one day after the last oral administration. A parallel study was performed in age-matched normal rats.

FCA treatment produced similar arthritis symptoms in normal and diabetic rats. Compared to vehicle control, standard dose NF3, high dose NF3, RR, and RA, all had insignificant effect on plasma glucose level, joint allodynia, joint swelling, or joint hyperaemia in diabetic rats, whereas, in normal rats, high dose NF3 moderately reduced plasma glucose level, joint swelling and allodynia, RA slightly reduced plasma glucose level, and RR slightly inhibited allodynia.

The present finding suggests that diabetes does not affect the development of arthritis. NF3 and its constituents do not have significant anti-inflammatory, analgesic, or hypoglycaemic action in diabetic rats. In normal rats, high dose NF3 may have small anti-inflammatory, analgesic, and hypoglycaemic actions, RA may have small hypoglycaemic action, and RR may have small analgesic action. Overall, NF3 and it constituents showed minor promises in their anti-arthritic and hypoglycaemic activities in both normal and diabetic rats.

S1-07

Effects of tanshinones on mitochondrial reactive oxygen species (ROS) in HepG2 cells

W.Y.W. Lee[1], X.L. Zhou[1], Y.W. Kwan[1], S.K. Kong[2], A.H.P. Ho[3], J.H.K. Yeung[1]

[1]School of Biomedical Sciences, Faculty of Medicine; [2]School of Life Sciences, Faculty of Science; [3]Department of Electronic Engineering, Faculty of Engineering, The Chinese University of Hong Kong.

Dihydrotanshinone is one of the abietane type-diterpene quinones (tanshinones) isolated from the Salvia miltiorrhiza (Danshen), which modulate the ROS-mediated p38 mitogen-activated protein kinase in dihydrotanshinone-induced apoptosis in HepG2 cells [1]. Reactive oxygen species (ROS) has been suggested to play an important role in its anti-cancer property. This study investigated the effects of tanshinones on mitochondria, the major site of ROS generation cells. Mitochondrial membrane potential and mitochondrial ROS after dihydrotanshinone treatment was monitored by confocal microscopy in HepG2 cells pre-probed with oxidized Mitotracker®Red CMXRos and reduced Mitotracker®Red CM-H_2XRos, respectively. Compared to tanshinone IIA, dihydrotanshinone produced a more significant reduction in mitochondrial membrane potential (65% vs 26%) and depletion of mitochondrial ROS (44% vs 24%). Pretreatment with N-acetylcysteine reduced the mitochondrial ROS depletion but not the loss of mitochondrial membrane potential, which may partly explain its protective effect on dihydrotanshinone-induced apoptosis. There was a simultaneous rise in nitric oxide (NO), monitored by difluorofluorescein diacetate, while blocking NO generation increased susceptibility towards dihydrotanshinone-induced cell death. This study indicated an important role of mitochondrial ROS level in regulating cellular response toward tanshinones treatment.

Reference

[1] Lee WYW et al., 2009 Cancer Letters 285:46-57.

Acknowledgement

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S1-08

Heteromeric coassembly of TRPV4, TRPC1 and TRPP2 to form a flow-sensitive channel

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Transient receptor potential (TRP) channels function as cellular sensors to perceive and respond to a variety of environmental stimuli including temperature, pain, pressure and fluid flow. These channels can be divided into seven subfamilies, including TRPV, TRPC, TRPP, and four others. Functional TRP channels are tetrameric complexes consisting of four pore-forming subunits, which could be identical (homotetrameric channels) or different (heterotetrameric channels). In the present study, we found that TRPV4, -C1, and -P2 associate together to form a physical complex. In function study, this TRPV4-C1-P2 complex mediates flow-induced Ca^{2+} influx in HEK293 cells over-expressing TRPV4, -C1, and -P2 and in rat mesenteric artery endothelial cells (MAECs). Pore-dead mutant of each of these three TRP isoforms abolished or markedly reduced the flow-induced cation currents and Ca^{2+} rises, suggesting all three TRPs contribute to the ion permeation pore of the channels. Taken together, we identified the first heteromeric TRP channels composed of subunits from three different TRP subfamilies, namely heteromeric TRPV4-C1-P2 channels. Functionally, heteromeric TRPV4-C1-P2 are the main channels that mediate flow-induced Ca^{2+} rises in native vascular endothelial cells, thus plays a key role in the control of vascular tone and blood pressure.

Roles of Ca^{2+}-sensing receptors in mediating the bone anabolic effects, *ex vivo*, of CU409B1 and 1,25α-dihydroxyvitamin D_3 in osteoblast-like cells of rats

C.C.W. Poon[1#], W.Y.W. Lee[2#], L.C.M. Lau[1#], M.W.K. Tse[1], P.P.Y. Lui[2], C.K.M. Chan[2], S.K. Kong[3], Y.W. Kwan[1]

[1]School of Biomedical Sciences; [2]Department of Orthopaedics and Traumatology, Faculty of Medicine; and [3]School of Life Sciences, Faculty of Science, The Chinese University of Hong Kong, Hong Kong.

Background: Osteoporosis affects ~15% of the population, of whom more than 75% are post-menopausal women. Current therapy (e.g. estrogen replacement therapy) is associated with unwanted side effects. The extracellular Ca^{2+}-sensing receptor (CaSR) is a G-protein-coupled receptor, expressed in parathyroid, kidneys, intestine and bone, which responses to changes in extracellular Ca^{2+}. In this study, we tested the hypothesis that CU409B1, alone and in combination with Vitamin D, provides anabolic effects of osteoblasts via CaSR modulation.

Methods: Primary osteoblasts were harvested from trabecular bones in iliac crests of Sprague Dawley (normal and ovariectomized (OVX)) rats. Osteoblasts (~90% confluence) were treated with either 1,25α-dihydroxyvitamin D_3 (Vit. D_3, 10 nM), CU409B1 (10, 30 and 100 nM) or a combination of Vit. D_3 plus CU409B1 for 7 days before subjecting to osteogenic biomarkers (osteocalcin (OCN) and alkaline phosphatase (ALP)) (qRT-PCR) and extracellular CaSR (immunocytochemistry) determination.

Results: Before drug treatments, a lower CaSR expression was detected in osteoblasts of OVX rats when compared to that of normal rats using immunocytochemistry. There was a trend of, but non-significant, increase of CaSR expression in osteoblasts of OVX rats 7 days after Vit. D_3 (10 nM) plus CU409B1 (10 nM) treatment. At Day 7, CU409B1 (10 nM), alone or in combination with Vit. D_3, increased OCN mRNA levels in osteoblasts of OVX rats. However, CU409B1 (30 and 100 nM), with and without Vit. D_3, resulted in a decrease of OCN mRNA levels in osteoblasts of OVX rats. ALP mRNA levels were not altered by CU409B1 (10, 30 and 100 nM), alone or in combination with Vit. D_3, in osteoblasts of OVX rats.

Conclusion: Our results demonstrate, for the first time, a lower expression of CaSR in the osteoblasts of OVX rats compared to normal rats. Incubation with CU409B1 (10 nM) plus Vit. D3 (10 nM) resulted in an increase in CaSR expression in osteoblasts of OVX rats in a time-dependent manner after treatment. In addition, OCN but not ALP mRNA level of osteoblasts of OVX rats was increased 7 days after CU409B1 plus Vit. D3 treatments.

[#] Contributed equally to the project.

Acknowledgement: This project is financially supported by GRF Grant HKSAR (Ref: 2140565). Fund provided to Mr. Lawrence CM Lau (summer of 2010) by the School of Biomedical Sciences (Faculty of Medicine, CUHK) is acknowledged.

Control of emesis: Modulation of tachykinin systems using TRPV1 ligands

J.A. Rudd

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Substance P NK_1 receptor antagonists are used for the treatment of chemotherapy-induced emesis. An alternative strategy may be to reduce neuropeptide release from emetic circuits. The ultrapotent capsaicin analogue, resiniferatoxin, is known to deplete substance P from sensory nerves, and antagonizes emesis induced by diverse challenge; however it also has undesirable side effects. In the present studies, we examine the potential of olvanil, a non-pungent TRPV1 activator, to antagonize cisplatin-induced acute and delayed emesis in ferrets.

