School of Biomedical Sciences Research Day 2010

15 June, 2010

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

Welcome Message from the Director of School of Biomedical Sciences

I am most delighted to welcome you to *Research Day 2010* of the School of Biomedical Sciences.

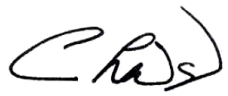
"A journey of a thousand miles starts from where one stands" (千里之行始於足下). We start this journey of making our School an international center of excellence in biomedical research together. Our School had her first year birthday 2 weeks ago. It is the opportune time for us to reflect on what we have achieved together this past year in this "Research Birthday Party" of the School.

With the reorganization and new environment, the commotion among colleagues is understandable. It makes me proud to see aside from those who happened to have prearranged out-of-town trips or other personal reasons which prevent them from attending, we have 100% participation in this event. This truly reflects the dedication of our colleagues towards research excellence.

A party is not a party without relatives and friends. I am most delighted by the participation of a number of our clinical associate members and friends from the Hong Kong University of Science and Technology. Their presence makes this event even more joyous. It is our hope that this event will develop into an interactive platform which facilitates closer and more frequent collaborations between colleagues of the School of Biomedical Sciences, our Associate Members and friends. This is the first ever Research Day. Our goal is to make it the annual flagship event of the School of Biomedical Sciences.

On behalf of all participating members, I would like to thank the hard work of Professor CH Cho and members of the Organizing Committee. Without them, *Research Day 2010* would not have been possible. I am also very grateful for the generous support provided by the various sponsoring companies.

Hope you enjoy the chatting and sharing with friends and colleagues, and the vibrant discussions during the sessions.



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

School of Biomedical Sciences Research Day 2010

Members of the Organizing Committee

Professor Chan Sun On

Professor Chan Wai Yee

Professor Cho Chi Hin

Professor Ko Wing Hung

Professor Tsui Kwok Wing Stephen

Professor Wan Chao

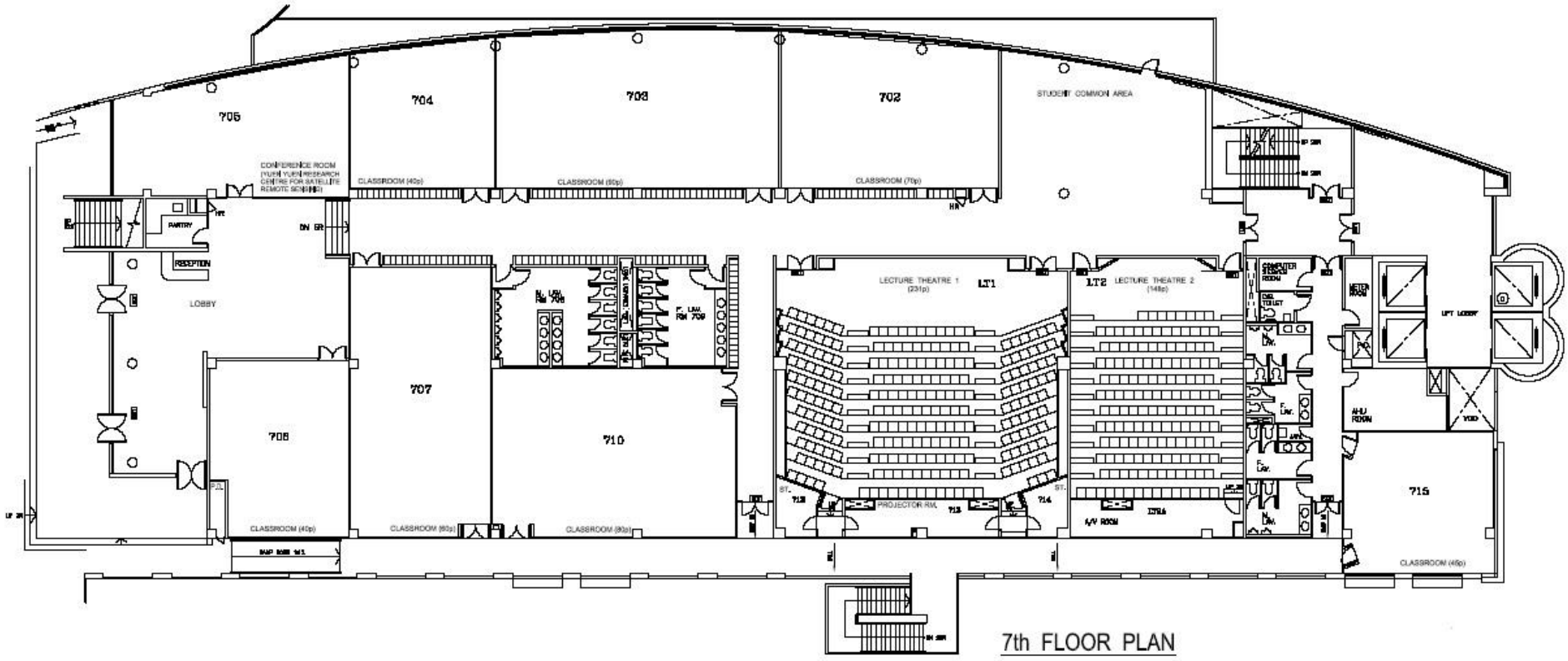
Professor Yao Xiaoqiang

Programme of SBS Research Day

9:15-9:30am Opening Ceremony (Dean and Director)

9:30-10:30	**Stem Cells**	**Receptors & Transporters**	**Cancer**
	Prof. Li Gang	Prof. Chan Hsiao Chang	Prof. Fung Kwok Pui
	Prof. Jiang Xiaohua	Prof. Ko Wing Hung	Prof. Yu Jun
	Prof. Wan Chao	Prof. Wise Helen	Prof. Ng Tzi Bun
	Prof. Lam Fu Yuen Francis	Prof. Yu Siu Bun Sidney	Prof. Chan Leung Franky
10:30-11:15	**Tea Break**		
11:15-12:00	**Reproduction & Endocrinology**	**Neurobiology (1)**	**Drug Toxicity & Drug Development**
	Prof. Cheng Hon Ki Chris	Prof. Waye Miu Yee Mary	Prof. Lin Ge
	Prof. Shum Sau Wun Alisa	Prof. Kwong Wing Hang	Prof. Rudd John A
	Prof. Leung Po Sing	Prof. Cho Yu Pang Eric	Prof. Baum Larry
12:00-12:20	**Photo Session**		
12:30-14:00	**Lunch Break**		
14:00-15:00	**Development**	**Neurobiology (2)**	**Cancer & Drug Targeting**
	Prof. Chan Wai Yee	Prof. Yung Wing Ho	Prof. To Kin Wah
	Dr. Wang Chi Chiu Ronald	Prof. Ke Ya	Prof. Tam Siu Cheung Michael
	Prof. Chan Wood Yee Woody	Prof. Chan Sun On	Prof. Wan Chi Cheong David
	Prof. Zhao Hui	Prof. Yew Tai Wai David	Prof. Cho Chi Hin
15:00-15:45	**Tea Break**		
15:45-16:45	**Bioinformatics and Genetics**	**Vascular Biology**	**Cell Biology and Cancer**
	Prof. Tsui Kwok Wing	Prof. Huang Yu	Prof. Liu Wing Keung Ken
	Prof. Pang Chi Pui Calvin	Prof. Kwan Yiu Wa	Prof. Lau Hang Yung Alaster
	Prof. Ma Ching Wan Ronald	Prof. Yao Xiaoqiang	Prof. Chen Yangchao
			Prof. Mak King Lun Kingston

Map of the Meeting Venue



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

Time	Title of Presentation	Name of Speaker	Abstract No.
09:15-09:30	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		
9:30-10:30	**Stem Cells** **Chairpersons: Prof. Poon Wai Sang & Li Gang**		
09:30-09:45	Targeting tumours using genetically modified mesenchymal stem cells	Prof. Li Gang	S1-1
09:45-10:00	Stem cells as a powerful tool for studying human development and diseases	Prof. Jiang Xiaohua	S1-2
10:00-10:15	Molecular and cellular mechanisms of oxygen sensing in mesenchymal stem cells	Prof. Wan Chao	S1-3
10:15-10:30	A comparative study of human mesenchymal stem cells derived from bone marrow and induced pluripotent stem cells on limb ischaemia in mice	Prof. Lam Fu Yuen Francis	S1-4
10:30-11:15	Coffee break and poster viewing		
11:15-12:00	**Reproduction & Endocrinology** **Chairpersons: Prof. Tang Leung Sang Nelson & Cheng Hon Ki Christopher**		
11:15-11:30	Discovery of a novel IGF in zebrafish: gonad-specific gene expression and role in oocyte maturation	Prof. Cheng Hon Ki Christopher	S1-5
11:30-11:45	Dysregulation of retinoic acid catabolism in diabetic pregnancy increases the risk of birth defects	Prof. Shum Sau Wun Alisa	S1-6
11:45-12:00	Pancreatic research	Prof. Leung Po Sing	S1-7
12:00-12:20	Photo session		
12:20-14:00	Lunch		
14:00-15:00	**Development** **Chairpersons: Prof. Chow King Lau & Chan Wood Yee Woody**		
14:00-14:15	Characterization of differentially methylated genes in normal and abnormal developing male germ cell	Prof. Chan Wai Yee	S1-8
14:15-14:30	Monoamine oxidase A modulates apoptotic neuronal death by controlling serotonin levels during murine embryonic brain development	Dr. Wang Chi Chiu Ronald	S1-9
14:30-14:45	Sacral neural crest cells: A distinct population of migratory cells contributing to enteric ganglia in mouse embryos	Prof. Chan Wood Yee Woody	S1-10
14:45-15:00	Molecular mechanism of early embryonic patterning	Prof. Zhao Hui	S1-11
15:00-15:45	Coffee break and poster viewing		
15:45-16:30	**Bioinformatics & Genetics** **Chairpersons: Prof. Pang Chi Pui Calvin & Tsui Kwok Wing Stephen**		
15:45-16:00	Integrated genomic & bioinformatic platform in the School of Biomedical Sciences of the Chinese University of Hong Kong	Prof. Tsui Kwok Wing Stephen	S1-12
16:00-16:15	Genomic mapping for eye disease genes	Prof. Pang Chi Pui Calvin	S1-13
16:15-16:30	Genetics of diabetes and obesity in Chinese	Prof. Ma Ching Wan Ronald	S1-14