In short-term experiments (4-24 h), olvanil (0.05-5 mg/kg, s.c.) antagonized apomorphine (0.25 mg/kg, s.c.)- and high-dose acute cisplatin (10 mg/kg, i.p.)-induced emesis ($P<0.05$) without affecting blood pressure, temperature, or generalized behaviour; it also antagonized emesis following i.c.v. administration ($P<0.05$). In long-term experimentation (up to 72 h), repeated administration of olvanil (0.05-5 mg/kg, s.c. three times per day) failed to antagonize cisplatin (5 mg-kg, i.p.)-induced acute and delayed emesis; it also interfered with the antiemetic action of ondansetron (1 mg/kg, i.p. three times per day).

In conclusion, olvanil has anti-emetic properties in some models of emesis in ferrets. However, its failure to improve the control of cisplatin-induced acute and delayed emesis limits its development for use in man.

Acknowledgement: These studies were supported by the Research Grants Council of Hong Kong (CUHK4527/05M).

S2-01

Mechanism of GABA ionic plasticity: the tale of two proteins

W.H. Yung

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The ability to regulate inhibitory synapses is a critical feature of the nervous system and a growing body of evidence indicates that brain-derived neurotrophic factor (BDNF) acutely modulates the efficacy of GABA synaptic transmission. Although the neuronal potassium-chloride co-transporter KCC2 has been implied in this BDNF-induced ionic plasticity, the reports about actions of BDNF on GABA signaling remain conflicting. By examining the effect of BDNF on GABA-A receptor signaling in cerebellar Purkinje neurons, we discovered that BDNF induces GABA ionic plasticity by acutely decreasing the strength of GABA-A synapses. This effect disappears in the absence of KCC2 activity. Under this condition, BDNF augments evoked GABA-A currents suggesting a direct facilitatory effect of BDNF on GABA-A receptor. The effect of BDNF is highly localized at the GABA-A synapse and is secured by physical coupling between GABA-A receptor and KCC2, revealed by co-immunoprecipitation studies. While both the effects of BDNF on GABA-A receptor and KCC2 are dependent on TrkB, only the effect of BDNF on KCC2 activity is dependent on a rise of intracellular calcium ion while the facilitatory action of BDNF on GABA-A receptor is dependent on activation of Cdk5. Based on these results, we hypothesize that the interaction between KCC2 and specific subunit of GABA-A receptor represents a fundamental mechanism rendering the rapid induction of ionic plasticity in individual or input-specific GABA synapses possible.

S2-02

Effect of Ginsenoside Rg1 on some Alzheimer processes and learning in ovariectomized rats

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Ginsenoside Rg1 has been reported to possess neuroprotective properties and also identified as a potent phytoestrogen. It has been demonstrated that estradiol influences the processing of amyloid β precursor protein (APP) and the phosphorylation of tau protein, the two hallmarks of AD pathology. Hence the neuroprotective and estrogenic effects of Rg1 may be related. However, it has been reported that the effect of Rg1 might be independent of estrogen receptors, and it is unknown how Rg1 might intervene the two 'hallmarks' processes in estrogen withdrawal states. In this study, adult female rats were ovariectomized and immediately treated with Rg1 by daily i.p. injection. At 2-3 weeks post operation, the rats were either tested on their learning ability using the Morris water maze, or were sacrificed for measurement of sAPPα, p-Akt, and p-Erk1/2. sAPPα is produced in the normal processing of APP, and Akt and Erk1/2 are correlated with neurofibrillary pathology (1,2). Preliminary results showed that the Rg1-treated ovariectomized rats, compared with the untreated ovariectomized rats, had a stronger spatial memory of the maze, a higher level of sAPPα, a higher p-Erk1/2 activity, and a lower p-Akt activity.

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S2-03

Regulation of dorsal root ganglion glial cell proliferation by dorsal root ganglion neurons

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The soma of dorsal root ganglion (DRG) neurons are enwrapped by satellite glial cells *in vivo*, and inhibiting neuron-glial cell communication appears to present a new approach to controlling chronic pain resulting from nerve damage or neuroinflammation. From our studies on purified DRG glial cells and DRG neuron-enriched cell cultures, we proposed that the presence of neurons affected the rate of proliferation of glial cells. To test this hypothesis, we monitored cell numbers, cell viability, and total cellular protein in mixed DRG cell cultures, neuron-enriched cell cultures and pure glial cell cultures over a period of 7 days. In cell cultures containing neurons, there was a significant increase in glial cell numbers and cellular protein by Day 7 ($p < 0.05$). There are several candidate molecules responsible for neuron-glial cell communication: ATP, glutamate, nitric oxide and prostanoids. Of these candidates, glutamate had little effect while ATP had the most dramatic effect inhibiting cell proliferation/viability in all 3 cell groups. Inhibiting nNOS and COX also appeared to inhibit cell proliferation. In conclusion, damaged/recovering DRG neurons may generate nitric oxide and/or prostanoids to facilitate DRG glial cell proliferation. This study was supported by a direct grant from The Chinese University of Hong Kong (CUHK2041584).

S2-04

The role of microRNA-29 in Duchenne Muscular Dystrophy

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MicroRNAs (miRNAs) are non-coding RNAs that regulate gene expression in post-transcriptional fashion, and emerging studies support their importance in a multitude of physiological and pathological processes. Here we describe the regulation and function of miR-29 in Duchenne Muscular Dystrophy (DMD) and its potential use as therapeutic treatment approach. Results demonstrate that miR-29 expression is down-regulated in dystrophic muscles. Restoration of its expression by intramuscular and intravenous injection improved dystrophy pathology by both improving regeneration and inhibiting fibrogenesis. Mechanistic studies revealed that loss of miR-29 in myoblasts promotes myoblasts transdifferentiation into myofibroblasts thus contributing to muscle fibrogenesis. We further demonstrated that miR-29 is under negative regulation by TGFbeta-Smad3 signaling via dual mechanism of both inhibiting MyoD binding and enhancing YY1-recurited Polycomb association. Together, these results identify TGFbeta-Smad3-miR-29 as a novel regulatory circuit during myoblast conversion into myofibroblasts which may be a contributing event in muscle fibrogenesis.

S2-05

DW-IN15, a small molecule, functions as a novel anti-HIV-1 inhibitor targeting the interaction between integrase and cellular *Lens epithelium-derived growth factor*

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Translocation of viral integrase (IN) into nucleus is a critical precondition of integration during the life cycle of HIV, a causative agent of Acquired Immunodeficiency Syndromes (AIDS). As the first discovered cellular factor to interact with IN, Lens epithelium-derived growth factor (LEDGF/p75) plays an important role in the process of integration. Disruption of the LEDGF/p75-IN interaction has been suggested to be exploited as target for drug development as against HIV infection. We have recently established a cell-based imaging platform to screen for compounds that disrupt IN/LEDGF interaction. Here, we reported that one small molecular compound, DW-IN15, could potently inhibit the IN-LEDGF/p75 interaction and affect the HIV-1 IN nuclear distribution at 1μM. DW-IN15 suppressed viral replication by measuring HIV-1 P24 antigen production in HIV-1IIIB acute infected C8166 cells with EC50 value of 11.19μM. DW-IN15 is considered to be a suitable lead compound for novel anti-AIDS drug discovery and development.

P2X7 receptor blockade by Brilliant Blue G promotes retinal ganglion cell survival and regeneration after optic nerve injury

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Purpose
Massive ATP release and activation of the P2X7 purinergic receptor after injury has been linked to neuronal degeneration. However, the role played by P2X7 in retinal ganglion cell (RGC) death after optic nerve transection (ONT) remains unknown. We tested whether P2X7 blockade by a relatively specific antagonist Brilliant Blue G (BBG) could enhance RGC survival and axonal regeneration after ONT.

Methods
Adult hamsters were subjected to ONT 2 mm behind the globe and injected intravitreally with different doses of BBG solution or its vehicle 0.9% NaCl. RGC survival was quantified at different post-injury periods and compared to the survival promoted by BDNF. Another P2X7 antagonist, Ox-ATP, as well as two non-specific P2 antagonists suramin and PPADS, were also assessed for their effects on RGC survival. RGC axonal regeneration was studied by grafting a peripheral nerve to the cut ON together with intravitreal BBG or vehicle injection. In separate experiments, BBG was delivered as eye-drops after ONT to compare its effects on RGC survival to that of intravitreal BBG.