17:00-18:00	Theme-based discussion on RGC-GRF submissions for 2010
18:30	Dinner

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

Time	Title of Presentation	Name of Speaker	Abstract No.
09:15-09:30	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		
09:30-10:30	**Receptors & Transporters** **Chairpersons: Prof. Huang Ping Bo & Chan Hsiao Chang**		
09:30-09:45	From epithelial cells to body function and disease: roles of CFTR	Prof. Chan Hsiao Chang	S2-1
09:45-10:00	$P2Y_6$ receptor-mediated Ca^{2+} and cAMP signaling pathways in cultured human bronchial epithelia, 16HBE14o-	Prof. Ko Wing Hung	S2-2
10:00-10:15	How to control the cell signalling activity of G protein-coupled receptors?	Prof. Wise Helen	S2-3
10:15-10:30	Transport protein particle: one tether, multiple functions	Prof. Yu Siu Bun Sidney	S2-4
10:30-11:15	Coffee break and poster viewing		
11:15-12:00	**Neurobiology (1)** **Chairpersons: Prof. Lam Chiu Wa Linda & He Jufang**		
11:15-11:30	Interaction of the dyslexia candidate protein Kiaa0319-Like with the Nogo Receptor 1 (NgR1)	Prof. Waye Miu Yee Mary	S2-5
11:30-11:45	Observations on the effects of tat protein and methamphetamine on cultured dopaminergic neuroblastoma cells	Prof. Kwong Wing Hang	S2-6
11:45-12:00	Hepatocyte Growth Factor promotes the survival and regeneration of retinal ganglion cells after optic nerve injury	Prof. Cho Yu Pang Eric	S2-7
12:00-12:20	Photo session		
12:20-14:00	Lunch		
14:00-15:00	**Neurobiology (2)** **Chairpersons: Prof. Xia Jun & Yung Wing Ho**		
14:00-14:15	Chronic intermittent hypoxia impairs synaptic plasticity and expression of brain-derived neurotrophic factor in mouse hippocampus	Prof. Yung Wing Ho	S2-8
14:15-14:30	Effect of hepcidin on brain iron homeostasis in iron-overloaded and parkinsonian rats	Prof. Ke Ya	S2-9
14:30-14:45	The role of Nogo on the guidance of commissural axons in the mouse spinal cord	Prof. Chan Sun On	S2-10
14:45-15:00	Some insights into the development, ageing and degeneration on the central nervous system of mammals including human	Prof. Yew Tai Wai David	S2-11
15:00-15:45	Coffee Break and poster viewing		
15:45-16:30	**Vascular Biology** **Chairpersons: Prof. Yip Wai Kwok Gabriel & Huang Yu**		
15:45-16:00	Is hydrogen sulfide important in vascular tone regulation?	Prof. Huang Yu	S2-12
16:00-16:15	Acute simvastatin inhibits K_{ATP} channels of porcine coronary artery myocytes	Prof. Kwan Yiu Wa	S2-13
16:15-16:30	TRPC5 is a pressure-sensor in aortic arch baroreceptor	Prof. Yao Xiaoqiang	S2-14

17:00-18:00	Theme-based discussion on RGC-GRF submissions for 2010
18:30	Dinner

Site 3 Room 703, 7th Floor, Mong Man Wai Building

Time	Title of Presentation	Name of Speaker	Abstract No.
09:15-09:30	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		
9:30-10:30	**Cancer** **Chairpersons: Prof. Lai Bo San Paul & Chan Leung Franky**		
09:30-09:45	Traditional Chinese Medicines in the treatment of cancer	Prof. Fung Kwok Pui	S3-1
09:45-10:00	Epigenetic inactivation of paired box gene 5 is associated with poor prognosis in gastric cancer patients	Prof. Yu Jun	S3-2
10:00-10:15	Apoptosis in breast cancer cells induced by French Bean hemagglutinin	Prof. Ng Tzi Bun	S3-3
10:15-10:30	Orphan nuclear receptors, estrogen-related receptors, in prostate cancer	Prof. Chan Leung Franky	S3-4
10:30-11:15	Coffee break and poster viewing		
11:15-12:00	**Drug Toxicity & Development** **Chairpersons: Prof. Chan Chung Ngor Juliana & Rudd John A**		
11:15-11:30	Cytochrome P450-mediated metabolic activation of pyrrolizidine alkaloids and biomarker for the assessment of pyrrolizidine alkaloids-induced hepatotoxicity	Prof. Lin Ge	S3-5
11:30-11:45	Drug responsiveness of the GI tract in the senescence-accelerated mouse	Prof. Rudd John A	S3-6
11:45-12:00	Testing drugs in transgenic mouse models of Alzheimer's disease and tauopathies	Prof. Baum Larry	S3-7
12:00-12:20	Photo session		
12:20-14:00	Lunch		
14:00-15:00	**Cancer & Drug Targeting** **Chairpersons: Prof. Poon Randy & Fung Kwok Pui**		
14:00-14:15	HuR: a novel molecular target for modulating ABCG2-mediated multidrug resistance in cancer chemotherapy	Prof. To Kin Wah	S3-8
14:15-14:30	The antiviral mechanism of trichosanthin	Prof. Tam Siu Cheung Michael	S3-9
14:30-14:45	Characterization of HIV-1 integrase nuclear translocation for the development of new class of anti-AIDS drugs	Prof. Wan Chi Cheong David	S3-10
14:45-15:00	Peptides targeting tumors for imaging and anti-cancer drug delivery	Prof. Cho Chi Hin	S3-11
15:00-15:45	Coffee Break and poster viewing		
15:45-16:45	**Cell Biology & Cancer** **Chairpersons: Prof. Ng Siu Man Simon & Liu Wing Keung Ken**		
15:45-16:00	Organotypic cultures for skin research	Prof. Liu Wing Keung Ken	S3-12
16:00-16:15	Regulation of human mast cells	Prof. Lau Hang Yung Alaster	S3-13
16:15-16:30	Functional study of cancer related genes by RNAi	Prof. Chen Yangchao	S3-14
16:30-16:45	Hedgehog signaling potentiates the development of osteosarcoma	Prof. Mak King Lun Kingston	S3-15

17:00-18:00	Theme-based Discussion on RGC-GRF submissions for 2010
18:30	Dinner

Abstracts

S1-1

Targeting tumours using genetically modified mesenchymal stem cells

C. Song, J.W. Zhou and G. Li

Stem Cell and Regeneration Program, School of Biomedical Sciences, Li Ka Shing Institute of Health Sciences and Department of Orthopaedics and Traumatology, Faculty of Medicine, Chinese University of Hong Kong.

Mesenchymal stem cells (MSCs) are capable of homing to many tissues after injurious insults and participate in the healing processes. MSCs interact with tumour cells and can specifically home to tumours. We investigated the distribution of systemically delivered MSCs in tumor bearing animals and evaluated the anti-tumour effects of HSV-TK (herpes simplex virus thymidine kinase) gene modified MSCs. Rat MSCs were transfected with luciferase or HSV-TK gene by lentiviral vector. PC3 or RIF-1 cells were used to establish tumor bearing animal models in nude (intra-dermal tumor) or C3H-HEN (lung metastasis model) mice. $1x10^6$ luciferase-MSCs were injected through tail vein into the tumour bearing animals. The distributions of luciferase-MSCs were confirmed using in vivo image system (IVIS 200, Xenogen, USA). To test the effect of systemic administrated HSV-TK-MSCs on tumor growth, Luciferase-PC3 intra-dermal model and luciferase-RIF-1 lung metastasis model were injected intravenously with $1x10^6$ HSV-TK-MSCs twice together with the pro-drug GCV. Tumor size were measured and compared. 72 hours after luciferase-MSCs injection, MSCs were found in the tumor sites and they were present till 30 days after. In the HSV-TK experimental group, significant inhibition of tumor growth of 41.27% was observed in PC3 subcutaneous tumor model at day 25, and of 98.44% in RIF-1 lung metastasis model at day 27. No obvious side effect was observed in the animals received MSCs-TK/GCV treatment. MSCs have the capability to home to the tumour microenvironments. MSCs-HSV-TK, with its expression is controlled by pro-drug, may be used for site and time specific anti-cancer gene therapy.

S1-2

Stem cells as a powerful tool for studying human development and diseases

X.H. Jiang

School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong

Although model organisms have been valuable for studying human disease, the cellular and physiological differences between model organisms and human have made it increasingly important to develop more relevant human disease models for mechanistic studies. Human embryonic stem cells (hESC) hold great promise as sources of tissue for regenerative medicine and therapeutics. In addition, their utility as tools to study the origins and biology of human disease must not be underestimated. HESC give rise to tissue-specific adult stem cells and, ultimately, to all mature tissues in the body. Disruptions to normal stem cell function can have catastrophic consequences and result in life-threatening or devastating disease. Understanding how such diseases arise will afford novel insights into how we can better prevent and treat them.

We harness the power of *in vitro* differentiated hESC to investigate the function of an oncogene, EWS-FLI1 in neural crest development and oncogenesis and, in so doing, provided unique and novel insights into how deregulated-oncogene can disrupt normal human development. We would also like to present the data showing how we use human mesenchymal stem cells to study stem cell aging and their improved efficacy in cell based therapy for CNS injury.

Molecular and cellular mechanisms of oxygen sensing in mesenchymal stem cells

C. Wan

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

During organogenesis low oxygen tension influences proliferation and differentiation of stem/progenitor cell populations. Hypoxia is a major stimulus for initiation of the angiogenic cascade during organogenesis and tissue regeneration. Our recent studies show that hypoxia inducible factor-1α (HIF-1α) is an essential mediator of the osteoblast response to hypoxia and serves to couple angiogenesis to osteogenesis. Overexpression of HIF-1α in mature osteoblasts produces marked changes in the vascularization and formation of long bones but did not influence development of flat bones of the skull. This suggests a different relationship between angiogenesis and osteogenesis, and unique osteoblast development at this skeletal site. Flat bones of the skull and long bones form by two different mechanisms. Long bones form through an endochondral process where messenchymal cells condense and form a cartilage anlagen, while flat bones of the skull form through a process called intramembranous bone formation. Whether and to what extent HIF-1α might also function in early cranial bone development remains a question. To investigate the requirement of HIF-1α in early cranial bone development we used the Cre/loxp strategy to generate conditional deletion of HIF-1α in condensing mesenchyme. Immunohistochemical analysis of cranial bones confirmed complete loss of HIF-1α immunoreactivity in the Cre expressing skulls. And deletion of HIF-1α impairs cranial bone development. Further studies are under way to characterize the phenotypes of the mutant mice and define the role of HIF-1α in regulating mesenchymal stem cell function.