Results
Intravitreal BBG promoted RGC survival dose-dependently, with maximal survival of >90% at 7 days post-injury. Except at 7 days post-ONT when the rescuing effect of BBG slightly fell short of BDNF, BBG injection resulted in higher survival than BDNF at longer post-ONT times. Ox-ATP promoted comparable survival as BBG whereas suramin and PPADS were only slightly better than vehicle, suggesting that P2X7 blockade enhanced RGC survival. RGC axonal regeneration into a peripheral nerve graft was increased significantly in BBG versus vehicle treatment. Interestingly, BBG given as eye-drops also promoted RGC survival to a similar extent as intravitreal BBG.

Conclusions
P2X7 blockade by intravitreal BBG promotes long term RGC survival and increases axonal regeneration. BBG eye-drops also improve survival and may be useful as a prolonged non-invasive therapy for chronic diseases like glaucoma.

S2-07

Study of two dyslexia candidate genes: KIAA0319 and KIAA0319L

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Developmental dyslexia is a common learning disability that affects 5-13% of the population depending on the stringency of categorization. Several candidate genes have been reported, including KIAA0319, DCDC1, DCDC2, Robo1, MRPL19, C2orf3 and more recently KIAA0319L. Our laboratory have collected DNA samples from local dyslexic children and confirmed some of the genetic associations. KIAA3019 is considered to be the most well-established candidate gene for susceptibility to developmental dyslexia. KIAA0319L is of particular interest because it is a homologue of KIAA0319, and has been recently identified as a candidate gene for DD (rs7523017; p=0.042). We have obtained a monoclonal antibody against Kiaa3019 and its availability should enhance further study of its function and its protein interaction partners. Furthermore, we have demonstrated the physical interactions of Kiaa0319L and NgR1 proteins in a yeast two-hybrid screen and confirmed their interactions using immunoprecipitation of transfected mammalian cells. The colocalization of these proteins in cytoplasmic granules of cortical neurons in human brain cortex is consistent with their physical interaction. Results with the knock down of “KIAA0319 homologue” flies are defective in visual memory by flight simulator experiments - showing that this gene is likely to be important in developmental dyslexia through it effects on memory.

S2-08

The role of Nogo on the guidance of axons in the mouse optic chiasm

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The axon growth inhibitory molecule Nogo consists of at least 3 isoforms, Nogo-A, -B and -C, of which A and B are the major isoforms in the central nervous tissues. Earlier studies in our laboratory have showed that Nogo is expressed on the midline glia and contributes to the bilateral routing of axons at the chiasm of mouse embryos. These results differ from other studies which show a neuronal localization of Nogo. To resolve this discrepancy, we have revisited the question of whether Nogo is expressed in the radial glial cells at the chiasmatic midline, using antibodies with well-defined binding specificity. Two antibodies Bianca and 11C7 were used, which recognize -A/B and -B isoform, respectively. We found both Nogo-A and Nogo-B exclusively on the radial glial cells in the E13 retina, when optic axons first leave the eye and arrive at the optic chiasm. At E14-15, Nogo-A and -B became localized predominantly on neurons and axons in the retina, suggesting an age specific expression in the glia and neurons. At the optic chiasm, both isoforms were localized on the radial glial cells and the optic axons. However, labels on the glia processes were more obvious when stained with Bianca than with 11C7, suggesting that Nogo-B is the predominant form in the midline glia. The existence of Nogo-B at the midline was further supported by the presence of staining with Bianca in Nogo-A knockout chiasm; such labels were not observed with 11C7. Furthermore, the absence of Nogo-A did not affect the routing of early axons at the chiasm and at later stage formation of ipsilateral projection, supporting that Nogo-B is the major player that guides optic axon growth at the mouse optic chiasm.

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S2-09

Subnormal level of retinoic acid increases the risk of birth defects in diabetic pregnancy

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Diabetic pregnancy increases the risk of birth defects. Using a mouse model, we have found that the retinoic acid (RA) catabolizing enzyme *Cyp26a1*, which expresses in specific tissues in the embryo to protect against the deleterious effect of ectopic RA signaling, is significantly down-regulated in diabetic pregnancy. Since *Cyp26a1* can be positively regulated by RA, and RA in the embryo is synthesized from retinol taken from the mother, we therefore hypothesize that there is subnormal maternal retinol level that can lead to an overall reduction in embryonic RA concentrations, which down-regulates *Cyp26a1* and thereby predisposing embryos to specific malformations.

First, retinol and RA levels were compared between diabetic and non-diabetic pregnancies. Indeed, we found that retinol level from maternal supply and embryonic RA concentrations were significantly reduced in the diabetic group. Conversely, when subnormal RA level in embryos of diabetic mice was restored by maternal supplementation with *sub-teratogenic* dose of RA, *Cyp26a1* expression level was normalized. Concomitantly, upon challenged with a *teratogenic* dose of RA, the susceptibility of *Cyp26a1*-normalized embryos of diabetic mice to various types of malformations was significantly reduced to a level similar to that of the non-diabetic group.

Our findings support the hypothesis that subnormal RA level will down-regulate *Cyp26a1* expression in the embryo, but supplementation with *sub-teratogenic* dose of RA can normalize *Cyp26a1* expression and reduces the risk of birth defects.

S2-10

Phase I/II randomized controlled trial of autologous bone marrow-derived mesenchymal stem cell therapy for chronic intracranial hemorrhagic stroke

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Intracranial hemorrhagic (ICH) stroke is a common neurological disorder and associated with permanent neurological deficits. Currently, there is no effective treatment for restoring the lost neurological functions. The discovery of stem cell plasticity and neurogenesis in adult brain have raised the hope that stroke might become amenable to cellular therapy to replenish the loss and degeneration of functional neural cells. Mesenchymal stem cell (MSC) therapy has been shown to be safe and effective in animal stroke models and a phase I/II trial for acute stroke. In March 2007 to January 2009 we conducted a double-blind, randomized, controlled phase I/II trial to examine the safety and efficacy of MSC therapy in a cohort of 9 patients (4 females and 5 males) of mean age of 52 years (range: 41 – 59 years) who had undergone ICH stroke for a year. MSCs were ex vivo expanded from 29 mL (17 – 42 mL) autologous bone marrow. Patients with severe disability were randomized to have two intravenous injections of autologous MSCs or placebos in 4 weeks. Neurological functions and clinical outcome were monitored pre-treatment and at 4, 12, 24 and 48 weeks post-treatment. In the treatment group of 3 female and 2 male patients, 8.67×10^5 ($2.65 \times 10^5 – 1.31 \times 10^6$) and 8.41×10^5 ($2.65 \times 10^5 – 1.45 \times 10^6$) MSCs up to passage-8 per Kg body weight were administered in two occasions. The control group of 1 female and 3 male patients received placebos in an identical manner. The cell viability was 95.3% (88.5-99.0%). Derived cells were immunophenotypically positive for CD29, CD44, CD73, CD90, CD105 and CD166, but negative for HLA-DR, CD45, CD33, CD38, CD3, CD19, CD16, CD34 and CD133. No adverse event was noted. Significant or trend of improvement in motor and cognitive functions was only observed in the treatment group (Functional Independence Measure (FIM) of motor components: p=0.068, 0.068 and 0.043 at 8, 16 and 28 weeks after treatment; cognitive functions: p=0.038, 0.041, 0.042 and 0.066 at 8, 16, 28 and 52 weeks; modified Barthel index: p=0.043 and 0.068 at 28 and 52 weeks). However, no difference in the Extended Glasgow Outcome Scale (GOSE) score was found. The study findings suggest that MSC therapy is safe and may improve both cognitive and functional recovery after ICH stroke, but beneficial clinical outcome was not apparent. Further phase III trials may need to attest the findings.