A comparative study of human mesenchymal stem cells derived from bone marrow and induced pluripotent stem cells on limb ischaemia in mice

F.F.Y. Lam[1], Q. Lian[2], Y. Zhang[2], J. Zhang[3], H.K. Zhang[4], X. Wu[2], Y. Zhang[2], S. Kang[2], J. C. Xia[5]; W.H. Lai[2], K.W. Au[2], Y.Y. Chow[2], C.W. Siu[2], C.N. Lee[6] and H.F. Tse[7]

[1]School of Biomedical Sciences, The Chinese University of Hong Kong, Shatin, Hong Kong, China. [2]Cardiology Division, Department of Medicine, University of Hong Kong, Hong Kong, China; [3]Institute of Medical Biology, Singapore; [4]Central Laboratory of Shenzhen Maternity and Child Healthcare Hospital, Shenzhen, China; [5]State Key Laboratory of Oncology in Southern China, Guangzhou, China; and [6]Department of Surgery, National University Hospital, National University of Singapore, Singapore. [7]Research Centre of Heart, Brain, Hormone, and Healthy Aging, University of Hong Kong, Hong Kong, China

Despite the initial encouraging results of preclinical and clinical studies, mesenchymal stem cells (MSCs) derived from adult tissue such as bone marrow have a limited proliferation and differentiation potential for tissue repair in ischaemic disease. More important, aging significantly impairs their survival and differentiation potential and thus limits their therapeutic efficacy. Recent breakthrough in the generation of induced pluripotent stem cells (iPSCs) offers the possibility to obtain a high yield of patient-specific MSCs. This study aimed to compare the effects of MSCs derived from human bone marrow (BM-MSCs) and iPSCs (iPSC-MSCs) on limb ischaemia in mice. Adult human BM-MSCs were purchased from Cambrex Bioscience (Rockland, USA) and human iPSC-MSCs were generated by Dr. Qizhou Lian (Hong Kong University). Limb ischaemia was induced by ligation of femoral artery in severe combined immunodeficient (SCID) mice. Intramuscular injection of BM-MSCs or iPSC-MSCs significantly attenuated severe hind-limb ischaemia, and promoted myogenesis and neovascularization. The benefits of iPSC-MSCs on limb ischaemia were superior to those of BM-MSCs, which may be attributed to iPSC-MSCs being more robust in enhancing vascular and muscle regeneration via direct de novo vascular differentiation and paracrine mechanisms. This study confirmed that functional MSCs can be generated from human iPSCs and they have greater potential than BM-MSCs for treatment of ischaemic diseases.

Discovery of a novel IGF in zebrafish: gonad-specific gene expression and role in oocyte maturation

J.Z. Li, Z.H. Liu, P. Zhu, K.W.Y. Sham and C.H.K. Cheng

School of Biomedical Sciences, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong.

The insulin-like growth factor (IGF) system plays an important role in reproduction. Distinct from the conventional IGFs (IGF-1 and IGF-2), a novel IGF (herein called IGF-3) encoded by a separate gene was identified in zebrafish. Using RT-PCR and *in situ* hybridization, the expression of this novel IGF-3 was found to be gonad-specific and starts early in zebrafish development. Using a specific antibody developed for zebrafish IGF-3, the IGF-3 protein was confirmed to be expressed in the zebrafish gonads only with a very high expression in the ovary. Real-time PCR and *in situ* hybridization revealed that the *igf-3* mRNA is relatively low in early follicles but is significantly increased after the mid-vitellogenic stage (midstage III) and is high in fully grown follicles. Within the follicles, *igf-3* mRNA was detected in both the oocyte and follicular cells. Intriguingly, IGF-3 immunoreactivity was confined to the follicular cells during development of the follicles. The expression of *igf-3* was upregulated upon treatment with human chorionic gonadotropins in both ovarian fragments and isolated follicles in dose- and time-dependent manners. Incubation of follicles with recombinant zebrafish IGF-3 significantly enhanced the final maturation of follicle-enclosed oocytes *in vitro* in time-, dose- and stage-dependent manners. These results suggest a significant role of IGF-3 in zebrafish ovary, especially in oocyte maturation.

Dysregulation of retinoic acid catabolism in diabetic pregnancy increases the risk of birth defects

L.M.Y. Lee[1], P. McCaffery[2], A.J. Copp[3], R.C.C. Wang[1,4] and A.S.W. Shum[1]

[1]School of Biomedical Sciences, The Chinese University of Hong Kong, HK; [2]Institute of Medical Sciences, University of Aberdeen, UK; [3]Institute of Child Health, University College London, UK; [4]Department of Obstetrics & Gynaecology, The Chinese University of Hong Kong, HK.

Pregnancies complicated by maternal diabetes are associated with an increased rate of birth defects. Interestingly, alterations in the levels of vitamin A, or its bioactive metabolite all-*trans* retinoic acid (RA), give rise to developmental abnormalities that are strikingly similar to those observed in diabetic pregnancies. This observation prompts us to hypothesis that vitamin A, RA and their downstream effectors are involved in diabetic embryopathy. As a key signaling molecule in embryonic development, the concentration of RA is tightly regulated, with both excess and deficiency leading to malformation. One of the key mechanisms to control RA concentrations is via catabolic inactivation.

Using the mouse as a model system, we show that the main RA catabolizing enzyme *Cyp26a1*, which is expressed in specific tissues to protect against inappropriate exposure to RA, is significantly down-regulated in embryos of diabetic pregnancy. Concomitantly, the tissues have reduced ability to inactivate RA, and are more susceptible to the teratogenic effect of aberrant concentrations of RA. Comparison of mutant embryos with haploinsufficiency of *Cyp26a1* and their wild-type littermates in diabetic and non-diabetic pregnancies show that there is a direct correlation between *Cyp26a1* expression levels, RA inactivating ability and susceptibility to RA teratogenicity. When *Cyp26a1* expression levels were normalized, the increased susceptibility to RA teratogenicity caused by diabetic pregnancy was abolished. Taken together, our findings support that dysregulation of RA catabolism via down-regulation of *Cyp26a1* increases the risk of anomalies in diabetic pregnancy. This discovery of the involvement of retinoid pathway in diabetic embryopathy may lead to the design of preventive measures to reduce the risk of birth defects other than controlling blood glucose level.

Pancreatic research

P.S. Leung

Program in Reproduction, Development and Endocrinology, School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong

The human pancreas consists of two organs in one: the exocrine gland consisting of acinar cells and duct cells, and the endocrine gland consisting of islet cells (e.g. beta-cells). While the physiological role of exocrine pancreas is to secrete digestive enzymes for digestion and absorption, the endocrine pancreas is to secrete islet hormones, such as insulin, for glucose homeostasis. The pancreatic functions are regulated by neurocrine, endocrine, paracrine and/or intracrine mechanisms; derangement of these regulatory pathways thus has negative impacts on our health and disease, notably diabetes. The underlying mechanisms whereby pancreatic functions are modulated have yet to be elucidated.

Pancreatic islets are central to current and future treatment of diabetes that include islet transplantation, pharmacological stimulation of insulin secretion and sensitivity, as well as pancreatic stem cell and regeneration approaches. While investigations into the islet cell biology provide extensive, as yet unavailable data, elucidation of the developmental biology of islet cell and pancreatic stem cell is critical for basic and clinical diabetic research. This knowledge will provide functionally-driven islet cells for clinical transplantation as well as establishing a platform for researchers and pharmaceutical companies to study and formulate novel drugs for diabetes and metabolic syndrome. As such, we have established a pancreatic research, particularly focusing on islet cell biology; these research areas are (i) Islet cell function and diabetes; (ii) Intestinal glucose uptake and diabetes; (iii) Pancreatic stem cells and islet cell development; (iv) Acinar cell function and pancreatic inflammation; and (v) Natural products as an alternative to treating pancreatic inflammation. Our pancreatic research should open up an innovative approach to the management of diabetes.

S1-8

Characterization of differentially methylated genes in normal and abnormal developing male germ cell.

H.H. Cheung and W.Y. Chan

Program in Reproduction, Development and Endocrinology, School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China.

DNA methylation is known to affect chromatin structure, genomic stability and/or altered transcriptional activity. Aberrant DNA methylation is known to be a hallmark of tumorigenesis, particularly in testicular germ cell tumors. In order to understand the various genes that regulate male germ cell development and whose abnormal regulation leads to testicular tumorigenesis, we compared global DNA methylation between normal and testicular tumor cells using methylated DNA immunoprecipitation and tiling array hybridization (MeDIP-chip). A high resolution cytosine methylation map of human normal testicular and germ cell cancer (TGCT) cells was obtained. Promoter methylation of annotated genes accounted for ~9% in both cases. About 1/3 (27%) of these genes demonstrated the perceived relationship between promoter methylation and gene expression, i.e. hypermethylation associated with suppression of gene expression. Comparison of the methylaiton map of normal and TGCT cells yielded more than 6000 differential methylated regions (DMRs). More than 70% of DMRs resided in intergenic regions. A focal analysis of DMRs located in the regulatory regions of annotated genes yielded 207 differentially methylated genes. Four candidate genes were selected for further characterization. These four genes were Apolipoprotein L domain containing 1 (*APOLD1*), Retrotransposon gag domain containing 1(*RGAG1*), Protocadherin 10 (*PCDH10*), and *Zswim2*. The function of the protein product of these genes is not known, with the exception of *PCDH10* which encodes a membrane protein for cell adhesion. Expression and functional analyses of these genes showed that they may play important roles in developing male germ cells. Aberrant methylation leading to abnormal expression of these genes could a cause of testicular tumor.

Monoamine oxidase A modulates apoptotic neuronal death by controlling serotonin levels during murine embryonic brain development

C.C. Wang[1,2,3], A. Borchert[4], A. Ugun-Klusek[5], L.Y. Tang[1], W.T. Lui[1], C.Y. Chu[1], E. Billett[5], H. Kuhn[4] and C. Ufer[4,5]

[1]Department of Obstetrics and Gynaecology; [2]Li Ka Shing Institute of Health Sciences, and [3]Neuro-degeneration and -development and Repair, Institute of Biomedical Sciences, The Chinese University of Hong Kong, Shatin, Hong Kong; [4]Institute of Biochemistry, University Medicine Berlin - Charité, Monbijoustr. 2, D-10117 Berlin, Germany; [5]School of Science and Technology, Nottingham Trent University, Clifton Lane, Nottingham, NG11 8NS, United Kingdom

Monoamine oxidases (MAO-A, MAO-B) metabolize biogenic amines and have been implicated in neuronal apoptosis. Unfortunately, the role of these enzymes in cerebral embryogenesis has not been investigated in detail. We found that MAO isoforms are expressed in mouse embryos as early as E6.5 and that expression silencing of MAO-A during in vitro embryogenesis induced developmental defects in the brain, which were paralleled by elevated serotonin levels. Similar abnormalities were observed when embryos were cultured in vitro in the presence of a MAO-A inhibitor or exogenous serotonin and when a transcriptional silencer of endogenous MAO-A expression was overexpressed. Inhibition of embryonic serotonin biosynthesis as well as interference with serotonin signaling rescued the knockdown phenotype. In the brain of MAO-A deficient embryos the number of apoptotic cells was reduced and this data was confirmed by impaired caspase-3/9 activation. These alterations were prevented by inhibition of embryonic serotonin biosynthesis suggesting a crucial role of serotonin as mediator in apoptotic signaling. Elevated serotonin levels activated the protein kinase ERK, which has previously been implicated in serotonin-induced apoptosis. Taken together this data indicates that embryonic knockdown of MAO-A expression impairs serotonine dependent developmental apoptosis by preventing the intrinsic path of cellular suicide.

Sacral neural crest cells: A distinct population of migratory cells contributing to enteric ganglia in mouse embryos

X. Wang, L. Bao and W.Y. Chan

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In infants with Hirschsprung's disease (HSCR), also known as congenital megacolon, part of the colon is dilated because of the regional absence or reduction of enteric ganglia. It is known that all enteric ganglia are derived from neural crest cells (NCCs) during embryonic development, and therefore HSCR is regarded as a developmental disorder. Our previous studies indicated that NCCs at the sacral level contribute a significant number of enteric ganglia to the hindgut, where aganglionosis is usually detected in HSCR patients. In this study, we aimed to determine the migration and roles of sacral NCCs in normal and mutant embryos with HSCR. We found in normal mouse embryos that (1) sacral NCCs took about 4 days to migrate from the neural tube to the hindgut; (2) sacral NCCs exhibited distinct migratory behaviours and migrated in a caudal-to-rostral manner within the hindgut to give rise to enteric neurons and glia; and (3) neural crest stem cells with self-renewal and multi-potency properties could be isolated from the E14.5 gut tube. In an animal model of HSCR, we also found that (4) migration of sacral NCCs was severely affected. The possibility of restoring the normal development of enteric ganglia in the mutant hindgut will be explored using neural crest stem cells.

Molecular mechanism of early embryonic patterning

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Our laboratory is engaged in studies of molecular-genetic mechanisms of early vertebrate development, using the frog *Xenopus laevis* as experimental system. Currently, two major projects are being carried out in my lab.

Function of Lrig3 in regulating receptor tyrosine kinase

The receptor tyrosine kinase constitute of high-affinity cell surface receptors for many polypeptide growth factors, cytokines, and hormones, which regulate many different developmental and physiological processes, and mutations are associated with several conditions in humans. Previously, we reported the isolation of a novel inhibitor of FGF signalling pathway named Lrig3. Lrig3 is a transmembrane protein, which is enriched in the organizer during the gastrulation. Lrig3 binds to FGFR1 and attenuate the FGF pathway through an unknown mechanism. In addition to FGF signalling, we found Lrig3 can bind to the EGFR. During the embryonic development, Lrig3 enhance EGF signalling perhaps through the PI3 kinase cascade.

The role of dhrs3 in early embryonic development

During early embryonic development, the retinoic acid signaling pathway coordinates with other signaling pathways to regulate body axis patterning and organogenesis. Through DNA microarrays, we have identified a gene called *short-chain dehydrogenase/reductase member 3* (*dhrs3*), which was activated in the organizer at the on set of gastrulation. By gain-of-function and loss-of-function studies, we found dhrs3 regulates embryonic patterning by mediating the production of retinoic acid. Our data suggest that dhrs3 converts all-*trans*-retinal to all-trans-retinol (vitamin A) to reduce the level of retinoic acid in developing embryos.

S1-12

Integrated genomic & bioinformatic platform in the School of Biomedical Sciences of The Chinese University of Hong Kong

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With the emergence of high-throughput genome sequencers from various companies including Applied Biosystems, Illumina and Roche, the conventional approach of genomic investigation has been revolutionized and new applications involving these sequencers have been designed and adopted not only in genome centres, but also in general laboratories working on biological and biomedical research. These genome sequencers are capable of generating terabytes of raw data daily, posing a big challenge to various aspects of bioinformatics and computational biology, e.g. data quality assurance, data storage, cross platform data analysis standards, data comparison, data annotation and gene network analysis. In this talk, the integrated framework and workflow in the Centre for Microbial Genomics & Proteomics and the Hong Kong Bioinformatics Centre will be introduced. Moreover, results of some of the projects conducted in these two centres will be presented.

Genomic mapping for eye disease genes

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High throughput technologies, information from the Human Genome and HapMap Projects, and powerful data analysis programs have led to identification of genetic risk factors for many eye diseases. We have searched for genes that implicated in major genetic eye diseases including primary open-angle glaucoma (POAG), age-related macular degeneration (AMD), and high myopia by using whole genome linkage and association approach, resequencing, and microarray analysis. In POAG we have established chromosome regions 5q22.1-q32 (*GLC1M*) and 15q22-24 (*GLC1N*) as independent linkage loci for juvenile-onset POAG. Interactive analysis has supported the monogenic and polygenic nature of POAG. And our microarray analysis has identified genes for ocular hypertension. For high myopia our whole-genome linkage analysis with fine mapping has rendered identification of critical regions, with one narrowed region at 12q21.31-12q22 overlapping with the known locus *MYP3*, and one novel locus at 5p15.33-15.2 spanning 17.45cM. For exudative AMD we completed a genome-wide association study and identified a promoter polymorphism rs11200638 in *HTRA1* conferring strong risk to the disease and acting additively with the *CFH* rs800292 (Val62Ile) to confer a 23.3-fold of increased risk and a population attributable risk of 78%. Our results largely enriched the knowledge of the genetic architectures of these common eye disorders.

Genetics of diabetes and obesity in Chinese

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Asia is in the midst of an epidemic of diabetes. Diabetes develops at a younger age in Asian populations than white populations, hence the morbidity and mortality associated with diabetes and its complications are also common in young Asian people. There have been much recent advances in our understanding of the genetic basis of type 2 diabetes. Large-scale genome-wide association studies, predominantly in European populations, have led to the identification of several genetic variants associated with type 2 diabetes. Our studies in Chinese suggest that the same genetic factors are also implicated in Asians, though ethnic differences in the frequency of risk variants result in differences in population-attributable risk, highlighting the need for population-specific studies. Identification of genetic predictors of type 2 diabetes has highlighted the possibility of prevention in high-risk individuals. At present, prediction of diabetes using identified genetic factors alone is marginally superior compared to prediction using clinical factors alone. Most currently identified type 2 diabetes genes are implicated in pancreatic beta-cell function, with surprisingly little overlap with identified obesity genes. Using the Hong Kong Diabetes Registry, a prospective cohort of more than 7000 patients with diabetes, we have also identified several genetic factors implicated in the development of diabetic complications. The identification of at-risk individuals can facilitate structured care delivery and intensive treatment to achieve blood pressure, glycaemic and lipid targets in order to reduce the progression of microvascular complications as well as cardio-renal end-points.

From epithelial cells to body function and disease: roles of CFTR

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The epithelial linings of various vital organs, including the airways, GI tract and reproductive tracts, are not merely a barrier protecting the internal environment but play important roles in regulating and/or modulating the physiological functions of various organs, defect of which could have pathological consequences. The importance of epithelial cells can be highlighted by cystic fibrosis, a disease with a multitude of clinical manifestations, which is caused by mutations of a gene encoding an epithelial cell anion channel, the cystic fibrosis transmembrane conductance regulator (CFTR). Our research aims to understand how epithelial cells affect body functions and disease processes. Our findings show that CFTR plays a vital role in regulating the cellular processes of a wide array of epithelial cells, affecting many organ functions and disease processes, including infertility, infection and cancer.

$P2Y_6$ receptor-mediated Ca^{2+} and cAMP signaling pathways in cultured human bronchial epithelia, 16HBE14o-

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Apical and/or basolateral membranes of polarized epithelia express P2Y receptors which regulate the transport of fluids and electrolytes. In the airway, P2Y receptors modulate Cl^- secretion through the phospholipase C (PLC) and calcium signaling pathways. However, the presence and function of apical versus basolateral $P2Y_6$ receptors in airway epithelium remains controversial. Recent evidence suggests that $P2Y_6$ receptors are coupled to the cAMP/PKA pathways. In this study, we examined P2Y receptor subtype expression and the effect of $P2Y_6$ receptor agonist, UDP, on basal short-circuit current (I_{SC}), real-time changes in intracellular calcium ($[Ca^{2+}]_i$) and cAMP concentration in a human bronchial epithelial cell line (16HBE14o-). Real-time PCR and Western blot analysis demonstrated $P2Y_1$, $P2Y_2$, $P2Y_4$ and $P2Y_6$ receptor expression. Using a simultaneous measurement technique of bioelectric and fluorescent signals, it was found that UDP stimulated a concomitant increase in I_{SC} and $[Ca^{2+}]_i$ in a concentration-dependent manner. Real-time cAMP changes in living cells were monitored by using CFP-Epac-YFP, an Epac-based polypeptide FRET reporter. The cAMP elevating agent, forskolin, or UDP evoked an increase in CFP/FRET emission ratio, which indicated an increase in intracellular cAMP level. These data suggest that activation of $P2Y_6$ receptors by UDP stimulates real-time increase in $[Ca^{2+}]_i$ and [cAMP].