Poster Presentations

Abstracts

Poster Session

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Library screening identified small molecule activators of microRNA-34a with anti-cancer activity Z.G. Xiao, C.H.K. Cheng, Y.C. Chen	P-02
Mitotic catastrophe in cancer cells Y.H. Ling, F.W.K. Cheung, H.C. Lee, J. Guo, B.P. Liu,W.K. Liu	P-03
Novel biomarkers for predicting response to chemotherapy in colorectal cancer K.K.W. To, W.W. Leung, S.S.M. Ng	P-04
Nuclear receptor estrogen-related receptor alpha promotes hypoxic growth of prostate cancer cells C. Zou, S. Yu, D.T. Yew, F.L. Chan	P-05
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P-01

A new Phaseolus vulgaris lectin induces selective toxicity on human liver carcinoma Hep G2 cells

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We describe here the purification and characterization of a new Phaseolus vulgaris lectin that exhibits selective toxicity to human hepatoma HepG2 cells and lacks significant toxicity on normal liver WRL 68 cells. This polygalacturonic acid-specific lectin (termed BTKL) was purified from seeds of P. vulgaris cv. Blue tiger king by liquid chromatography techniques. The 60-kDa dimeric lectin showed strong and broad-spectrum hemagglutinating activity toward human, rabbit, rat, and mouse erythrocytes. Bioinformatic analysis unveils substantial N-terminal sequence similarity of BTKL to other Phaseolus lectins. Among a number of tumor cells tested, BTKL exhibits potent anti-HepG2 activity which is associated with (1) induction of DNA fragmentation, (2) production of apoptotic bodies and chromatin condensation, (3) triggering of cell apoptosis and necrosis, and (4) depolarization of mitochondrial membrane (low $\Delta\Psi m$). Furthermore, BTKL could induce inducible nitric oxide synthase (iNOS) expression and subsequent nitric oxide production in vitro in mouse macrophages, which may contribute to its antitumor activity. In addition, BTKL could bring about a significant dose-dependent increase in the production of mRNAs of proinflammatory cytokines including interleukin-1 beta, interleukin-2, tumor necrosis factor alpha, and interferon-gamma. In sum, the antitumor activity and mechanism of BTKL provided here suggest that it has potential therapeutic value for human liver cancer.

P-02

Library screening identified small molecule activators of microRNA-34a with anti-cancer activity

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MicroRNAs (miRNAs) play critical roles in various biological processes through regulating gene expression. Aberrant expression of miRNAs has been well documented in a variety of cancers. miRNAs function as oncogenes or tumor suppressors and represent promising therapeutic targets for cancer. Small molecules modulating miRNAs expression represent potential novel anti-cancer agents. miR-34a is downregulated and function as tumor suppressor in various cancers including hepatocellular carcinoma (HCC). In this study, we established the miR-34a reporter construct and developed an assay for screening small-molecule activators of miR-34a. The natural product library (NPL, TimTec) containing 640 pure compounds was screened to identify miR-34a activators. We identified two compounds with significant activation on miR-34a expression. We further demonstrated the growth inhibitory effect of these two compounds on HCC cells in vitro. The in vivo anti-cancer efficacy is under investigation.

P-03

Mitotic catastrophe in cancer cells

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Apoptosis is a form of programmed cell death with active participation of caspases for DNA cleavage. It is characterized by cell shrinkage, membrane blebbing and formation of apoptotic bodies, and is considered a major target for chemotherapy. Evasion of apoptosis results in resistance to anticancer therapies, and increasing attention is therefore directed towards other types of cell deaths, such as autophagy and mitotic catastrophe. Autophagy is a stress-related catabolic process in which proteins and organelles are degraded by lysosomal enzymes, while mitotic catastrophe is a form of aberrant mitosis which is characterized by cell enlargement and multinucleated and unequal DNA separation. In a continuous program searching for bioactive anticancer candidates from natural sources, we have identified two potent aberrant mitotic inducers, a β-carboline alkaloid and an isomalabaricane triterpene, that activated expression of cyclin kinase inhibitor p21, and arrested growth of human colon HT29 cells and melanoma cells at the G1 phase of the cell cycle. Interestingly some cells escaped from prolonged growth arrest without proper cell division, resulting in binucleated or polyploidy G1 cells. These small molecules provide potential development of new drugs for chemotherapeutic applications.

P-04

Novel biomarkers for predicting response to chemotherapy in colorectal cancer

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Multidrug resistance (MDR) remains a major unresolved obstacle to successful cancer chemotherapy. It is usually associated with an increased efflux of cytotoxic drugs by ATP-binding cassette (ABC) transporters including ABCG2. Overexpression of ABCG2 is frequently observed in drug-selected resistant cancer cell lines and is also suggested to be involved in clinical drug resistance in cancer patients. Adjuvant chemotherapy composing of ABCG2 substrate anticancer drugs such as 5-fluorouracil and irinotecan is routinely used in colon cancer patients after surgical resection of the primary tumor to reduce the risk of recurrent and metastasis. Unfortunately, the prognosis and response to chemotherapy of the more advanced stage colon cancers remain very poor. An effective way to predict drug response could lead to better therapeutic outcome.

MicroRNAs (miRNAs) represent a large class of short endogenous non-coding RNA playing an important role in regulating mRNA and protein expression. Evidence pointing to the role of miRNAs in determining drug sensitivity and resistance is emerging. We have recently identified a novel regulatory pathway by which low levels of miR-519c promote upregulation of a mRNA-binding protein HuR and subsequently leading to ABCG2 overexpression and increased drug resistance. Results from our pilot study analyzing colon cancer tissues and their matched adjacent non-cancerous colon tissues revealed a good correlation between low miR-519c expression and high levels of HuR and ABCG2 in the tumor, and poor patients' response to chemotherapy. This miR-519c/HuR/ABCG2 axis may hold promise to guide treatment selection with the ultimate goal of individualizing therapy for patient with cancer.

P-05

Nuclear receptor estrogen-related receptor alpha promotes hypoxic growth of prostate cancer cells

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Estrogen-related receptors (ERRα,β,γ) which share significant homology with estrogen receptors, are orphan nuclear receptors and constitutively transactivated without estrogen-binding. Among the three subtypes, ERRα has been characterized to play a pivotal role in diverse aspects of energy homeostasis, including oxidative phosphorylation and mitochondrial biogenesis, via its transcriptional regulation of target genes. Expression studies in clinical tumor tissues show that ERRα expression is positively associated with a few hormone-dependent cancers, including breast, ovarian and endometrial cancers, and has been proposed to be useful as a prognostic marker for these cancers. We have previously reported that ERRα displays a ubiquitous expression pattern in various prostatic cell lines and an apparent elevated expression in high-grade prostate cancer. However, its exact functional roles and prognostic value in prostate cancer still remains unclear. In this study, we analyzed the functional significance of ERRα in prostate cancer cell growth regulation by its stable expression in a hormone-sensitive prostate cancer cell line LNCaP. Our results showed that stable expression of ERRα in LNCaP cells could enhance their in vitro cell proliferation, adhesion to fibronectin, invasion and colony formation capacities of LNCaP cells under both normoxia and hypoxia condition (1% O2), while such in vitro growth phenotypes could be suppressed by an ERRα specific antagonist XCT790, suggesting that ERRα-induced growth phenotypes were ERRα specific. Moreover, under hypoxia condition, ERRα could stabilize HIF1α protein, up-regulate HIF1α target genes involved in angiogenesis (VEGF) and hypoxic glycolysis in both prostate cancer cell line and immortalized prostatic epithelial cell line.

Taken together, our results showed that ERRα over expression promotes hypoxic growth of prostate cancer cells.

(This study is supported by a RGC General Research Fund CUHK461009)

P-06

The Wnt/DVL signaling antagonist DACT2 is a pro-apoptotic tumor suppressor epigenetically silenced in multiple tumors and inhibits tumor cell proliferation and migration

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Wnt/Dvl/β-Catenin signaling pathway is aberrantly activated in a wide range of human cancers by frequently disrupted its negative regulators, hence identifying novel tumor suppressor as the antagonist to this signaling pathway is needed. Here, we identified a novel DACT (Dpr/Frodo) family member DACT2 downregulated in multiple human tumors. Epigenetic study revealed that in contrast to its broad expression in normal tissues and cell lines, *DACT2* was silenced or downregulated by promoter methylation in multiple tumor cell lines. Pharmacologic demethylation reactivated *DACT2* expression along with concomitant promoter demethylation. *DACT2* promoter methylation was also frequently detected in multiple primary tumors, suggesting *DACT2* methylation is tumor specific. Ectopic expression of DACT2 dramatically inhibited tumor cell colony formation in silenced tumor cell lines, as well as induced tumor apoptosis, thus functioning as a tumor suppressor. DACT2 is a cytoplasm protein. Overexpression of DACT2 suppressed β-Catenin activation and accumulation in nucleus by downregulating the expression of Dishevelled (Dvl) and the phosphorylation of glycogen synthase kinase 3β (GSK-3β). Aberrant Wnt/Dvl/β-Catenin signaling disrupted by DACT2 further inhibited its target oncogenes such as *c-Myc*, *CCND1* and *fibronectin* by preventing the binding to the Lef/Tcf family of transcription factors, thus inhibiting tumor cell proliferation and migration. DACT2 also inhibited tumor cell epithelial-mesenchymal transition by upregulating epithelial cell marker E-cadherin as well as downregulating mesenchymal markers fibronectin and vimentin. Thus, our work demonstrate that DACT2 is a functional tumor suppressor acting as a negative regulator of the Wnt/Dvl/β-Catenin pathway, epigenetically silenced in multiple tumors and could be a promising biomarker for human tumors.