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How to control the cell signalling activity of G protein-coupled receptors

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While classical pharmacological approaches to drug development tend to focus on developing more potent and specific receptor agonists and antagonists, an alternative approach is to influence factors regulating production and cell surface expression of G protein-coupled receptors (GPCRs). Here we provide two examples of factors affecting cell surface expression of GPCRs:

(1) Having discovered that the ghrelin receptor polypeptide (GHS-R1b) behaves as a dominant-negative mutant of the appetite-stimulating ghrelin receptor (GHS-R1a), we have now shown that GHS-R1b forms heterodimers with GHS-R1a and thus traps it within the endoplasmic reticulum. Therefore, GHS-R1a cannot progress through the Golgi apparatus, and cell surface expression of GHS-R1a is decreased. Given that GHS-R1a is a constitutively active GPCR, then factors elevating GHS-R1b production will attenuate GHS-R1a-dependent signalling and affect growth.

(2) GPCRs can be regulated by cell-cell contact in a receptor-specific manner. Inflammatory stimuli such as prostaglandin E_2 (PGE_2) and prostacyclin are well known as hyperalgesic agents, capable of intensifying pain sensations mediated through activation of Gs-coupled EP4 and IP receptors in the dorsal root ganglia (DRG). Using cells isolated from adult rat DRG, we have discovered that the glial cells in DRG cultures also express EP4 and IP receptors and are far more responsive than DRG neurons with regard to activation of adenylyl cyclase. However, when DRG glial cells are co-cultured with DRG neurons, their ability to respond to EP4 and IP receptor agonists is significantly attenuated. This contact-dependent interaction between neurons and glial cells is not a universal response, since glial cell responses to other Gs-receptor agonists (e.g., β2-adrenoceptor agonists) and receptor-independent activators of adenylyl cyclase (e.g., forskolin) are not inhibited by DRG neurons. In addition, the absence of neurons generates an activated phenotype in the glial cells reminiscent of glial cell activation observed *in vivo* following nerve injury. In the future, we hope to determine the physiological relevance and the mechanisms underlying the inhibitory effect of DRG neurons on EP4 and IP receptor-dependent activity in DRG glial cells.

Transport protein particle: One tether, multiple functions

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In eukaryotic cells, the secretion of most hormones and growth factors to the outside of the cells is carried out by small transport vesicles. Before these transport vesicles deliver protein to the cell surface or other membrane compartments, the vesicles need to be tethered to the correct place. A protein complex called TRAPP (Transport protein particle) has been found to be important for the vesicle tethering between the Endoplasmic Reticulum and the Golgi complex. As TRAPP is one of the important cellular machineries that control the secretion of protein hormones and other important proteins, genetic defects that severely hamper the functions of TRAPP are lethal. Mild defects usually cause a variety of diseases as exemplified by the mutations in TRAPP subunit, Trs20, in patients with recessive X-linked spondyloepiphyseal dysplasia tarda (SEDT). Recently, mutations causing defect in neurodevelopment, and hence, mental retardation, have been identified in human TRAPP subunit Trs120. These results further reinforce the idea that vesicle tethering by TRAPP is involved in many fundamentally important physiological functions.

S2-5

Interaction of the dyslexia candidate protein Kiaa0319-Like with the Nogo Receptor 1 (NgR1)

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Developmental dyslexia is a neurobehavioral disorder affecting 5-10% of school age children. KIAA0319-LIKE is a novel gene recently identified as a candidate gene located at the susceptibility loci 1p34 with unknown biological function. To identify the putative function of Kiaa0319-LIKE, we performed a yeast -2-hybrid screen for potential interacting partners and found that the axon guidance receptor, the Nogo Receptor 1 (NgR1) interacts with Kiaa0319L. Anti-KL antibody was used successfully in co-precipitating the endogenous Kiaa0319L and the endogenous NgR1 protein from adult mouse brain lysate. Similar result can be observed by overexpressing Kiaa0319L and NgR1 protein in Human Embryonic Kidney cells. We have thus provided evidence for the physical interaction between Kiaa0319L and NgR1 which is an important axon guidance molecule involved in inhibition of axon regeneration in the central nervous system.

Observations on the effects of tat protein and methamphetamine on cultured dopaminergic neuroblastoma cells

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The HIV-1 trans-activator tat protein, apart from its crucial role in the viral replication, is known to produce a multitude of cytotoxic effects, both within and outside the affect cells. Methamphetamine (MA) disrupts the vesicular storage of DA, raising the cytosolic DA concentration, and hence the concentration of its toxic metabolic products[1]. Recent publications suggest that Tat and MA together result in greater neuronal damage than when administered separately,[1,3] and it has been suggested that they act through the glial cells.[2,3] It is not clear whether Tat and MA have a direct synergistic effect on neurons and what organelles are involved. This study aimed to observe the effects of these two molecules on cultured SH-SY5Y neuroblastoma dopaminergic cells. The cells were incubated with Tat (100 nM), MA (4 mM), or Tat+MA for 24 to 48 hours, and then harvested for electron microscopy, fluorescent microscopy, and Western blotting study. EM showed many autophagic vacuoles of different morphologies in the MA and MA+Tat treated cells, but not in the Tat cells. MTT method showed significant reduction in cell viability only after MA+Tat treatment. Fluorescent signals for cell injuries, namely TUNEL, JC-1, cytoplasmic cytochrome C, as well as cleaved caspase 3 activity, were much stronger in the MA+Tat cultures than in the MA and Tat cultures. The MA and Tat induced cell death was not extensive. The autophagic vacuoles in the MA-treated cells might not necessarily signify that the cells were near their terminal stage. The combined effect of both molecules leads to extensive cell death, and apoptosis is a possible pathway for this event.

References:

1. Pugliese A, Vidotto V, Beltramo1 T, Petrini S, Torre D. 2005. A review of HIV-1 Tat protein biological effects. Cell Biochem Funct. 23: 223–7.
2. Thomas DM, Kuhn DM. 2005. MK-801 and dextromethorphan block microglial activation and protect against methamphetamine-induced neurotoxicity. Brain Res. 1050:190-8.
3. Theodore S, Cass WA, Nath A, Maragos WF. 2007. Progress in understanding basal ganglia dysfunction as a common target for methamphetamine abuse and HIV-1 neurodegeneration. Curr HIV Res. 5:301-13.

Hepatocyte growth factor promotes the survival and regeneration of retinal ganglion cells after optic nerve injury

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Injury of the adult central nervous system carries a poor prognosis due to the limited survival and regeneration of damaged neurons. Both intrinsic constraints within neurons to mount an appropriate regenerative response, and the abundance of growth-inhibitory molecules elaborated by glial cells, are responsible for the negative outcome. One of the strategies to combat these negative factors is to supply neurotrophic factors to boost up the neurons' intrinsic growth machinery and to neutralize the effects of glial-derived inhibitors. Many neurotrophic factors are, however, quite specific in terms of their signaling actions as well as target cell specificities, such that it is difficult to satisfy the diverse requirements of multitude neuronal populations. It has been found that hepatocyte growth factor (HGF), a protein originally known for its ability to stimulate the growth of hepatocytes and other tissues, possesses the ability to support the differentiation and growth of the developing nervous system. Because of the remarkable ability of HGF to influence diverse neuronal types, it may be a good candidate to target damaged neurons in different parts of the nervous system. We report on the efficacy of HGF in promoting the survival of retinal ganglion cells after optic nerve injury, as well as its ability to stimulate damaged axons to regenerate. HGF was able to promote ganglion cell survival for a longer time period than brain-derived neurotrophic factor, one of the most potent survival-promoting factors for ganglion cells identified so far. In a model of grafting a peripheral nerve to the cut optic nerve to induce axonal regeneration, HGF stimulated more ganglion cell axons to regenerate into the graft compared to the control. Understanding the mechanisms by which HGF promotes ganglion cell regeneration will provide a basis for testing its effect on other regions of the injured central nervous system, as well as in other eye diseases like glaucoma and retinal ischemia.

S2-8

Chronic intermittent hypoxia impairs synaptic plasticity and expression of brain-derived neurotrophic factor in mouse hippocampus

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Obstructive sleep apnea (OSA) is a common sleep disorder, characterized by repeated episodes of airway obstruction during sleep resulting in intermittent hypoxemia. Previous studies found that OSA causes neurocognitive deficits including impairment in perception and memory. It is well known that learning and memory involves a cellular process known as long-term potentiation (LTP). In this study, we examined the effect of intermittent hypoxia (IH) on the magnitude of early phase LTP (E-LTP) in hippocampal CA3-CA1 pathway. Four groups of adult male mice were exposed to 3-day IH, 7-day IH, 14-day IH and normoxia environment respectively. The paradigm of IH consisted of cycles of oxygen levels between 10% and 21% every 90 s during the daytime for 8 hrs. We found that there was a significant decrease in E-LTP in both 7-day IH group and 14-day IH group compared with the control group, but no significant change in 3-day IH group. A significant decrease in mature BDNF monomer was found in 3-day, 7-day and 14-day IH group. Similar trends were observed in mature BDNF dimer, while there was no significant change in pro-BDNF in both groups. Overall, these data indicate that neurocognitive deficits observed in OSA may be caused by IH-induced impairment in hippocampal LTP. A possible cause is the reduced expression of mature forms of BDNF.

Effect of hepcidin on brain iron homeostasis in iron-overloaded and parkinsonian rats

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Abnormal iron accumulation was demonstrated in many neurodegenerative diseases such as Parkinson's disease. Currently very little is known about the mechanisms of brain iron homeostasis. We speculated that hepcidin, an iron-regulatory hormone in the periphery, also regulates iron homeostasis in the CNS. We have shown previously that in cultured neurons and astrocytes, treatment with hepcidin peptide or hepcidin expressing-adenovirus (ad-hepcidin) reduced iron uptake and altered the expression of various iron transporters. Here we show that increasing brain hepcidin expression by an ad-hepcidin over-expression system decreased iron content and ROS level in the brain of iron over-loaded rats. Also, increased level of hepcidin down-regulated the expressions of the uptake transporters transferrin receptors, divalent metal transporter 1 and the iron exporter ferroportin 1 in different brain regions. Further *in vivo* studies showed that hepcidin over-expression significantly slowed down the rate of both transferrin-bound (TBI) and non-transferrin-bound (NTBI) iron uptake and promoted iron elimination from brain. Finally, in the 6-OHDA model of Parkinson's disease, increased hepcidin expression prevented the iron increase in the substantia nigra. Consistent with these observations, behavioral tests showed that hepcidin reduced apomorphine-induced rotation and protected dopamine neurons against 6-OHDA toxicity. It is concluded that hepcidin plays a crucial role in brain iron homeostasis and reducing the iron level by manipulating hepcidin expression could be beneficial in iron accumulation-associated neurodegenerative diseases.