P-07

Natural mutants of hepatitis B virus X protein increase the level of hypoxia-inducible factor-1α by interfering with von-Hippel-Lindau in hepatocellular carcinoma

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Background: Mutations in the hepatitis B virus X (HBx) gene are frequently found in HBV-associated hepatocellular carcinoma (HCC). Activation of hypoxia-inducible factor-1α (HIF-1α) in response to both hypoxic stress and oncogenic signals has important implications for the development and progression of cancer.

Objective: In this study, we explore the relationship between HIF-1α and naturally occurring mutants derived from human HCC.

Methodology: Forty mutation hotspots were identified from 101 HCC tissue samples. Four frequently detected mutants (A257G, G348A, A389T, and G391A) are located in the HBx transactivation domain. The DNA of these mutants was then isolated and cloned for functional studies on HepG2 and MIHA cells.

Results: The natural HBx mutants were found to retain a similar ability to wild-type HBx to increase the expression and transcriptional activity of HIF-1α in HepG2 and MIHA cells. Immunoprecipitation analysis further revealed that these natural HBx mutants inhibited the binding between HIF-1α and von-Hippel-Lindau protein (pVHL), which may prevent HIF-1α degradation.

Conclusion: We have identified 40 HBx mutation hotspots and revealed a potential association between the four most frequently occurring mutants and HIF-1α. Analysis of these mutants demonstrated that they function in the same way as wild-type HBx in inhibiting the binding between HIF-1α and its degrading molecule, pVHL, thus suggesting a novel mechanism by which mutant HBx may stabilize HIF-1α protein.

P-08

Platforms for searching anti-cancer therapeutics from Traditional Chinese Medicine

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The development of invasiveness, metastasis and multidrug resistance are threatening problems in late stage cancer. To develop new therapeutic strategies against these problems, various *in vitro* and *in vivo* models have been set up in our laboratory. These platforms include (i) detection of apoptosis, cell cycle analysis and autophagy activity assay for examining anti-proliferation; (ii) Scratch assay, capillary tube formation and zebra fish model for examining anti-angiogenesis; (iii) Boyden chamber migration/invasion assay and nude mice bearing green fluorescence protein (GFP)-expressed metastatic tumour model for examining anti-metastasis; (iv) phagocytic activity assay and measurement of immune marker for examining the potential of cancer vaccination. Two natural products Pheophorbide a and Polyphyllin D, purified from Traditional Chinese Medicine, will be demonstrated for their anti-tumour mechanisms by applying these platforms. Moreover, promoter methylation analysis and gene expression analysis have been applied to understand the development of multidrug resistance and related differential gene expression in tumour cells.

P-09

GPER-1 mediates the inhibitory actions of estrogen on adipogenesis in 3T3-L1 cells through perturbation of mitotic clonal expansion

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Estrogen has been shown to inhibit adipogenesis. Estrogen replacement therapy therefore affects fat metabolism in post-menopausal women. A novel transmembrane estrogen receptor, GPER-1, is recently identified in various animals including mouse, rat, human and zebrafish. GPER-1 has been demonstrated to mediate various estrogenic actions in vertebrates, but the exact roles of GPER-1 in adipogenesis remain to be resolved.

We have observed an up-regulation in the expression of GPER-1 in the mouse preadipocytes cell line 3T3-L1 during induced adipogenesis. In addition, perturbation of cell differentiation was also observed in the presence of a specific GPER-1 agonist, G1, during mitotic clonal expansion (MCE) of the 3T3-L1 cells. By means of Oil-Red-O staining, the production of oil droplets in the G1-treated differentiated 3T3-L1 cells is shown to be reduced. FACS analysis and Western blotting analysis of cell cycle factors during MCE of the 3T3-L1 cells reveals an inhibition of cell cycle arrest at the G1 stage triggered by GPER-1 activation.

In conclusion, this study on the involvement of GPER-1 in mammalian adipogenesis reveals an elevated expression of GPER-1 during adipogenesis. In addition, an inhibition of adipogenesis by GPER-1 activation was also observed during MCE. It is therefore postulated that GPER-1 serves as a negative regulator of adipogenesis in adipose tissues. The results provide insights into the possible development of therapeutic agents for the treatment of obesity by targeting GPER-1.

Pancreatic islet cell biology in health and disease

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Pancreatic islet cells are known for maintaining glucose homeostasis; reduced islet β-cell function and/or cell-mass induces hyperglycemia, observed in both types 1 and 2 diabetes mellitus. Pharmacological and non-pharmacological approaches targeting islet β-cells are thus central to current and future diabetes management. Treatment/curing modalities include islet cell transplantation, stimulation of β-cell insulin secretion and regeneration and pancreatic stem cell research. Investigating islet cell biology provides extensive and novel information elucidating the biology of islet cell development. This provides data for basic and clinical diabetes research and a platform for developing novel drugs, and functionally responsive β-cells for islet transplantation, for treating diabetes.

In light of this perspective, our research focus is on islet cell biology and development in relation to diabetes. In the past decade, we have established *in vitro*, *ex vivo* and *in vivo* models for islet cell and pancreatic stem/progenitor cell research. Of particular interest are our recent investigations into the roles and interactions of several novel regulatory factors (angiotensins and incretins as well as vitamin D and vitamin A) on the function, growth and differentiation of β-cells and/or pancreatic progenitor cells. Better understanding of islet cell biology, including their development, should provide insights into normal and abnormal functioning of islet β-cells and their abnormalities in diabetes.

Expression of angiotensin type II receptor: A systematic review

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Angiotensin type II (AT2) receptor displays agonist-dependent and -independent activities, coupling to a spectrum of signalling molecules that include phosphatases, kinases, G proteins and Na^+, K^+-ATPase. Despite the large volume of work on AT2 receptor, consent cannot be reached on the cellular distribution, signalling cascade and receptor function. In the present study, rat AT2 receptors tagged with GFP, myc, HA or FLAG were transiently or stably expressed in HEK293, CHO-K1, and PC12 cells, evaluating the effects of species and tissue specificity on AT2 receptor expression. AT2 receptor was mainly detected on cell membrane and perinuclear region, and existed as monomer (40 kDa) as well as oligomers (70 - 250 kDa). However, levels of expression, cellular distribution and receptor oligomerization depended on cell type and tagging. Stably transfected HEK293 cells gave the highest expression of N-terminal myc-tagged AT2 on the cell surface. Intriguingly, AT2 receptor oligomers were enriched in cell membrane, and glycosylation promoted membrane localization of myc-AT2 but had no effect on AT2-GFP. Functionally, AT2 receptor mediated different cellular responses in different cell types. Serum starvation induced apoptosis in stably AT2-expressing CHO cells but exerted no effect on HEK293 and PC12 cells. However, AT2-expressing HEK293 and PC12 cells exhibited a partial cell cycle blockage with cells accumulating at G1 and S phases, respectively. Although showing similar cellular distribution, transiently expressed myc-AT2 receptor existed predominantly as monomer with ladder of high molecular weight forms in HEK293 cells, suggesting a posttranslational modification. Co-expression analysis indicated transiently expressed AT2 receptor was ubiquitinated. Unexpectedly, western analysis of day 19 mouse embryo suggested the embryonic AT2 receptor expression pattern was similar to that of transiently expressed AT2 receptor and putatively ubiquitinated.

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P-12

Cathelicidin protects against *Helicobacter pylori* colonization and the associated gastritis in mice

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Helicobacter pylori (*H. pylori*) infection is one of the most prevalent infectious diseases in the world. *H. pylori* infection causes chronic active gastritis in the majority of infected patients. Although there are antibiotics which are effective to eradicate the bacteria, the development of drug resistance is common and is also the major cause of treatment failure. It is therefore needed to establish a new form of drug therapy through different mechanisms of action. This would provide an alternative of treatment for *H. pylori* infection. In this study we would like to demonstrate such approach by using a pleiotropic host defense peptide "cathelicidin" naturally occurred in the gastrointestinal tract and expressed in the circulating immune cells to reduce infection and perhaps inflammation in the stomach. This peptide was shown to possess microbicidal activity against a broad spectrum of microorganisms including *H. pylori*. In this project we propose to adapt an animal model to infect cathelicidin-knockout mice with *H. pylori* in order elucidate the roles of endogenous cathelicidin as an essential host defense factor in the control of *H. pylori* colonization in the stomach and protection against gastritis. Secondly we used the bioengineered technology to incorporate cathelicidin gene into a probiotic to increase the expression and secretion of the peptide in the stomach in order to affirm the modulatory role of cathelicidin and probiotic in *H. pylori* infection and gastritis formation.