S2-10

The role of Nogo on the guidance of commissural axons in the mouse spinal cord

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Nogo is a member of reticulon superfamily proteins that consists of 3 isoforms, Nogo-A, -B and –C. It inhibits axon regeneration in the adult central nervous system. Previous studies in our laboratory showed Nogo is an inhibitory molecule regulating axon divergence in the mouse optic pathway. Here we found that this inhibitory molecule was also expressed in the developing spinal cord and involved in commissural axon guidance. Using Nogo specific antibodies, both Nogo-A and Nogo-B were localized on neurons and radial glial cells. In the floor plate, only Nogo-B was localized on the radial glia processes when commissural axons are crossing the midline. Nogo-66 receptor (NgR) was expressed weakly by commissural axons initially but was upregulated after crossing. After blocking NgR activity with NEP1-40, midline crossing was dramatically reduced in open-book preparations of the spinal cord, both at E11 and E12. Growth cones stalled in the midline region were more complex in morphology and larger in size when compared with those in controls treated without the blocking peptide. In co-culture assays, E13 floor plate inhibited extension of post-commissural axons, and this inhibition was abolished by NEP1-40. Outgrowth of pre-commissural axons was enhanced in the presence of floor plate. Western-blot assays of conditioned medium collected from floor plate cultures showed that a 37-kDa form of Nogo that was secreted by the floor plate. These results suggest that Nogo is involved in repelling commissural axons out of the floor plate through NgR, and that this repulsion may be mediated by contact inhibition through membrane bound Nogo and long-range influence through diffusible form of the protein.

S2-11

Some insights into the development, ageing and degeneration on the central nervous system of mammals including human

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This presentation summaries and highlights some of the results from our research group on the development, ageing and ketamine induced degeneration of the central nervous system in the past twenty years. Developmental studies on the human revealed that prenatal developmental events had different timings for the different areas of the human CNS. Using a pig neonatal model, fMRI recordings further demonstrated a diffused response in the neonatal cortex which subsequently changed into an orderly pattern in the adult. In the aged human, more cortical areas were activated for a specific motor act and/or sensory stimulation than the young adult. These results could even be repeated in the mice as well. In abnormal ageing, the apoptotic patterns in the human cortex of presenile dementia patients in comparison with those of senile dementia of the Alzheimer type had significant differences. The cells survived in these individuals, appeared to be BDNF positive. In the Alzheimer disease patients, not only the cortical areas were involved, but also some nuclei in the brainstem, notably the trigeminal nucleus. In the ketamine treated monkeys and mice, positive hyperphosphorylated tau sites were observed in the cortices, although in different layers of the cortices of the two different animals. fMRI in these intoxicated monkeys showed activated areas in the cortices closely related to the documented signs and symptoms of ketamine addiction in the human. Apoptotic cell deaths were evident as well in the cortices of these monkeys and mice, confirming a real detrimental episode of neurodegeneration in these animals after addiction.

Is hydrogen sulfide important in vascular tone regulation?

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In addition to nitric oxide and carbon monoxide, hydrogen sulfide (H_2S) is the third gaseous molecule that may play a role in the physiology and pathophysiology of several organs. H_2S was first found to relax rat mesenteric arteries in a glibenclamide-sensitive manner (Cheng et al., 2004) and may also utilize different cellular and ionic mechanisms in other vascular bed. NaHS, the H_2S donor produces acute vasodilatations in rat coronary, cerebral and renal arteries. Endothelium plays little role in the effect of NaHS. The relaxant effect of NaHS is inhibited in arteries contracted by 60 mM KCl. NaHS reduces $CaCl_2$-induced tension of arteries bathed in a depolarizing solution. Glibenclamide does not affect NaHS-induced dilatations in these arteries but it abolishes the relaxation in rat aortas. By contrast, NaHS does not relax rat pulmonary arteries. NaHS at low concentrations inhibits endothelium-dependent contractions in renal arteries without affecting phenylephrine- induced contraction. The present results show that NaHS could relax systemic arteries but not pulmonary arteries and may inhibit calcium influx as the main mechanism responsible for the vasodilatations. The effect of ATP-sensitive K^+ channel blocker shows vascular bed difference and glibenclamide is without effect in most arteries. The inhibitory effect of NaHS on endothelium-dependent contractions suggests that H_2S may interfere with some steps in a cascade of cellular events leading to the release and action of endothelium-derived contracting factors. The physiological relevance and potential vascular benefit of endogenous H_2S remains controversial and certainly require further in-depth examination.

Reference

Cheng et al., (2004) Am J Physiolo Heart Circ Physiol 287:H2316-23.

Acute simvastatin inhibits K_{ATP} channels of porcine coronary artery myocytes

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Statins (3-hydroxy-3-methyl-glutaryl coenzyme A (HMG CoA) reductase inhibitors) consumption provides beneficial effects on cardiovascular systems. However, effects of statins on vascular K_{ATP} channel gatings are unknown. The aim of this study is to elucidate the underlying mechanisms involved in vascular K_{ATP} channels modulation by simvastatin. In porcine isolated coronary artery, the cromakalim (10 nmol/L to 10 μmol/L)- and pinacidil (10 nmol/L to 10 μmol/L)-induced concentration-dependent relaxation was inhibited by simvastatin (3 and 10 μmol/L). Simvastatin (1, 3 and 10 μmol/L) suppressed (okadaic acid (10 nmol/L)-sensitive) cromakalim (10 μmol/L)- and pinacidil (10 μmol/L)-mediated opening of whole-cell K_{ATP} channels of human left internal mammary artery and porcine coronary artery myocytes. Simvastatin (10 μmol/L) and AICAR (1 mmol/L) elicited a time-dependent, compound C (1 μmol/L)-sensitive [^{3}H]-2-deoxy-glucose uptake and an increase in $[ATP]_i$ levels. Simvastatin caused a time (2 - 30 min)- and concentration (0.1 - 10 μmol/L)-dependent increase of p-AMPKα-Thr^{172} and p-PP2A-Tyr^{307} expression, with no effect on p-LKB1-Ser^{428} and p-HMG CoA reductase-Ser^{871} expression. The enhanced p-AMPKα-Thr^{172} expression was inhibited by compound C, ryanodine (100 μmol/L) and KN93 (10 μmol/L). Simvastatin-induced p-PP2A-Tyr^{307} expression was suppressed by okadaic acid, compound C, ryanodine, KN93, phloridzin (1 mmol/L), ouabain (10 μmol/L), and in $[glucose]_o$-free or $[Na^+]_o$-free conditions. Simvastatin causes ryanodine-sensitive Ca^{2+} release which is important for AMPKα-Thr^{172} phosphorylation via Ca^{2+}/CaMK II. AMPKα-Thr^{172} phosphorylation causes $[glucose]_o$ uptake (and a $[ATP]_i$ increase), closure of K_{ATP} channels, and phosphorylation of AMPKα-Thr^{172} and PP2A-Tyr^{307} resulted. Phosphorylation (inactivation) of PP2A-Tyr^{307} occurs at a site downstream of AMPKα-Thr^{172} phosphorylation.

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TRPC5 is a pressure-sensor in aortic arch baroreceptor

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Arterial baroreceptors contain mechanosensor and mechanotransducer that can detect the change in blood pressure, and then transduce the mechanosignals into electrical signals, eventually coding into action potential frequency in sensory nerves. In the present study, we recorded a stretch-activated channel in the soma and the neurite terminals of aortic arch baroreceptor neurons. This channel was identified to be TRPC5, because its activity was abrogated by an anti-TRPC5 blocking antibody T5E3 and a dominant-negative TRPC5 construct T5DN. Disruption of TRPC5 function in baroreceptor neurons *in vivo* impaired the baroreceptor action potential firing in response to blood pressure elevation, and it also impaired the baroreceptor-mediated reflex control of heart rate. We conclude that TRPC5 is a mechanosensitive channel participating in the mechanosensing and mechanotransducing at the arterial baroreceptor.

Traditional Chinese Medicines in the treatment of Cancer

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Surgery, chemotherapy and radiotherapy have been using as the basic ways for cancer treatment. More recently, there had been increased comprehensive researches on the application of traditional Chinese medicine (TCM) in cancer therapy. During the application of TCM, concerns have been put on the sources of herbal materials as well as the preparation and fractionation of the herbs. In the presentation, bioassay-guided fractionation method and structure-activity-relationship study of the active component of TCM will be discussed. Moreover, the concerns in using herbs as research materials such as authentication and standardization will be presented. In our laboratory, various *in vitro* and *in vivo* models have been set up for screening and mechanistic studies of compounds isolated from TCM. *In vitro* platforms covering different areas of cancer research included proliferation, immunomodulation, gene expression, angiogenesis and metastasis. *In vivo* models such as xenograft on nude mice, zebrafish and Green Fluorescence Protein (GFP)-expressed metastatic tumour model have been used to further verify the efficacy of potential compounds. In the presentation, pheophorbide a isolated from *Scutelleria barbata* (半枝蓮) and polyphyllin D isolated from *Paris polyphylla* (七葉一枝花) will be given as examples to illustrate how these platforms are used in our studies.

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Epigenetic inactivation of *paired box gene 5* is associated with poor prognosis in gastric cancer patients

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Background & Aims: Using methylation-sensitive representational difference analysis, we identified that *paired box gene 5* (*PAX5*) is involved in human cancer. We analysed its epigenetic inactivation, biological functions and clinical application in gastric cancer.

Methods: *PAX5* promoter methylation was evaluated by bisulfite genomic sequencing. The effects of *PAX5* re-expression were determined in proliferation, cell cycle, apoptosis, migration and invasion assays. Immunoprecipitation (ChIP) and cDNA expression arrays were performed to reveal the molecular basis of the *PAX5* functions.