In this study we had successfully constructed a cathelicidin-secreting *Lactococcus lactis* (a kind of probiotic) which was shown to survive and colonize in the gastric epithelium in the wild type and cathelicidin-knockout mice. *H. pylori* was also found to successfully colonize in the gastric tissues in both types of animals and proven by PCR and ELISA. This bacterium infection induced marked gastric inflammation as shown by increases in inflammatory score and neutrophil infiltration three months after infection. Different inflammatory cytokines including TNF-α and IL-6 were markedly increased. There was no significant difference in inflammatory responses in the stomach between the wild type and the cathelicidin-knockout mice indicating that endogenous cathelicidin may not play an important role in the prevention of *H. pylori*-induced gastritis. However, alternative day treatment with cathelicidin-encoded *Lactococcus lactis* but not the probiotic alone for two months significantly reduced the gastric levels of both inflammatory cytokines examined in this study. There was a trend in the reduction of inflammatory score by this bioengineering preparation in both wild type and knockout mice. Also there was an indication that the level of *H. pylori* infection was also decreased. Moreover, this preparation did not affect the hepatic and renal functions in mice after two months of treatment. Taken together, it is likely that cathelicidin-encoded probiotic could be an effective and safe therapeutic agent for the treatment of *H. pylori*-induced gastritis. This could serve as an alternative therapy for *H. pylori* patients who are intolerant and resistant to conventional antibiotic treatment.

P-13

Potential mechanism-based biomarker for the diagnosis of hepatotoxicity induced by pyrrolizidine alkaloids

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Pyrrolizidine alkaloids (PAs) are widely distributed worldwide. More than 660 PAs have been identified in over 6,000 plants, and about half of them are hepatotoxicity, leading to acute hepatotoxicity known as hepatic sinusoidal obstruction syndrome (HSOS) or liver cancer in human exposed to PA-containing medicinal herbs and/or PA-contaminated foodstuffs. PAs require cytochrome P450-mediated metabolic activation to generate the corresponding pyrrolic metabolites, which are chemically reactive and can rapidly react with cellular macromolecules, such as proteins or DNAs to form pyrrole-protein or pyrrole-DNA adducts leading to hepatic toxicity or tumor, respectively. However, to date, these are no specific method for the diagnosis of PA-induced HSOS.

Recently, our studies have demonstrated that the formation of hepatic pyrrole-protein adducts correlated well with the hepatotoxicity in laboratory animals treated with toxic PAs. Therefore, we anticipate that the pyrrole-protein adducts can be developed as a potential biomarker of PA-induced hepatotoxicity. In the present study, we developed two approaches, namely UPLC-MS analysis with pre-derivatization to release pyrroles from the adducts and immunoassay with the raised antibody specifically recognizing the adducts, to determine such pyrrole-derived protein modification. Using the developed methods, such adducts were successfully determined in both liver and blood samples of rats treated with both PA and PA-containing herb, and the quantity of such adducts in biological samples correlated to the severity of hepatotoxicity. Moreover, the UPLC-MS method was applied to analyze patient samples, and the pyrrole-protein adducts were successfully determined, for the first time, in blood samples of several HSOS patients poisoned by a PA-containing Chinese medicinal herb Tushanqi (*Gynura segetum*). Our results revealed that the pyrrole-protein adducts has a potential to be developed as a biomarker for the assessment of PA-induced hepatotoxicity.

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P-14

Adenosine potentiates toll-like receptor 2 induced IL-8 release from human mast cells

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Adenosine, an endogenous nucleoside, modulates a large number of cellular systems in inflammation. In this study, we pharmacologically characterized the actions of adenosine on toll like receptor (TLR) induced IL-8 release from human cultured mast cells (HCMC). Treatment of bacterial peptidoglycan (PGN) and lipopolysaccharides (LPS), specific ligands of TLR2 and TLR4 respectively, significantly increased IL-8 production from HCMC. Adenosine and NECA at sub-nanomolar concentrations (10^{-8} – 10^{-7} M) potentiated PGN-mediated IL-8 production which was not observed in LPS treated cells. A_1 receptor agonists, CCPA and 2'MeCCPA, both markedly potentiated the IL-8 production by PGN dose-dependently. Moreover, a similar effect was also found with A_3 receptor selective agonists 2Cl-IB-MECA and HEMADO. A_2 receptor was not involved in the enhancing effect as A_2 receptor specific CGS21680 and CV1808 were found not to affect the PGN effect. The potency of adenosine agonists was found in rank order of 2'MeCCPA > NECA > CCPA > HEMADO > 2-Cl-IB-MECA >> CGS21680/CV1808. When the effects of specific adenosine receptor antagonists on the potentiating action of NECA were investigated, significant inhibition was observed with antagonists of A_1 receptor, CGS15943 and PSB36, and A_3 receptor, MRS3777, but not that of A_{2B} receptor, PSB1115. These results suggest that A_1/A_3 receptors are responsible for the potentiating effect of adenosine on mast cell activation by PGN in human innate immune reactions.

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P-15

Spga-lncRNA3, a novel long non-coding RNA that regulates developmental programs of spermatogonial stem cells

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Spermatogonial stem cell**s** (SSCs) are male germ line stem cells that control spermatogenesis by their ability to both self-renew and generate subsequent germ cell types into spermatozoa through rounds of differentiation. Although factor like Glial cell line-Derived Neurotrophic Factor (GDNF) is known to be important in self-renewal of SSCs, the exact mechanisms that govern SSCs differentiation and pluripotency remain largely unknown. To identify the differentiation and self-renewal mechanisms, we performed large-scale transcriptome studies on male germ cells by whole-genome tiling microarrays and sequencing approaches. The findings suggested majority of SSC genome is transcribed and is far more than current gene annotations. We found a huge number of transcript species are known as long non-coding RNAs (lncRNAs). By applying various bioinformatics pipelines, we have identified a number of SSC-specific lncRNA candidates and examined their effects on cellular differentiation using C18–4 SSC *in vitro* differentiation model. We found a potential candidate, code-named as *Spga-lncRNA3*, demonstrated significant differentiation inhibition, suggesting it may be important in maintaining stem cell state of SSCs. Our contribution in this study is expected to provide detailed understanding of how lncRNAs regulate cell differentiation and proliferation in spermatogenesis. Dysregulation of these developmental programs is known to associate with gametogenesis defects and disease states such as cancer. The findings are expected to substantially change the concepts in developmental and cancer biology, which could lead to the development of novel therapeutic strategies.

P-16

Mesenchymal stem cells improve plasticity and cognition of ischemic hippocampus

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Background and objective: Transplantation of bone marrow mesenchymal stem cells (BMSC) improves the motor function in rats with traumatic brain injury and ischemic stroke, but its effects on learning and memory have not been systematically studied. This study investigated the cellular behavior of BMSC in the ischemic brain and its effect on cellular plasticity and cognitive ability in a murine ischemic stroke model.

Materials and methods: CD45- CD11b- primary Green Fluorescence Protein (GFP) bearing BMSC was intracerebroventricular administrated at the same time of the induction of ischemia. Neurobehavioral recovery was measured by the multiple water-T-maze system. At sacrifice, CA1 tissues were harvested for analysis of apoptosis and inflammation molecular markers. The migration and differentiation of the transplanted cells were examined at different time points post transplantation.

Results: The transplanted group had a significantly higher performance in special relearning ability after transplantation. The apoptosis related molecular markers in the hippocampus CA1 in the untreated group increased significantly compared with the treated group at translational level. The expression of numerous proinflammatory markers was downregulated at transcriptional levels after stem cell treatment. Along the nerve fiber tract, the transplanted BMSC migrated to the CA1 area and differentiated into neurons at 15 weeks.

Conclusion and discussion: In this murine ischemic stroke model, BMSC have been characterized to protect CA1 neurons from ischemic injury, the early function improvements from MSC treatment is partly due to an anti-inflammation mechanism.