Results*:* *PAX5* was frequently silenced or down-regulated in gastric cancer cell lines and primary gastric cancers. The downregulation of *PAX5* was closely linked to the promoter hypermethylation status and could be restored with demethylation treatment. Re-expression of *PAX5* in gastric cancer cell lines reduced cell growth, arrested cell cycle in G_0/G_1 phase, induced cell apoptosis, repressed cell migration/invasion and retarded tumor growth in nude mice. The anti-tumorigenic function of *PAX5* were at least by up-regulating downstream targets *P53, P21, BCL2-associated X protein, metastasis suppressor 1* and *tissue inhibitors of metalloproteinase 1*; and down-regulation of *BCL2, CyclinD1, mesenchymal epithelial transition factor (MET)* and *matrix metalloproteinase 1*. ChIP assay demonstrated that PAX5 directly bound to the *p53* and *MET* promoters. Moreover, *PAX5* hypermethylation was detected in 90% (145 of 161) of primary gastric cancers compared with 16% (3 of 19) of non-cancer tissues ($P < .0001$). Gastric cancer patients with *PAX5* methylation had a significant poor survival compared with the unmethylated cases.

Conclusions: *PAX5* is a novel functional tumor suppressor in gastric carcinogenesis. Detection of methylated *PAX5* can be utilized as an independent prognostic factor.

Apoptosis in breast cancer cells induced by French Bean hemagglutinin

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Hemagglutinins and lectins are carbohydrate-binding proteins produced by a variety of organisms including human, other animals, plants and fungi. They exhibit many activities including antitumor, immunomodulatory, antibacterial, antifungal, antiviral and insecticidal activities.

A dimeric 64-kDa hemagglutinin was isolated with a high yield from French beans using a chromatographic protocol that involved Blue-Sepharose, Q-Sepharose, and Superdex 75. Its N-terminal sequence resembled those of other *Phaseolus* hemagglutinins. It inhibited proliferation of hepatoma HepG2 cells and breast cancer MCF-7 cells with an IC_{50} of 100 μM and 2 μM, respectively. On the other hand, it did not affect proliferation in normal embryonic liver WRL68 cells. After exposure of MCF-7 cells to the hemagglutinin for 24 hours, a number of changes were detected in the cells. Growth arrest in the G0/G1 and G2/M phases was observed. The number of cells undergoing early apoptosis and late apoptosis increased. A disruption of mitochondrial transmembrane potential and disorganization of the inner mitochondrial membrane were induced. It appears that apoptosis was induced by the hemagglutinin due to the release of cytochrome c from the mitochondria. Western blot analysis disclosed that the hemagglutinin induced apoptosis through the death receptor-mediated pathway.

Orphan nuclear receptors, estrogen-related receptors, in prostate cancer

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We have been studying on members of ligand-independent orphan nuclear receptors **estrogen-related receptors** (**ERRs**) in prostate cancer, based on a working hypothesis that these nuclear receptors may play roles in growth regulation of prostate cancer cells. Recent studies indicate that ERRs are involved in diverse metabolic, developmental and cellular differentiation processes in many target cells and tissues. ERRs consist of three closely related members (**ERRα,β,γ**), which share high homology with estrogen receptors (ERs) particularly in their DNA-binding domain. However, ERRs are constitutively active without estrogen binding and are genuine orphan nuclear receptors. Therefore, it has been hypothesized that the ligand-independent ERRs may functionally crosstalk with the ligand-dependent ERs in target cells including prostatic cells. However, recent advances also reveal that ERRs may function independently via novel signaling pathways. (1) In the study of ERRγ (**Yu** et al., *Cancer Research* 2007), we showed that its stable expression suppressed significantly both *in vitro* cell growth and *in vivo* tumorigenicity of prostate cancer cells (LNCaP and DU145) via a cell-cycle arrest at G1/S phase and also induction of two cyclin-dependent kinase inhibitors p21 and p27. We further showed by reporter assay that induction of p21 and p27 by ERRγ was mediated through direct transactivation of their gene promoters. Our findings suggest for first time that ERRγ performs an anti-proliferative or tumor-suppressing function in prostate cancer cells. Importantly, our results suggest that ERRγ could be a potential novel therapeutic target for prostate cancer treatment as its anti-proliferating activity could be potentiated by an ERRβ/γ agonist. Similarly, in the study of another ERR subtype ERRβ (**Yu** et al., *Oncogene,* 2008), we demonstrated that ERRβ also performs a similar tumor suppressor function in prostate cancer partly via its inhibitory role in cell-cycle regulation and direct induction of p21. We further confirmed that p21 is a direct ERRβ-target gene, and its induction by ERRβ is responsible for its growth inhibitory effect in prostate cancer cells. Similar to ERRγ, ERRβ-mediated growth inhibition could be potentiated by *in vitro* treatment with an ERR-agonist (DY131) and its specific inhibitory effect was confirmed by shRNA-knockdown of ERRβ.

S3-5

Cytochrome P450-mediated metabolic activation of pyrrolizidine alkaloids and biomarker for the assessment of pyrrolizidine alkaloids-induced hepatotoxicity

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With the rapidly growing global interest in the use of natural products as medical remedies, dietary supplements, and functional foods, natural products have gained more attentions with regard to their curative properties as well as their potential health hazards. The growing awareness of safety issues of natural products, including Chinese medicines (CM), consequently leads to more stringent government regulations and public concerns. Among various phytotoxins, pyrrolizidine alkaloids (PA), which naturally and diversely distribute across the plant kingdom, are one of the most common causes of food/herb poisonings in foraging animals and humans. One major area of our current research involves the study of cytochrome P450-mediated metabolic activation and tissue-selective toxicity of natural products, and the current focus in this area is on the investigation of the mechanism of PA-induced hepatotoxicity and development of a mechanism-based biomarker for the assessment of such toxicity. Most of the naturally occurring PAs are hepatotoxic and may induce cancer. Regardless of structural differences all hepatotoxic PAs undergo P450-mediated bioactivation to generate the corresponding pyrrlic metabolites, which further react with proteins to form pyrrole-protein adducts leading to toxicity. We have developed a novel and specific LC-MS method, and also raised an antibody and developed immunoassay to specifically determine pyrrole-protein adducts. Using these methods we have, for the first time, unequivocally identified pyrrole-protein adducts in biological samples of patients and rats exposed to PA-containing CMs. Our study demonstrated that the pyrrole-derived protein adduction correlates well with PA-induced hepatotoxicity and has a potential to be developed as a biomarker for the assessment of PA-induced hepatotoxicity. These findings further our understanding of the mechanism of PA intoxication and may provide better methods for determining the risk associated with PA exposure and possible preventive measures for PA-containing CM-induced hepatotoxicity and carcinogenicity.

Drug responsiveness of the GI tract in the senescence-accelerated mouse

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There is generally a reduction in functionality of the gastrointestinal (GI) tract during aging. Interstitial cells of Cajal have been identified as pacemaker cells for contractile activity of the GI tract. In the present studies, we investigate their 'slow wave' activity using radiotelemetric recordings of gastric myoelectric activity (GMA) in 12-month old senescence-accelerated mice (SAMP8) and aged matched controls (SAMR1) during feeding and following treatment with the muscarinic agonist, bethanechol. The postprandial dominant frequencies (DF) of the gastric slow waves in SAMR1 and SAMP8 were 6.80 ± 0.45 (n=8) and 6.79 ± 0.55 (n=7), respectively ($P>0.05$). Bethanechol (6 mg/kg, s.c.) enhanced bradygastria and reduced tachygastria in SAMR1 ($P<0.05$), but no change in DF or normogastria was observed. Bethanechol could not be studied in SAMP8 as 7 out of 10 animals had to be excluded from the analysis since no DF could be observed during the 1h baseline recordings ($P<0.05$). Further studies in other age groups are required to assess the significance of the present findings. SAMP8 are also used to model Alzheimer's disease, so future studies will also track changes of GI function with mechanisms involved in cognition.

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Testing drugs in transgenic mouse models of Alzheimer's disease and tauopathies

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Abnormally aggregated proteins may cause most neurodegenerative disease. Examples are oligomers of A peptide in Alzheimer's disease (AD) and phosphorylated tau protein in tauopathies (including AD and frontotemporal dementia). Several transgenic mouse models mimic the pathological and behavioral changes of AD. Amongst these, JNPL3 mice overexpress mutant tau and develop neurofibrillary tangles (NFT) and motor deficits, and Tg2576 mice overexpress mutant APP (that produces A) and develop amyloid plaques and memory loss. Double transgenic JNPL3xTg2576 mice have both plaques and NFT. APP and tau may interact in contributing to AD. We breed JNPL3, Tg2576, and JNPL3xTg2576 mice, which we treated with curcumin, a phenolic compound found in turmeric (*geung wong*) that disaggregates A , or 17-AAG, which induces heat shock protein degradation of aggregated proteins. The number of NFT was decreased in JNPL3 mice fed curcumin. Curcumin reduced mortality in all mice ($p=0.05$) and in all males ($p=0.009$) but not females. Amongst strains, JNPL3xTg2576 males ($p=0.012$) had the most significant mortality reduction. 17-AAG tended to decrease NFT in males ($p=0.06$) but increase NFT in females ($p=0.04$), and tended to preserve motor function in all mice ($p=0.14$). Drugs decreasing aggregated tau show benefit in a mouse model of AD.

HuR: a novel molecular target for modulating ABCG2-mediated multidrug resistance in cancer chemotherapy

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ATP-binding cassette (ABC) drug efflux transporters play an important role in the regulation of intracellular drug concentrations, thereby determining cell sensitivity to chemotherapeutic agents. Overexpression of ABCG2, one of these transporters, is frequently observed in human cancers, conferring multidrug resistance (MDR) to a broad spectrum of anticancer drugs. The identification of molecular pathways and targets that can modulate ABCG2 expression will aid the development of novel therapeutic strategies for overcoming MDR.