P-17

Ischemia-preconditioned neurons protect astrocytes against ischemia via upregulation of erythropoietin

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It is well established that ischemia-preconditioned astrocytes and neurons can protect neurons against ischemia-induced injury. Since there is evidence suggesting the existence of bi-directional communications between neurons and astrocytes under physiological conditions, we speculate that preconditioned neurons, like astrocytes, can protect astrocytes from ischemia-induced injury. In this study, we tested this hypothesis based on both *in vitro* and *in vivo* models of ischemia. In the cultured system, we demonstrated that ischemia-preconditioned medium from neuron could significantly increase cell viability, and at the same time decrease LDH release and the percentage of apoptotic cells in astrocytes undergoing ischemia. We found that preconditioning of neurons increased the expression and release of erythropoietin (EPO). Further analyses revealed that activation of PI3K, ERK and STAT-5 signaling pathways by EPO attributed the anti-apoptotic action of ischemia-preconditioned neurons on astrocytes. In the *in vivo* model of forebrain ischemia-reperfusion, prior destruction of cortical neurons by kainic acid resulted in markedly increased apoptosis of astrocytes, which was accompanied by a reduction in preconditioning-induced increase in EPO content. We conclude from our studies that ischemia-preconditioned neurons can protect astrocytes against ischemia-induced injury confirming our hypothesis that bi-directional protective communications between neurons and astrocytes play crucial roles under pathophysiological conditions.

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Ets-1 regulated by FGF signaling is essential for neural crest differentiation

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The neural crest (NC) is a unique cell population that arises at the border of the neural plate and epidermis. The NC differentiates into various cell types including most of the peripheral nervous system, melanocytes, and the craniofacial skeleton. The clinical significance of neural crest is reflected in that almost one-half of all birth defects are caused by abnormal development of this developmental process. We previously indicated that Lrig3 was an essential factor for NC formation. We then identified Est-1 as the downstream target of Lrig3 using Affymetrix microarray. In line with the gene profiling assay, we found that Est-1 was activated during the NC formation, and specifically expressed in cranial NC. Knockdown of Lrig3 in NC tissue blocked the expression of Ets-1. Ectopic expression of Ets-1 before gastrulation caused inhibition of *Slug* and *FoxD3,* while knockdown of Est-1 up-regulated their expression. Ets-1 was essential for the migration of NC as the expression of twist was down-regulated by knockdown of *Ets-1*. Animal cap assay indicated that *Ets-1* was up-regulated by FGF signaling, and such activation was blocked by Su5402, the FGF inhibitor. Our study clarified the role of Ets-1 in NC formation, and shed light on the complexity of regulatory networks in NC differentiation.

P-19

The anti-herpetic activity of Trichosanthin via NF-κB and p53 pathways

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Trichosanthin (TCS) is a type I ribosome inactivating protein, which was found to inhibit human simplex virus type 1 (HSV-1) replication. The anti-HSV-1 ability of TCS is related to its selectivity to induce apoptosis in infected cells but the mechanism is unclear. In this study, we explored how the selectivity of TCS contributes to its anti-HSV-1 mechanism via nuclear factor-κB (NF-κB) and p53 pathways on human epithelial carcinoma HEp-2 cells. It was showed HSV-1 infection induced translocation of NF-κB to nucleus and binding of NF-κB to nuclear DNA to benefit the HSV-1 replication, but this NF-κB activation was negatively regulated by TCS. Meanwhile, compared to uninfected HEp-2 cells, TCS induced a significantly more p53 and BAX activation with no DNA damage and significantly less G_1 to S and G_2/ M phase arrest in HSV-1 infected cell, the BAX activation in infected cell correlated the cell death signaling of p53. Taken together, these results suggest that the anti-HSV-1 effect of TCS is related to the suppression of NF-κB activation and the regulation of p53-related cell death in infected cells by TCS.

Key words: Trichosanthin; human simplex virus type 1; nuclear factor-κB; p53

P-20

G1613A mutation on HBV core promoter suppresses e antigen secretion and enhances viral replication

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This study presents a follow-up investigation of a previous case control study which aimed to identify genomic markers in hepatitis B virus (HBV) associated with hepatocellular carcinoma (HCC) [J.J.Y. Sung, S.K.W. Tsui, C.H. Tse, Y.E.T. Ng, K.S. Leung, K.H. Lee, T.S.K. Mok, A. Bartholomeusz, T.C.C. Au, M.D. Zhang, H.L.Y. Chan. 2008. J Virol. 82(7):3604-11]. Our results showed that one of those markers, the G-to-A mutation at nucleotide 1613 (G1613A), can significantly increase the core promoter activity and the effect was reversible by the A-to-G back mutation. Moreover, the effect was genotype-dependent with the promoter activity higher in genotype C than in genotype B. In a study recruiting 255 chronic HBV carriers with subgenotype Cs suggested that the mutation was associated with serum HBV DNA level higher than 6 log copies/ml in female. Furthermore, *in vitro* full-length genome study of the mutation showed that the mutation decreased the extracellular HBeAg level significantly by 86-90%, while no significant change on the level of surface antigen (HBsAg). Real-time quantitative polymerase chain reaction showed that the mutation significantly increased the level of extracellular HBV DNA by 2-4 folds. Taken together, our results suggest that the G1613A mutation suppresses the HBeAg production and enhances virus production.

P-21

Functional relationship between vesicle tethering factor and cytoskeleton

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Protein transport in the early secretory pathway is mainly mediated by two forms of vesicles: COP (coat protein complex) II trafficking from Endoplasmic reticulum (ER) to Golgi and COPI recycling protein back to ER. Vesicles budding from donor compartments undergo budding, movement, tethering and fusion to deliver cargo proteins to the target compartments. Tethering plays a critical role for the specificity of vesicle targeting because it is the initial interaction between vesicles and target membranes. TRAPP (transport protein particle) complex acts as the tethering factor of COPII vesicles traffic from ER to Golgi. It has been well studied in yeast but its functions in mammalian cells are still not clear. We have recently uncovered an intimate relationship between COPII vesicle and the cytoskeleton. Such relationship is critical to the coordination between vesicle tethering and movement. Using co-immunoprecipitation, RNAi and fluorescence microscopy and other cell biological techniques, we analyzed the interaction of a cytoskeletal element with TRAPP. Our data suggest a mechanism that mammalian TRAPP functions in COPII vesicles tethering by binding with cytoskeleton.

P-22

Dab2 in early skeletal muscle development

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Dab2 is an intracellular adaptor protein and a potential tumor suppressor. In mouse embryos, our pilot study showed that Dab2 was first expressed in the medial aspect of the somite (one of the embryonic origins of muscle cells) at E9.5, and at E10.5 co-localized with the early muscle markers Pax3 and Myf5 at the ventrolateral lip of the somite. It has also been shown that Dab2 is involved in the MAPK signalling pathway which is known to be crucial to the muscle development. Taken together, we hypothesize that Dab2 is involved in the early skeletal muscle development. To prove this hypothesis, *Xenopus laevis* embryos and C2C12 myoblasts were employed as in *vivo* and in *vitro* models in this study. *In situ* hybridization showed that *XDab2* was expressed in somites of *Xenopus* embryos and co-localized with the muscle markers *XPax3* and *XMyoD.* Morpholino mediated knockdown of *XDab2* expression in somites down-regulated the expression of these two muscle markers. When C2C12 myoblasts were induced to differentiate into myotubes, Dab2 was simultaneously expressed. If Dab2 expression during myotube formation was suppressed by transfection with Dab2 miRNAs or shRNAs, significantly fewer myotubes and lower myoblast fusion indices were found comparing to myoblasts transfected with the control plasmid. Our results indicated that Dab2 plays an indispensable role in the early development of skeletal muscle.

P-23

Follistatin-like 1 (FSTL1) increases transepithelial resistance in kidney epithelial cells

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Follistatin-like 1 (FSTL1) is a member of the follistatin protein family, characterized by a structure unit found in follistatin, called the FS domain. Studies have suggested that FSTL1 regulates organ tissue formation in embryos and play a role in heart repair in response to harmful stimuli. However, the role of FSTL1 in kidney function and regeneration is unknown.