The stress-response protein HuR is an RNA-binding protein that regulates gene expression posttranscriptionally. Elevated cellular level of HuR has been observed in various cancer types and is correlated with advanced malignancy. We have developed several ABCG2-overexpressing drug-resistant cell lines by prolonged selection with anticancer drugs. Surprisingly, high HuR level is noted in these resistant sublines but not in the corresponding parental cell lines. We found that the elevated level of HuR in the resistant cells stabilizes ABCG2 mRNA, thereby increasing drug efflux and decreasing cytotoxicity of substrate drugs. Interestingly, HuR level is also affected by a microRNA previously found to regulate ABCG2. The coordinate regulation of ABCG2 by this microRNA through (1) a direct repressive effect and (2) an indirect pathway involving HuR could account for the different response between individual cancer patients to chemotherapy.

The antiviral mechanism of trichosanthin

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Trichosanthin is a plant protein with antiviral activity. The mechanism of its action is widely believed to be mediated through ribosome inactivation. It was recently discovered that trichosanthin selectively induce more apoptosis in HIV or HSV infected cells than the respective normal host cells. The apoptosis pathway appeared to be different. The more potent pathway involving mitochondria depolarization is activated in HSV infected cells. Selective apoptosis appears to be responsible in part to the antiviral mechanism of trichosanthin.

S3-10

Characterization of HIV-1 integrase nuclear translocation for the development of new class of anti-AIDS drugs

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Translocation of viral integrase from cytosol into nucleus is a critical precondition of integration during the life cycle of HIV, the causative agent of Acquired Immunodeficiency Syndromes (AIDS). It has been considered as an important target for the drug development against HIV infection. In order to understand the detailed mechanisms of integrase-host cell protein complex interactions, we have constructed an HIV-1 integrase-EGFP vector as visible tag to monitor the translocation process in a fluorescence-based cell imaging assay.. When transiently transfected this vector into HeLa cells, the EGFP: integrase is mainly localized in the nucleus. We hypothesize that any drugs that can inhibit the translocation process are novel class of drugs for treating patients with HIV infection. Using the method of virtual screening, we have screened ~ 60000 compounds from natural and synthetic drug databases. Eight synthetic compounds and one natural product were found to block integrase translocation significantly in our cell-based assay. Moreover, two of the compounds were found to show significant inhibition on P24 production in viral replication assay. The results of the present study demonstrated the validity to apply virtual drug screening to search for protein-protein interaction inhibitors as potential lead candidates for drug development.

Peptides targeting tumors for imaging and anti-cancer drug delivery

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Ligand-mediated diagnosis and targeted drug therapies have significant clinical implications in cancer patients. In this study, we biopanned and identified peptides specifically targeting cancer cells and tumor vasculature for image detection and drug delivery. We established an orthotopic colorectal cancer model in mice and applied *in vivo* phage library to isolate peptides specifically recognizing the vasculature and cancer cells in colorectal cancer tissues. Using a double immunofluorescent staining for phage (peptide) and blood vessels, a phage was identified. It homed to the colorectal cancer tissues by 11 to 90 folds more than other organs. Chemical synthetic peptide (TCP-1) displayed by the phage inhibited the homing ability of the phage to the tumor mass. Meanwhile, immunostaining analysis indicated that TCP-1 after injection at the first and second round of circulation in animals localized only in the vasculature of colorectal cancer tissues. This peptide could also internalize into colon cancer cells when incubated *in vitro*. TCP-1 when conjugated with a proapoptotic peptide or an anti-cancer drug specifically induced targeted apoptosis in tumor blood vessels and cell cycle arrest *in vitro*. This study strongly indicates the therapeutic potential of this compound for colorectal cancer and perhaps other solid tumors in the gastrointestinal tract.

Organotypic cultures for skin research

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The skin is the largest organ which covers and protects the body from environmental noxious stimuli, such as ultraviolet radiation, mechanical trauma, and chemical and biological insults. Although conventional monolayer cell cultures or static 2-dimentional cell-cell co-cultures have made major advances in understanding the biological and physiological properties of different skin epidermal cells, these cell models cannot reflect the complicated physiological and anatomical skin microenvironment. Since the use of animals for skin experiments is controversial because of ethical and legal reasons, an in-vivo like 3-dimentional organotypic culture (OTC) has become an alternative non-animal model to study normal skin development, contact allergen and drug screening, viral infection, wound healing, and skin carcinogenesis and invasion in both pharmaceutical and therapeutic research. Using various constituent cell populations, we have recapitulated many aspects of the differentiated structure and functions of the skin in our OTCs to study expression of certain proteins during skin development and differentiation.

Regulation of human mast cells

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The mast cell is an important effector of the immune system due to its repertoires of preformed and newly synthesized chemical mediators and cytokines. Although it has been conventionally associated with type 1 hypersensitivity reaction, it is now recognized to participate in all pathophysiological conditions involving inflammation. Since mast cells mature locally in tissues, the studies of human mast cells have been hampered by the lack of a convenient source. We have developed a protocol for culturing mature human mast cells from circulating progenitors isolated from human buffy coat preparations to overcome this problem. These cultured mast cells demonstrated similar phenotypic characteristics of primary mast cells isolated from human lungs. However, addition of IL4 and IL-9 altered the phenotypic characteristics to those expressed by mast cells found in skin. Pharmacological studies of actions of locally produced inflammatory chemical mediators, such prostaglandin E_2 and adenosine, provide new directions for the design of mast cell modulating drugs for the management of inflammatory conditions. The identification of toll-like receptors by these cells suggested that they are also involved in the defense of microbial infections. They have been a useful *in vitro* model for us to study the roles of mast cells in clinical conditions, such as asthma and aspirin hypersensitivity. The roles of mast cells in other inflammation associated conditions such as cancer remain to be further explored.

S3-14

Functional study of cancer related genes by RNAi

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My active research interest is functional genomics and cancer molecular biology. RNAi and lentiviral vector are two technology platforms used in my team. We also aim to develop RNAi-based antiviral therapeutics for HIV and Avian flu. We have developed an inducible RNAi system for mammalian cells and demonstrated the critic role of CXCR4 gene in tumor metastasis. We also established lentiviral RNAi system. We found that EZH2 played an important role in hepatocellular carcinoma as well as in liver metastasis of pancreatic cancer. We further investigated the mechanism underlying EZH2 leading to cell proliferation. Most recently, we tested the potential of small molecule pharmacologic inhibitor of EZH2 for cancer therapy.

Hedgehog signaling potentiates the development of osteosarcoma

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Osteosarcoma is one of the most common primary malignant bone cancers. However, the initiation of this disease remains largely unclear. Our previous studies demonstrated that Hedgehog (Hh) signaling severely perturbed bone turnover rate during adult bone homeostasis and showed a site-specific bone overgrowth in the long bones. Since Hh signaling has been implicated in the development of multiple cancers such as basal cell carcinoma and medulloblastoma, we hypothesize that Hh signaling plays an important role in the development of osteosarcoma. Here, we generated a genetic engineered mouse model with heterozygous deletion of *p53* and partially upregulated Hh signaling specifically in mature osteoblasts by removing a *Ptch1* allele. We found that osteosarcoma development is dependent on partial loss of p53 and significantly potentiated by upregulation of Hh signaling. We established cell lines from the mutant osteosarcomas and found that they are highly proliferative. This increased proliferation is significantly inhibited when treated with cyclopamine, an Hh specific inhibitor. In addition, these immortalized cell lines possess some degree of plasticity that they can be induced to differentiate into either osteoblasts or adipocytes. This multipotency is correlated to a significant portion of tumor cells express markers associated with stem/progenitor cells. The tumorigenic potential and their metastatic properties will be further tested in immunocompromised mice and the underlying mechanism for the development of osteosarcoma will be investigated.

What have we learnt from orphan GPCR *Mas*?

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Mas was originally isolated from a human epidermoid carcinoma and predicated to be an orphan GPCR. Pharmacological studies suggested that *mas* may be an angiotensin receptor, but contradictory results have been reported. To search for a surrogate agonist for mas, a phage-displayed random peptide library was panned against *mas*-overexpressing cells and a peptide agonist MBP7 was identified. Functional studies indicated that MBP7 induced inositol phosphate accumulation, calcium mobilization and Erk1/2 phosphorylation in cells stably overexpressing *mas*. Furthermore, responses to MBP7 critically depended on levels of *mas* expression and availability of the putative C-terminal tail of mas. However, it is of interest to note that while there was no significant difference in the basal intracellular calcium levels, basal Erk1/2 phosphorylation was suppressed in *mas*-transfected cells in comparison with the empty vector-transfected controls.

Despite GPCRs having been implicated to play an active role in tumourigenesis, the underlying mechanism of how GPCRs orchestrate tumour formation is largely unknown. To characterize mas-induced tumour formation, a series of stably *mas*-transformed cell lines, overexpressing mas at different levels, were constructed using DHFR-mediated *mas* transgene amplification. Consistent with *in vitro* cellular responses, frequency and rate of xenograft tumour formation were positively correlated with levels of *mas* expression while no significant difference was found in tumour weight. Cellular studies suggest *mas* overexpression enabled anchorage-independent growth. mRNA profiling indicated an upregulation of chemokine genes.

Taken together, these results suggest that *mas* encodes a conventional GPCR with agonist-independent intrinsic activity which may contribute to the oncogenic development following *mas* overexpression.

Manipulating BRE, a TNF modulator, expression to improve the function of human umbilical cord perivascular (HUCPV) mesenchymal progenitor cells

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Human umbilical cord perivascular (HUCPV) progenitor cells behave like stem cells and have been touted for therapeutic use in regenerative medicine. We have recently discovered a gene called BRE that is highly expressed in these cells but we do not know the reason. Therefore, we propose to investigate whether BRE plays a role in regulating the self-renewal of HUCPV cells. In addition, the gene's role is in controlling HUCPV cell transdifferentiation and homing/migration. We have previously reported that BRE can bind TNFR1 and over-expressing BRE can protect cancer cells from TNFa-triggered apoptosis. This finding is highly significant to HUCPV cells since these cells might eventually be implanted into damaged tissues to elicit tissue repair. Proinflammatory cytokine TNFa is always present at high concentrations in damaged tissues, which potentially could be detrimental to implanted HUCPV cells. Hence, we want to determine whether over-expressing BRE in HUCPV cells could protected them from the deleterious effects of TNFa. This will be first investigated in vitro and then in vivo, using a freeze-damage leg muscle model. Furthermore, we will also examine BRE's role in the ubiquination of TNFR1 and proteins associated with the TNFR1 signaling pathway. The results generated from this study will indicate the usefulness of manipulating BRE expression to enhance some of the biological properties of HUCPV.