Here, we show that FSTL1 expression in interstitial cells was dramatically increased in the obstructed kidney compared to the contralateral kidney in mice. Our in vitro studies show that FSTL1 increased transepithelial resistance (TER), a measure of tight junction complexity and function, in mouse inner medullary collecting duct (mIMCD3) cells, while it did not have any effect on mIMCD3 cell proliferation, migration and TGF-β1-induced epithelial-to-mesenchymal transition (EMT). In addition, FSTL1 increased Akt phosphorylation levels in mIMCD3 cells, and inhibition of Akt activity abolished stimulation of TER by FSTL1. Taken together, these results suggest that increased FSTL1 expression in interstitial cells may play a role in renal tubular regeneration following kidney injury.

P-24

CFTR-dependent signaling cascade in reproduction

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The cystic fibrosis transmembrane conductance regulator (CFTR) is known to be a cAMP-activated Cl^- channel. It has also been shown to be involved in HCO_3^- transport in various cell types, either directly or indirectly. While mutations of CFTR are known to cause CF with a wide range of clinical presentations, including lung disease, pancreatic insufficiency and infertility, the importance of the CFTR-medicated HCO_3^- transport in cellular signaling linking to various physiological functions, apart from electrolyte transport, remains largely unexplored. A soluble form of adenylyl cyclase (sAC) is known to be the sensor of HCO_3^- and distributed in a variety of cell types. Our recent studies have found that sAC is also expressed in male and female germ cells, as well as the embryos. A number of reproductive events, including sperm capacitation, estrogen production and embryo development are found to be critically dependent on HCO_3^-, CFTR and sAC. We also investigated the CFTR-HCO_3^- activated signaling pathway downstream of sAC and found that this pathway may trigger different signaling cascades leading to genetic and epigenetic regulation of different reproductive and developmental processes. The CFTR/ HCO_3^-/sAC signaling pathway is also found to cross-talk and modulate FSH receptor-mediated signaling pathway and estrogen production in ovarian granulosa cells. Therefore, it appears that the CFTR-mediated HCO_3^- -dependent pathway may be an important signaling mechanism regulating ovarian functions, defect of which may result in ovarian disorders associated with cystic fibrosis and polycystic ovarian syndrome.

P-25

Differential effects of two missense mutations of Asp-578 in human luteinizing hormone/chorionic gonadotropin receptor (hLHR)

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Constitutive activating mutations of the human luteinizing hormone/chorionic gonadotropin receptor (hLHR) have been associated with familial male-limited precocious puberty (FMPP), a noncentral form of gonadotropin-independent precocious puberty due to abnormal production of testosterone. Recent findings suggested hLHR mutations is also a risk factor for the development of testicular tumors. Two FMPP patients with the Asp578Gly mutation developed Leydig cell neoplasia, while a somatic Asp578His activating mutation of the hLHR was identified in a number of testicular tumor patients. The underlying mechanisms for such phenotypic difference were poorly understood. We hypothesized that hLHR mutations may participate in testicular tumor development by adapting molecular signals unique to each mutation. A MA-10 Leydig cell model was established by stably transfecting with two hLHR mutations, D578G and D578H, to study the potential mutation specific effect in testicular tumor development. By examining gene expression profile using cDNA microarray and systems biology approach, the wild type and two mutants could be distinguished by hierarchical clustering and multi-dimensional scaling analysis. Novel regulatory pathways unique to each mutation were also identified, which consists of 9 networks in D578G and 12 in D578H. Data suggested that c-Myc and c-Src were the key regulators in D578G and D578H mutants, respectively. The involvement of these two factors was confirmed by molecular and functional assays. The results open a new dimension and provide novel explanation for the role of hLHR mutation in testicular tumorigenesis.

P-26

Cellular mechanisms for stimulation of Cl^- secretion in cultured human bronchial epithelial cells by *Cordyceps militaris* extract

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Cordyceps militaris (CM), also known as the caterpillar fungus, is a well-known traditional Chinese medicine that can be artificially cultivated on a large scale. In recent decades, CM extract has been reported to have different biological activities, such as anti-tumor activity (Park et al., 2009) and immuno-modulation (Shin et al., 2010). In this study, 16HBE14o-, a human bronchial epithelial cell line, was used as a model to study the regulation of ion transport by CM water extract (Yue et al. 2008). The 16HBE14o- cells were grown on Transwell-COL membranes until confluent (Wong et al., 2009). The monolayers were mounted in Ussing chambers, in which they were bathed in normal Krebs-Henseleit solution with a basolateral-to-apical Cl^- gradient. An increase in short-circuit current (I_{SC}) was measured by electrophysiological technique.

Apical or basolateral application of CM extract stimulated a dose-dependent increase in I_{SC}. The I_{SC} responses were inhibited by apical pretreatment of 10 μM cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel blocker, $CFTR_{-inh172}$. Apical addition of 300 μM DIDS, a calcium-activated Cl^- channel (CaCC) blocker, suppressed the increase in CM-induced I_{SC} by 50.3% and 40.6% at the apical and basolateral sides, respectively. The apical I_{SC} response was reduced by 38.1% after the basolateral application of 10 μM TRAM-34, an intermediate conductance Ca^{2+}-activated K^+ channel blocker. The basolateral application of chromanol 293B, a cAMP-activated K^+ channel blocker, reduced the I_{SC} response by 44.4%. Furthermore, the CM extract-induced I_{SC} could be significantly inhibited by adenylate cyclase (AC) inhibitor MDL-12033A, PKA inhibitor H-89, and intracellular Ca^{2+} chelator BAPTA-AM. Compared with untreated control epithelia, CM extract stimulated an increase in PKA activity in the treated epithelial cell line when measured by Pep-Tag® non-radioactive cAMP-dependent protein kinase assay (Promega). In conclusion, CM extract stimulated transepithelial Cl^- secretion in 16HBE14o- cells through apical CFTR Cl^- channels or CaCC. The basolateral cAMP- or Ca^{2+}-activated K^+ channels were activated by CM extract to provide a driving force for apical Cl^- secretion. The underlying signal transduction mechanisms involve both AC/cAMP/PKA- and Ca^{2+}-dependent pathways.

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3-year experience on animated courseware study in physiology teaching

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The use of animations is generally viewed as an effective e-learning tool by enabling students to visualize complicated molecular processes with both clearer and graphic-rich features when compared to text or static graphics. The objective of the study is to determine how animations help support students' learning. Two animated courseware namely "General Physiology" and "Cardiovascular Physiology" were developed and provided to a group of undergraduate students who took a physiology course with animations provided as a supplementary learning medium primarily for their self-study at home. Selected animations were also played in lectures to introduce important concepts of the specific areas. Feedback from students about the usefulness and the role of animations for learning was collected through surveys (269 and 320 students were surveyed in 2008-2009 and 2009-2010 respectively) and interviews (12 students in 2009-2010). Positive and encouraging evaluations from students were well received and that provided strong and supportive reasons for continuous improvement of existing courseware and also development of new animated courseware in other physiology subjects.

Pharmacological enhancement of eNOS expression by AVE3085 restores endothelial function in type 2 diabetic db/db mice

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Background- Reduced nitric oxide (NO) bioavailability is associated to diabetes-related vascular complications. The present study investigated whether up-regulation of endothelial NO synthase (eNOS) expression by eNOS enhancer AVE3085 can improve the endothelial dysfunction in type-2 diabetic db/db mouse.

Methods- AVE3085 was added to mouse aortas for overnight culture or was orally administrated to twelve-week-old db/db mice (10 mg/kg/day) for 7 days. Aortas were mounted in myography to study vascular reactivity. Reactive oxygen species (ROS) levels in the vascular wall were determined by DHE staining. Changes of nitric oxide (NO) level in aortic endothelial cells were detected with a DAF fluorescence dye. Protein expression was detected by Western blotting.

Results- Culturing with 1 μM AVE3085 for 18 hours and chronic treatment with AVE3085 improved acetylcholine-induced endothelium-dependent relaxations in aortas of db/db mice and reduced oxidative stress in vascular wall. AVE3085 increased the total eNOS in db/db mouse aortas. Furthermore, AVE3085 potentiated the NO production in cultured endothelial cells isolated from db/db mouse aortas. More importantly, AVE3085 prevented the high glucose (30 mM)-induced impairment of endothelial function and ROS over-production in wild type mice but not in $eNOS^{-/-}$ mice.

Conclusion- The present study provides novel evidence that eNOS transcriptional enhancer AVE3085 improves endothelial function in type 2 diabetic mice through up-regulating eNOS expression and NO production, while reducing ROS over-production. Therapeutic strategy targeting eNOS expression to increase NO bioavailability may serve as a useful tool to combat against vascular dysfunction in diabetes.